Biosynthesis, Structural Characterization and Biological Evaluation of Silver Nanoparticles Using Some Medicinal Plants

Submitted in Partial fulfillment of the Requirement for

the Award of the Degree of

DOCTOR of PHILOSOPHY

In

PHARMACEUTICAL SCIENCES

By

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FACULTY OF HEALTH SCIENCES

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ALLAHBAD-211007 (U.P.), INDIA

2018

DECLARATION

I, Deepika Singh that the work presented in this thesis entitled "**Biosynthesis, Structural Characterization and Biological Evaluation of Silver Nanoparticles Using Some Medicinal Plants"** submitted to the Department of Pharmaceutical Sciences, SIHAS, Sam Higginbottom University Of Agriculture, Technology & Sciences, Allahabad, for the award of the Degree of Doctor of Philosophy in Pharmaceutical Sciences an original work. I have neither plagiarized nor submitted the same work for the award of any other degree. In case, this undertaking is found incorrect, my degree may be withdrawn unconditionally by the university.

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Content i
List of tables ix
List of figures xiii
List of abbreviation xviii
Abstract xxii

Chapter	Section	Title	Pg. No.
1.	**1.**	**INTRODUCTION**	1
	1.1	Medicinal plants	1
	1.2	Cancer	2
	1.2.1	Characteristics of cancer	3
	1.2.2	Histological types of cancer	4
	1.2.3	Hepatic cancer	4
	1.2.4	Renal Cancer	8
	1.3	Antioxidants: endogenous and exogenous	11
	1.3.1	Chemoprevention	13
	1.3.2	Benefits of natural products in cancer	14
	1.4	Nanoparticles	16
	1.4.1	Nanoparticles as a targeted drug delivery system	16
	1.4.2	Advantages of nanoparticles	17
	1.4.3	Metallic nanoparticle	17
	1.4.4	Method of Preparation of metal nanomaterial	19
	1.4.5	Silver nanoparticle	20
	1.5	Plant profile	29
	1.5.1	Amla	29
	1.5.2	Mahua	32

	1.5.3	Karonda	35
	1.6	Justification of study	38
	1.7	Objectives	39
2.	**2.**	**Review of Literature**	40
	2.1	Literature review of *Phyllanthus emblica*	40
	2.2	Literature review of *Madhuca longifolia*	45
	2.3	Literature review of *Carissa carandas*	49
3.	**3.**	**Materials and Method**	53
	3.1	Collection and authentication of selected plants	53
	3.2	Pharmacognostical study	53
	3.2.2	Morphological or organoleptic evaluation	54
	3.2.3	Microscopy evaluation	54
	3.2.4	Physio-chemical evaluation	57
	3.3	Extraction	59
	3.3.1	Preparation of aqueous plant extract	59
	3.4	Preliminary phytochemical analysis	62
	3.5	Preparation of the solution and plant extract	65
	3.5.1	Preparation of metal precursor solution	65
	3.5.2	Synthesis of silver nanoparticles	66
	3.6	Characterization techniques	66
	3.6.1	Ultraviolet–visible spectroscopy	66
	3.6.2	Fourier Transform Infrared spectroscopy	67
	3.6.3	X-ray diffractometry	68
	3.6.4	FESEM with EDX	68

	3.6.5	TEM	69
	3.7	*In vitro* cytotoxic activity	69
	3.7.1	Preparation of cell culture	69
	3.7.2	Study of anticancer property	69
	3.8	*In vivo* hepatic cancer and renal cancer activity	70
	3.8.1	Animals	70
	3.8.2	Chemicals	70
	3.8.3	LD_{50} determination by acute toxicity study	70
	3.8.4.	Study design	71
	3.8.5.	Preparation of liver homogenate	75
	3.8.6.	Biochemical analysis from serum	75
	3.8.7	Determination of lipid peroxidation	80
	3.8.8	Determination of enzymatic and non-enzymatic antioxidant parameter	81
	3.8.9	Histological examination	83
	3.9.9	*In vivo* renal cancer	84
	3.9.10	Post-mitochondrial supernatant (PMS) and microsomes preparation	84
	3.9.11	Assay of serum marker enzyme	84
	3.9.12	Estimation of tumour marker enzyme	85
	3.9.13	Estimation of lipid peroxidation	86
	3.9.14	Estimation of enzymatic and non-enzymatic antioxidant profile	87
	3.9.15	Evaluation of cytokines/inflammatory mediators	89
	3.9.16	Estimation of tumour promotion markers	89
	3.9.17	Histopathological analysis	90

	3.10	Statistical Analysis	90
4	**4.**	**Results**	91
	4.1	Pharmacognostical character of leaf of *Phyllanthus emblica*	91
	4.1.1	Macroscopical characters of leaf of *Phyllanthus emblica*	91
	4.1.2	Microscopic characters of leaf of *Phyllanthus emblica*	91
	4.2	Extraction of Aqueous extract of *Phyllanthus emblica*	92
	4.3	Preliminary phytochemical studies of aqueous extract of *Phyllanthus emblica*	93
	4.4	Biosynthesis of silver nanoparticles of extract of *P.emblica*	94
	4.5	Characterization of silver nano-drugs of PEAgNPs	95
	4.5.1	UV-Vis spectral analysis of PEAgNPs	95
	4.5.2	FTIR spectral analysis of PEAgNPs	96
	4.5.3	XRD analysis of PEAgNPs	99
	4.5.4	Field Emission Scanning Electron Microscope (FESEM) of PEAgNPs	100
	4.5.6	Energy dispersive X-ray (EDX) analysis of PEAgNPs	101
	4.5.7	TEM of PEAgNPs	102
	4.6	*In vitro* cytotoxic activity of PEAgNPs against HuH-7 Cell line	103
	4.7	*In vivo* hepatic cancer studies of PEAgNPs on rats	103
	4.7.1	Effect of green synthesized PEAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC in rats	104
	4.7.2	Effect of PEAgNPs on the development of liver nodules in control and DEN- induced HCC rats	104
	4.7.3	Effect of PEAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats	107
	4.7.4	Effect of PEAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats	108

	4.7.5	Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	110
	4.7.6	Effect of PEAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats	111
	4.7.7	Effect of PEAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats	113
	4.7.8	Effect of PEAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats	113
	4.7.9	Histopathological observation	113
	4.8	Renal cancer	118
	4.8.1	Effect of PEAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats	118
	4.8.2	Effect of PEAgNPs on serum marker enzymes of control and DEN induced RCC rats	119
	4.8.3	Effect of PEAgNPs on tumour marker enzyme in control and DEN induced RCC rats	120
	4.8.4	Effect of PEAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats	121
	4.8.5	Effect of PEAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats	121
	4.8.6	Effect of PEAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN-induced RCC in rats	122
	4.8.7	Effect of PEAgNPs on ODC activity and thymidine incorporation in kidney of control and DEN- induced RCC in rats	123
	4.8.8	Histopathology observation	126
	4.9	Pharmacognostical character of leaf of *Madhuca longifolia*	128
	4.9.1	Macroscopical characters of leaf of *Madhuca longifolia*	128

	4.9.2	Microscopic characters of leaf of *Madhuca longifolia*	128
	4.10	Extraction of Aqueous extract of *Madhuca longifolia*	129
	4.11	Preliminary phytochemical studies	130
	4.12	Synthesis of Silver nanoparticles of *Madhuca longifolia*	131
	4.13	Characterization of biosynthesized AgNPs of *Madhuca longifolia* extract	132
	4.13.1	UV spectral analysis of MLAgNPs	132
	4.13.2	FTIR spectra analysis of MLAgNPs	133
	4.13.3	XRD pattern of MLAgNPs	136
	4.13.4	Field Emission Scanning Electron Microscope (FESEM) of MLAgNPs	137
	4.13.5	Energy dispersive X-ray (EDX) analysis of MLAgNPs	138
	4.13.6	TEM study of MLAgNPs	139
	4.14	*In vitro* cytotoxic activity of MLAgNPs against HuH-7 Cell line	140
	4.15	*In vivo* hepatic cancer Studies of MLAgNPs on rats	141
	4.15.1	Effect of green synthesized MLAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats	141
	4.15.2	Effect of MLAgNPs on the development of liver nodules in control and DEN- induced HCC rats	142
	4.15.3	Effect of MLAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats	144

	4.15.4	Effect of MLAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats	145
	4.15.5	Effect of MLAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	148
	4.15.6	Effect of MLAgNPs on lipid peroxidation in liver of control and DEN- induced HCC in rats	150
	4.15.7	Effect of MLAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats	151
	4.15.8	Effect of MLAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats	151
	4.15.9	Histology of liver tissue	152
	4.16	Renal cancer	157
	4.16.1	Effect of MLAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats	157
	4.16.2	Effect of MLAgNPs on serum marker enzymes of control and DEN induced RCC rats	157
	4.16.3	Effect of MLAgNPs on tumour marker enzyme in control and DEN induced RCC rats	159
	4.16.4	Effect of MLAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats	160
	4.16.5	Effect of MLAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats	160
	4.16.6	Effect of MLAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN-induced RCC in rats	161

	4.16.7	Effect of MLAgNPs on tumour promotion markers in kidney of control and DEN- induced RCC in rats	161
	4.16.8	Histopathological studies of renal tissue	165
	4.17	Pharmacognostical character of leaf of *Carissa carandas*	167
	4.17.1	Macroscopical characters of leaf of *Carissa carandas*	167
	4.17.2	Microscopic characters of leaf of *Carissa carandas*	167
	4.18	Extraction of aqueous extract of *Carissa carandas*	168
	4.19	Preliminary phytochemical studies	169
	4.20	Synthesis of silver nanoparticles of *Carissa carandas*	170
	4.21	Characterization of silver nano-drugs of *Carissa carandas*	171
	4.21.1	Structural characterization of CCAgNPs by Absorbance spectroscopy	171
	4.21.2	FTIR spectroscopy of CCAgNPs	172
	4.21.3	XRD analysis of CCAgNPs	173
	4.22.4	FESEM with EDX analysis of CCAgNPs	174
	4.22.5	EDX analysis of CCAgNPs	174
	4.22.6	TEM analysis of CCAgNPs	175
	4.23	In vitro cytotoxic activity of PEAgNPs against HuH-7 Cell line	177
	4.24	In vivo hepatic cancer Studies of CCAgNPs on rats	178
	4.24.1	Effect of green Synthesized CCAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats	178
	4.24.2	Effect of CCAgNPs on the development of liver nodules in control and DEN- induced HCC rats	178
	4.23.3	Effect of CCAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats	178
	4.24.5	Effect of CCAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats	182

	4.24.6	Effect of CCAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	184
	4.24.7	Effect of CCAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats	186
	4.24.8	Effect of CCAgNPs on the levels of antioxidant enzymes in liver of rats	187
	4.24.9	Effect of CCAgNPs on the activities of membrane bound enzymes in rats	187
	4.24.10	Histopathology observation of hepatic tissue	187
	4.25	Renal cancer	192
	4.25.1	Effect of CCAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats	192
	4.25.2	Effect of CCAgNPs on serum marker enzymes of control and DEN induced RCC rats	193
	4.25.3	Effect of CCAgNPs on tumour marker enzyme in control and DEN induced RCC rats	193
	4.25.4	Effect of CCAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats	195
	4.25.5	Effect of CCAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats	196
	4.25.6	Effect of CCAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN-induced RCC in rats	199
	4.25.7	Impact of CCAgNPs on tumour promotion markers in kidney of rats	199
	4.25.8	Histopathology of CCAgNPs on renal tissue	199
5	**5**	**Discussion**	**203**
6.	**6.**	**SUMMARY & CONCLUSION**	228
7.	**7.**	**References**	234

		Appendix	
		List of publication	

List of tables

Table no.	Title	Page no.
1.1	Green synthesis of silver nanoparticles using plant extracts	25
3.1	Authentication of leaves of medicinal plants	53
4.1	Determination of leaf constant of *Phyllanthus emblica*	92
4.2	Physico-chemical parameters of leaves of *P.emblica*	92
4.3	Percentage yield of extract of leaves of *Phyllanthus emblica*	93
4.4	Preliminary phytochemical screening of aqueous extract of *Phyllanthus emblica*	93
4.5	XRD of PEAgNPs	99
4.6	in vitro cytotoxic activity of PEAgNPs on hepatic cancer (HuH-7 cell line)	103
4.7	Effect of green Synthesized PEAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats	105
4.8	Effect of PEAgNPs on the development of liver nodules in control and DEN induced HCC rats	106
4.9	Effect of PEAgNPs on the activities of marker enzymes in the serum of control and DEN induced HCC in rats	107
4.10	Effect of PEAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN induced HCC rats	109
4.11	Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN induced HCC rats	110
4.12	Effect of PEAgNPs on lipid peroxidation activity in control and DEN induced HCC rats	112
4.13	Effect of PEAgNPs on enzymatic and non-enzymatic antioxidant profile in control and DEN induced HCC rats	114
4.14	Effect of PEAgNPs on Membrane bound activities in control and DEN induced HCC rats	116
4.15	Effect of PEAgNPs on Macroscopic evaluation of DEN induced renal cancer	118
4.16	Effect of PEAgNPs on serum marker enzymes in rats	119
4.17	Effect of PEAgNPs on tumour marker enzyme in control and DEN induced RCC rats	120

4.18	Effect of PEAgNPs on lipid peroxidation in kidney of control and DEN-induced RCC in rats	121
4.19	Effect of PEAgNPs on the levels of antioxidant enzymes in kidney of control and DEN induced RCC in rats	123
4.20	Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in kidney of control and DEN induced RCC in rats	125
4.21	Effect of PEAgNPs on ODC and thymidine incorporation mediators in kidney of control and DEN induced RCC in rats	126
4.22	Determination of leaf constant of *Madhuca Longifolia*	129
4.23	Physico-chemical parameters of leaves of *Madhuca Longifolia*	129
4.24	Percentage yield of extract of leaves of *Madhuca Longifolia*	130
4.25	Qualitative chemical test on the aqueous extract of *Madhuca longifolia*	130
4.26	XRD pattern of MLAgNPs	136
4.27	*In vitro* cytotoxic activity of MLAgNPs on hepatic cancer (HuH-7 cell line)	140
4.28	Impact of MLAgNPs on the Body weight and relative liver weight in kidney of control and DEN induced RCC in rats	142
4.29	Effect of MLAgNPs on the development of macroscopic hepatic nodules in different groups of rats.	143
4.30	Effect of MLAgNPs on serum marker enzyme of hepatic parameter in liver of control and DEN induced HCC in rats	144
4.31	Effect of MLAgNPs on serum marker enzyme of non-hepatic parameter in liver of control and DEN induced HCC in rats	146
4.32	Effect of MLAgNPs on proinflammatory cytokines and inflammatory mediators control and DEN induced HCC in rats	148
4.33	Effect of MLAgNPs on lipid peroxidation activity in liver of control and DEN induced HCC rats	150
4.34	Effect of MLAgNPs on the levels of antioxidant enzymes in liver of control and DEN induced HCC in rats	153
4.35	Effect of MLAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats	155
4.36	Effect of MLAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats	157
4.37	Effect of MLAgNPs on serum marker enzymes of control and DEN induced RCC rats	158

4.38	Effect of MLAgNPs on tumour marker enzyme	159
4.39	Effect of MLAgNPs on lipid peroxidation in kidney of control and DEN-induced RCC rats	160
4.40	Effect of MLAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats	162
4.41	Effect of MLAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats	164
4.42	Effect of MLAgNPs on tumour promotion markers in kidney of control and DEN- induced RCC in rats	165
4.43	Determination of leaf constant of *Carissa carandas*	168
4.44	Physico-chemical parameters of leaves of *Carissa carandas*	168
4.45	Percentage yield of extract of leaves of Carissa *carandas*	169
4.46	Qualitative chemical test on the aqueous extract of *Carissa carandas*	169
4.47	XRD analysis of CCAgNPs	174
4.48	*In vitro* cytotoxic activity of CCAgNPs against HuH-7 Cell line	177
4.49	Effect of green Synthesized CCAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats	179
4.50	Effect of CCAgNPs on the development of macroscopic hepatic nodules in different groups of rats.	180
4.51	Effect of CCAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC in rats	181
4.52	Effect of CCAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats	183
4.53	Effect of CCAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	185
4.54	Effect of CCAgNPs on lipid peroxidation activity	186
4.55	Effect of CCAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats	188
4.56	Effect of CCAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats	190

4.57	Effect of CCAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats	192
4.58	Effect of CCAgNPs on serum marker enzymes of control and DEN induced RCC rats	193
4.59	Effect of CCAgNPs on tumour marker enzyme in control and DEN induced RCC rats	194
4.60	Effect of CCAgNPs on lipid peroxidation in kidney of control and DEN-induced RCC rats	196
4.61	Effect of CCAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats	197
4.62	Effect of CCAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats	200
4.63	Impact of CCAgNPs on tumour promotion markers in kidney of control and DEN- induced RCC in rats	201

List of figures

Figure no.	Title	Page no.
1.1	Characteristics of Cancer Cell	3
1.2	Different stages of development of tumors in Kidney	11
1.3	Possible mechanism of action of chemopreventive agents	14
1.4.	Different types of metal nanoparticles	18
1.5	Different method of preparation of metal nanoparticles	19
1.6	Different approach for the production of silver nanoparticles	21
1.7	Biosynthesis of silver nanoparticles	22
1.8	Leaves of *Phyllanthus emblica*	30
1.9	Tree of *Phyllanthus emblica*	31
1.10	Leaves of *Madhuca longifolia*	33
1.11	Tree of *Madhuca longifolia*	34
1.12	Leaves of *Carissa carandas*	36
1.13	Tree of *Carissa carandas*	37
3.1	Diagrammatic representation of extraction process	61
3.2	Treatment regimen for *Phyllanthus emblica*	72
3.3	Treatment regimen for *Madhuca longifolia*	73
3.4	Treatment regimen for *Carissa carandas*	74
4.1	Transverse section of leaf of *P.emblica*	91
4.2	Biosynthesis of silver nanoparticles of *P.emblica* aqueous extract	94

4.3	UV-Vis spectrum of PEAgNPs	95
4.4	FTIR spectrum: a) PEE b) Biogenic silver nanoparticles of *P.emblica*	97-98
4.5	XRD spectrum of synthesized PEAgNPs	100
4.6	FESEM images of Synthesized PEAgNPs	101
4.7	EDX spectrum of synthesized PEAgNPs	101
4.8	TEM images of synthesized PEAgNPs	102
4.9	*in vitro* cytotoxic activity of PEAgNPs on hepatic cancer cell line	103
4.10	Effect of PEAgNPs on serum marker enzyme of hepatic parameter	108
4.11	Effect of PEAgNPs on serum marker enzyme of non-hepatic parameter	109
4.12	Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	111
4.13	Effect of PEAgNPs on lipid peroxidation activity in liver of control and DEN- induced HCC in rats	112
4.14	Effect of PEAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats	115
4.15	Effect of PEAgNPs on Membrane bound activities in liver of control and DEN- induced HCC rats	116
4.16	Photomicrograph of eosin-haematoxylin stained histological liver section area for (a) Normal control group, (b) DEN induced liver cancer group (c) DEN+ PEE (d) DEN +PEAgNPs (10mg/kg bw) (e) DEN +PEAgNPs (20mg/kg bw), and (f) DEN+ Silymarin	117
4.17	Effect of PEAgNPs on serum marker enzymes	119
4.18	Effect of PEAgNPs on tumour marker enzyme	120
4.19	Effect of PEAgNPs on the levels of antioxidant enzymes	124
4.20	Effect of PEAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats	125
4.21	Effect of PEAgNPs on ODC activity and thymidine incorporation in kidney of control and DEN- induced RCC in rats	126

4.22	Photomicrograph of eosin-haematoxylin stained histological renal section area for (a) Normal Control group, (b) DEN induced renal cancer group (c) DEN+ PEE (d) DEN +PEAgNPs (10mg/kg bw) (e) DEN +PEAgNPs (20mg/kg bw)	127
4.23	Transverse section of leaf of *Madhuca Longifolia*	128
4.24	Biosynthesis of Silver nanoparticles of *Madhuca Longifolia* aqueous extract	131
4.25	UV-Vis spectrum of MLAgNPs	132
4.26	FTIR spectra a) MLE b) MLAgNPs	135
4.27	XRD spectrum of synthesized MLAgNPs	136
4.28	FESEM images of Synthesized MLAgNPs	137
4.29	EDX spectrum of synthesized MLAgNPs	138
4.30	TEM images of MLAgNPs	139
4.31	*In vitro* cytotoxic activity of MLAgNPs on hepatic cancer	140
4.32	Effect of MLAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats	145
4.33	Effect of MLAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats	147
4.34	Effect of MLAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	149
4.35	Effect of MLAgNPs on lipid peroxidation in liver of control and DEN-induced HCC in rats	151
4.36	Effect of MLAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats	154
4.37	Effect of MLAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats	155
4.38	Photomicrograph of eosin-haematoxylin stained histological liver section area of (a) Normal control group, (b) DEN induced liver cancer group (c) DEN+ Silymarin (d) DEN+ MLE (e) DEN +MLAgNPs (20mg/kg bw) , and (f) DEN +MLAgNPs (30mg/kg bw)	156

4.39	Effect of MLAgNPs on serum marker enzymes of control and DEN induced RCC rats	158
4.40	Effect of MLAgNPs on tumour marker enzyme	159
4.41	Effect of MLAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats	163
4.42	Effect of MLAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats	164
4.43	Effect of MLAgNPs on tumour promotion markers in kidney of control and DEN- induced RCC in rats	165
4.44	Photomicrograph of eosin-haematoxylin stained histological renal section area for (a) Normal Control group, (b) DEN induced renal cancer group (c) DEN+ MEE (d) DEN +MLAgNPs (20mg/kg bw) (e) DEN +MLAgNPs (30mg/kg bw)	166
4.45	Transverse section of leaf of *Carissa carandas*	167
4.46	Biosynthesis of Silver nanoparticles of Carissa *carandas* aqueous extract	170
4.47	UV-Vis spectrum of CCAgNPs	171
4.48	FTIR spectra a) CCE b) CCAgNPs	172-173
4.49	XRD analysis of CCAgNPs	173
4.50	FESEM with EDX analysis of CCAgNPs	175
4.51	TEM analysis of CCAgNPs	176
4.52	*In vitro* cytotoxic activity of CCAgNPs against HuH-7 Cell line	177
4.53	Effect of CCAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats	182
4.54	Effect of CCAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats	184
4.55	Effect of CCAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats	185
4.56	Effect of CCAgNPs on lipid peroxidation in liver of control and DEN-induced HCC in rats	186

4.57	Effect of CCAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats	189
4.58	Effect of CCAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats	190
4.59	Photomicrograph of eosin-haematoxylin stained histological liver section area for (a) Normal control group, (b) DEN induced liver cancer group (c) DEN+ Silymarin (d) DEN+ CCE (e) DEN +CCAgNPs (20mg/kg bw) , and (f) DEN +CCAgNPs (30mg/kg bw)	191
4.60	Effect of CCAgNPs on serum marker enzymes of control and DEN induced RCC rat	194
4.61	Effect of CCAgNPs on tumour marker enzyme in control and DEN induced RCC rats	195
4.62	Effect of CCAgNPs on the levels of antioxidant enzymes in kidney of rats	198
4.63	Effect of CCAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats	200
4.64	Impact of CCAgNPs on tumour promotion markers in kidney of control and DEN- induced RCC in rats	201
4.65	Photomicrograph of eosin-haematoxylin stained histological renal section area for (a) Normal Control group, (b) DEN induced renal cancer group (c) DEN+ CCE (d) DEN +CCAgNPs (20mg/kg bw) (e) DEN +CCAgNPs (30mg/kg bw)	202

List of Abbreviations

Abbreviation	Description
AST	Aspartate aminotransferase
DEN	Diethylnitrosamine
DLS	Dynamic light scattering
FTIR	Fourier transforms infrared spectroscopy
SEM	Scanning electron Microscope
TEM	Transmission electron microscope
^{13}C NMR	Carbon-13 nuclear magnetic resonance
A/G ratio	Albumin to globulins ratio
ABTS	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
ACS	American cancer of society
AD	Alzheimer diseases
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
b.w.	Body weight
BSA	Bovine serum albumin
BUN	Blood Urea Nitrogen

$CaC1_2$	Calcium chloride
CAT	Catalase
CCAgNPs	*Carissa carandas* silver nanoparticles
CCE	*Carissa carandas*
CCl_4	Carbon tetrachloride
CDNB	1-chloro -2, 4-dinitrobenzene
CNS	Central nervous system
CO_2	Carbon dioxide
CSCPEA	Committee for purpose of supervision of experiment on animals
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNPH	2,4- Dinitrophenyl hydrazine
DPPH	2,2-diphenyl-1-picrylhydrazyl
EAC	Ehrlich ascites carcinoma
EDS	Energy dispersive spectroscopy
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
G6PD	Glucose-6-phosphate dehydrogenase
GC-HRMS	Gas chromatography/high-resolution mass spectrometry
GC-MS	Gas chromatography–mass spectrometry
GGT	γ-glutamyl transpeptidase
GPx	Glutathione peroxidase
GR	Glutathione reductase
H_2O	Water

H_2O_2	Hydrogen peroxide
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HAEEO	Hydroalcoholic extract of *Emblica officinalis*
HBV	Hepatitis B virus
HCC	Hepatocellular cancer
HCCSC	Human colon cancer stem cells
HCL	Hydrochloric acid
HCV	Hepatitis C virus
HPLC-DAD	High performance liquid chromatography coupled with diode array detector
HPTLC	High-performance thin-layer chromatography
HR-TEM	High-Resolution Transmission Electron Microscopy
IEAC	Institutional animal ethics committee
LDH	Lactate dehydrogenase
LPO	Lipid peroxidation
MDA	Malondialdehyde
MELC	Methanol extract of *Carissa carandas* leaves
ML	*Madhuca longifolia*
MLAgNPs	*Madhuca longifolia* Silver nanoparticles
MLE	*Madhuca longifolia* extract
MLME	Methanolic extract of leaves of *M. longifolia*
MTT	-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
$NaBH_4$	Sodium borohydride

NaCl	Sodium chloride
NADH	Nicotamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcoholic fatty liver disease
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NCCS	National centre for Cell Science
NDDS	Novel drug delivery system
nm	Nanometer
ODC	Ornithine decarboxylase
OECD-423	Organization for Economic Co-operation and Development
PCA	Perchloric acid
PEE	Phyllanthus emblica extract
PEEP	Polyphenol extract of *Phyllanthus emblica*
PMS	Post-mitochondrial supernatant
RCC	Renal cell carcinoma
RCC	Renal cell carcinoma
Rpm	Revolution per minute
RT	Room Temperature
SIHAS	Shalom Institute of Health and allied sciences
SOD	superoxide dismutase
SPR	Surface plasmon resonance
TCA	Trichloroacetic acid
TNM	Tumor node malignant

TNM	Classification of Malignant Tumours
TP	Total protein
WHO	World health organization
XO	Xanthine oxidase
XRD	X-ray diffraction
RNA	Ribonucleic acid
TNF-α	Tumour necrosis factor-alpha
IL-1β	Interleukin 1-beta
IL-6	Interleukin-6
NF-kB	Nucleus factor kappa-light chain beta
ROS	Reactive oxygen species
SEM	Standard error of mean
pH	Hydrogen ion concentration
GSH	Glutathione
LD_{50}	Lethal dose to 50% population

Abstract

Oxidative stress and inflammation plays a pivotal role in the expansion and progression of hepatic cancer and renal cancer. Nanoparticle based drug delivery can quickly enhance the restorative capability of hepatic cancer and renal cancer. Silver nanoparticles synthesize from plant source of great importance due to their small size, economic, non- hazardous and different biomedical application. In the current study, the impacts of oxidative stress and proinflammatory markers of biosynthesized silver nanoparticles of *Phyllanthus Emblica*, *Madhuca longifolia*, *Carissa carandas* leaves against diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC) and renal cancer in wistar rats till 16 weeks with its underlying mechanism. The physico-chemical properties of biosynthesized silver nanoparticles were determined by Ultra-Visible spectroscopy, Fourier transform infrared spectroscopy, field emission scanning electron microscope, energy dispersive x-ray Analysis, x-ray diffraction studies and transmission electron microscopy. Silver nanoparticles significantly enhanced the process of recovery from hepatic cancer and renal cancer at both the dose level in animal models, which was ascertained by increased body weight, reduced hepatic knobs on the outer surface of liver and renal, reduced level of serum biochemical parameters, decreased lipid peroxidation, increased membrane bound enzymes, increased enzymatic and non-enzymatic antioxidants parameters viz. catalase, glutathione peroxidase, superoxide dismutase and alteration in the level of proinflammatory cytokines and mediators viz. tumor necrosis factor (TNF-α) and nuclear factor (NF-κB). Histopathological studies also affirmed the recovered hepatocellular and renal architecture with increased intercellular space and regained the shape of nuclei in cytoplasm in silver nanoparticles treated group. Our outcomes implicate successfully biofabrication of plants (*Phyllanthus Emblica*, *Madhuca longifolia, and Carissa carandas*) silver nano-particles and exhibited a chemoprotective potential in the prevention and intervention of hepatic cancer and renal cancer.

Keywords: *Phyllanthus Emblica*, *Madhuca longifolia, and Carissa carandas*), NF-κB,

Chapter-1

Introduction

1. Introduction

1.1 Medicinal Plants

It is well known that alternative medicines plays an important role in healthcare system around the globe. These alternative medicines are the products of plants, animals, synthetic substance and minerals in which plant based products have their own importance without any side effects. Plant serve as a rich source of medicines and near about 35 % of pharmaceutical industry based on the traditional and medicinal plant for their phytoconstituents which show potential therapeutic effects. Nature plays a promising role in providing us a variety of herbal drugs. The Indian subcontinent is a huge depository of medicinal plants that are utilize as a part of conventional therapeutic medications. India has folklore of botanical garden, these traditional plants has been used since ancient times and now become a part of a health care system (Bisht *et al*., 2010). There are variety of traditional and alternative system of medicines recognized in India and includes- ayurveda, unani, homeopathic, siddha, naturopathy, yoga and aromatherapy. Today WHO focuses on the standardization of herbal formulation for its safety, efficacy, purity and quality. Herbal plant possesses an active principle (chemical constituents) which has a tendency to combat with various diseases (Birdi *et al*., 2014). The use of medicinal plant still play an important role to cover the basic health needs in the developing countries and the industrialized societies have been traced to the extraction and development of several drugs of these plants as well as from traditionally used folk medicine. Today herbal extract is used to formulate into novel formulation to increase its potential (Majumder and Paridhavi, 2013).

In ancient time, it was supposed that life to be existed for last 6000 years ago in ayurveda. Ayurveda is considered as an oldest system of medicine worldwide. It was hypothesised that in the universe everything is composed of five basic elements i.e. space, air, liquid, solid and energy. In human body, they exist in mixed forms like vata, kapha, and pitta. medicinal herbs i.e. rasa, guna, virya, vipak and prabhava is used to treat the pathological

diseases (Joshi, 2004). Ancient medicine practitioners compiled a book on Ayurveda which is known as Samhita and proposes a number of dosage forms. Isolation of hypertensive alkaloids from *Rauwolfia Serpentina* (sarpaganda plant) was the first significant contribution in ayurveda "materica medica". The different medicinal plants were mentioned in Ayurveda are *Asparagus racemosus, Cassia angustifolia, Withania Somnifera, Piper longum, Amygdala amara, Solanum Khasianum, Acacia Chundra, Ricinus communis , Hedychium Spicatum, Apium Graveolens*. Ayurveda is not meant only for nutritional basis to abuse or rejuvenate the brain and body. It is a framework for strengthening, an arrangement of freedom and long life from diseases (Narayanaswamy, 1981).

1.2 Cancer

Hyperproliferation of cell and uncontrolled cell growth is termed as cancer. Cancer spread from single cell, changes by environmental conditions, genetic factors and it multiply continuously and invade surrounding tissue also. American cancer of society (ACS) defines that cancer is the common name for a collectively 100 diseases in which cell continuously grow in any part of the body which is out of control. It also spread throughout the blood and lymphatic system. When compare to normal tissue, the rate of new cell and old cells growth are in balance but this rate get disturbed in case of cancer cell (Marte, 2004). Cell suicide or Apoptosis is process in which old cell or destruct cell automatically undergoes self-destruct. When multiplication of cells take place, it generate growing mass of tissue is termed as tumour or neoplasm. There are two types of tumours benign or malignant. A benign tumour develops locally at the site of origin and does not spread whereas malignant tumours invade neighbour tissues and spread to other parts (Katheder *et al*., 2017).

1.2.1 Characteristics of cancer

There are major changes occur in cellular physiology which forms a malignant growth in cancer. Cancer Cell pro-survival traits are classified by five characteristics which are shown in figure 1.1.

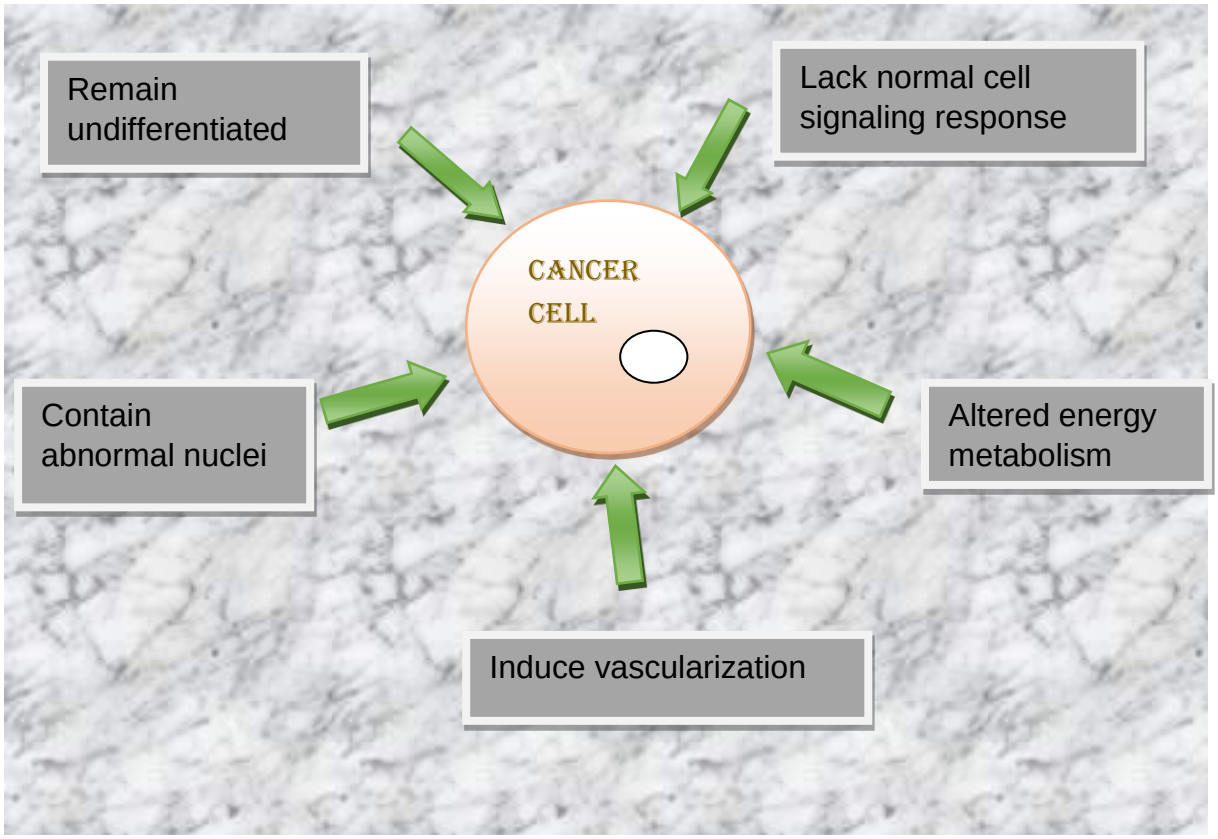


Figure 1.1 Characteristics of Cancer Cell

1.2.2 Histological types of cancer

Types of cancer classified on the basis of histological categories, which are as follows (CRUK, 2013)**:**

a) **Carcinoma** – This type of cancer originates in the skin or in tissues which cover internal organs. 90 % of cancers are considered as carcinoma.

b) **Leukaemia** – This cancer originates from blood-forming tissue such as bone marrow. It causes large numbers of abnormal blood cells which directly enter in the blood stream.

c) **Lymphoma** – This cancer starts it journey from the cells of the immune system. Lymphomas occur in the lymphocytes of lymph system.

d) **Myeloma** – This type of cancer originates in the cells of the immune system. They are the cancer of the bone marrow, especially those cells produces antibodies.

e) **Sarcoma** – This type of cancer occurs in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

f) **Central nervous system type**– This cancer located in the tissue of brain and central nervous system (CNS) and they exist in the form of glioma, blastoma, CNS lymphoma, etc.

g) **Mesothelioma** – This is a rare form of cancer. This cancer begins that develops from the mesothelium (protective lining of organs) of the internal organs. This cancer caused by the chemical toxicants such as asbestos.

1.2.3 Hepatic Cancer

Primary liver cancer is also known as hepaotocellular carcinoma. Hepatocellular cancer is the one of most common type of cancer and remains leading cause for deaths worldwide. It is increasing day by day and fifth largest cause in men and ninth largest in women. Its incidences were found more in western countries and in some country of Asia also (Bosetti *et al*., 2014). When abnormal cells overgrow and destroy other tissue of body and multiply continuously in liver leads to develop a liver cancer. Primary liver cancer directly originates in liver and spread to blood stream directly. Other factors which are responsible for causing HCC are hepatitis B & C, obesity, alcoholic and non-alcoholic cirrhosis and side effects or over dosage of drugs (Marengo *et al*., 2016).

It remain asymptomatic but detected by means of imaging, shows arterial phase enhancement and venous phase in patients with lesions greater than 2 cm in case of HCC and based on extent of tumor and vascular invasion.

1.2.3.1 Types of primary liver cancer:

It is very common in adult and children and most occurring cancers in the world. Others types of HCC begin in form of small nodules overall the liver. The different types of liver cancers are as follows (Cancer research UK, 2015):

a) **Intrahepatic cholangio carcinoma:** This cancer is develop in liver and in the bile duct which is outside the liver. It is most common in the United States in older people.
b) **Hepatoblastoma:** It is a very rare diseases occur only in small children. The survival rate is high, if diagnosed earlier with hepatoblastoma in children. If they are large and spread over the liver, the treatment is quiet difficult.
c) **Metastatic Cancer:** They are usually not known as liver cancer, but begin in other parts of body. Cancer originating from colon, lungs, pancreas, colon and breast and spread to liver. Lymphomas and leukemias, cancers that originate in the lymph nodes and bone marrow, respectively, can also invade the liver.

1.2.3.2 Molecular Tissue Markers of hepatic cancer

To diagnose the HCC, a large number of immunophenotypical markers viz HepPar1, albumin, fibrinogen, a1-anti-trypsin and Alfa-Fetoprotein are involved. When such specific markers are unable to diagnose poorly differentiated tumours additional markers are used. , Glypican-3 (GPC-3) which is an oncofetal protein is considered as more effective and highly specific markers which easily detect 80 % case of HCC (Toyoda *et al*., 2015). Immunophenotyping studies are based on the types of antibodies which easily diagnose the HCC and dysplastic nodules.

1.2.3.3 Pathogenesis of HCC according to aetiology

a) **Hepatitis B**: HBV infection arises on cirrhosis and normal liver and in turn to develop HCC and also related to consumption of more alcohol. Telomere shortening induce malignant transformation of cirrhotic nodules takes place when chronic inflammation, oxidative stress and replicative senescence of hepatocytes occurs. One of the mechanisms behind the carcinogenesis is insertion mutagenesis in which HBV DNA integrates into the human genome and continuous replicate in tumours cells. Insertions takes place near the fragile site of DNA and continuously repeats the sequence and transcribe the site of DNA throughout the human genome. Direct oncogenic properties of viral protein is another mechanism which is directly related the HBV carcinogenesis occur through viral integration and produce truncated protein such as pre S2/S protein & HBx.

b) **Hepatitis C**: HCV occur during telomere shortening of DNA which causes chronic inflammation, immune-mediated hepatocyte death, tissue damage, fibrosis synthesis by hepatic stellate cell and replicative senescence. Factors which are responsible for HCC is progression of fibrosis and steatosis.

c) **High alcohol intake:** During alcohol consumption there is high risk of occurrence of HCC along with cirrhosis. Acetaldehyde is a metabolite of ethanol which has a carcinogenic properties and bind DNA of cell. Alcohol dehydrogenase catalyses the production of acetaldehyde with polymorphisms and regulates the activity of this enzyme.

d) **Non-alcoholic fatty liver disease (NAFLD):** Chronic low-grade inflammation leads to develop liver injury and HCC and predisposes to obesity. HCC also stimulates with certain foreign chemical toxicants. These factors mostly linked with production of proinflammatory cytokines viz. IL6 and TNF-alpha. This inflammation occurs with

action of hepatocytes, Kupffer cells and adipocytes. NAFLD characterizes with imbalance between leptin and adipocyte predisposes to HCC.

e) **Genetic alterations in HCC:** Aflatoxin B1 is a type of mycotoxin that develops in dietary products and contaminates these products. It founds in some regions of Asia and mainly associated with HCC development. Aflatoxin B1 related HCC affects the codon 249 (R249S) of TP53 and cause specific mutation of this region in DNA. Codon 249 (R249S) of TP53 is consider as surrogate marker of aflatoxin B1 and predisposes to HCC development (Nault, 2014).

1.2.3.4 Overview of Treatment

Various forms of treatment for the HCC are as follows (De Lope *et al.*, 2012):

a) Surgery: It is process in which removal of tumours and corresponding healthy tissue of the liver take place during operation. Surgery is of two types to cure hepatic cancer:

- **Hepatectomy.** During hepatectomy, the part of the liver to be removed. This only occur if the tumours is present in only one part of the liver and other part of liver is working well. After surgery this liver comes back to its normal size within 2 to 3 weeks.
- **Liver transplantation.** Liver transplant is other option for the surgery in case of HCC. A suitable donor is required to implant the liver inpatient that have HCC and tumour size is of 5 cm. Sometimes body of patient shows signs that body might be reject the liver of donor body after transplantation. The patient needs a medication in order to avoid rejection.

b) *Radiofrequency ablation (RFA):* In this therapy heat is required to kill cancerous cell. Therapy is given through skin, laparoscopy or surgical operation.

c) *Percutaneous ethanol injection:* In this, cancerous cell is destroyed through ethanol injection in liver tumours. This therapy is effective in case when the tumour size is smaller than 3 cm.

d) *Radiation therapy:* High beam of X-Ray and other particles kills the cancerous cells. Stereotactic body radiation therapy in which high dose of radiation is delivering to tumours cells and not affected the normal tissues. It effectively treats the tumours size of 5 cm.

e) *Chemoembolization and radioembolization:* Chemoembolization include chemotherapy treatment in which drugs is used to destroy cancer cells which inhibit the growth and dividing of cells. The drugs are directly injected into the hepatic artery and its block the flow of blood to the artery for the shorter time. The chemotherapy drugs are used in the tumour cell of liver for a span of time to destroys its. Radioembolization use radioactive beads which deliver a radiation directly to tumour cells.

f) *Targeted therapy:* In targeted therapy, the specific genes, proteins and surrounding tissue of tumours cells are targeted by the drug treatment. It blocks the growth and proliferation of tumour cells anti-angiogenesis drugs are used in case of HCC. The most common drugs which are used in treatment of HCC are Sorafenib (Nexavar) and regorafenib (Stivarga).

g) *Immunotherapy:* They are also known as biologic therapy. It is under clinical trials for the standard treatment for HCC. In this the materials which are used is either made by body or develop in laboratory to restore the immune system to boost the natural's mechanism of the body to combat against the cancer.

1.2.4 Renal Cancer

Renal Cell Carcinoma (RCC) is the most well-known kind of kidney tumour, representing roughly 90% of all dangerous kidney tumours and 3% of all metastatic kidney tumours. It involves distinctive RCC sorts with particular histopathological and hereditary attributes. There is a 1.5:1 power of male over female, with peak rate happening in the vicinity of 60 and 70 years old. RCC is dangerous tumor and most deadly of urological malignancies. In RCC, cancerous cells produce in the coating of the kidney tubules and develop into a mass called a tumor. In the same way as other different malignancies, the development of tumour, starts slower and develops bigger after some time. RCC regularly develops as a solitary mass. There are situations where a kidney may contain more than one tumour, or tumors are found in both kidneys in the meantime (Crumley *et al*., 2013).

1.2.4.1 Factors which increases the chances of promoting kidney malignancy (Kazancioğlu, 2013):

a) **Smoking** – Individuals who smoke have double the danger of creating kidney malignancy as non-smokers. Up to 33% of all kidney malignancies are believed to be identified with smoking.
b) **Obesity** – Overabundance muscle to fat ratio may cause changes in specific hormones that can prompt kidney tumor.
c) **Hypertension** – Whether it is caused by being overweight, hypertension expands the danger of kidney tumor.
d) **Kidney failure**– Individuals with end-stage kidney ailments have a higher danger of creating kidney tumor.
e) **Family history** – Individuals who have relatives with kidney cancer cell growth, particularly a siblings, are at higher risk.

f) **Inherited conditions** – Around 3– 5% of kidney malignancies happen in individuals with specific acquired disorders, including von Hippel-Lindau ailment, inherited papillary RCC and Birt-Hogg-Dubé disorder.

g) **Introduction to harmful substances at work** – The hazard might be higher after normal presentation to specific chemicals, for example, some metal degreasers, arsenic or cadmium.

1.2.4.2 Types of RCC

a) There are five primary types of renal cell carcinoma that are recognized by analysing the tumor under a microscope (Cairns, 2011) which are as follow:

b) Clear Cell RCC: The individual cells that make up clear cell renal cell carcinoma seem extremely pale or clear. Clear Cell RCC is the most widely recognized type of renal cell carcinoma, representing around 80% of individuals with kidney malignancy.

c) Papillary RCC: These cancers frame little finger-like projections (called papillae Papillary RCC is the second most basic type - around 10% to 15% of individuals have this shape.).

d) Chromophobe RCC: Like clear cell carcinoma, the cells of these tumors are likewise pale, however, are significantly bigger and have certain other particular highlights. The third generally normal type of renal carcinoma is chromophobe RCC, representing around 5% of cases.

e) Collecting duct RCC: The rarest type of RCC is collecting duct renal carcinoma. The significant normal for collecting duct RCC is that the disease cells can shape sporadic tubes.

f) Unclassified RCC: Around 5% of renal malignancies are unclassified in light of the fact that their appearance does not fit into any of alternate classes (Eble *et al*., 2004).

1.2.4.3 Different stages of development of tumors in Kidney

In Australia, the TNM system is the technique frequently utilized for organizing kidney malignancy. The TNM offers numbers to the span of the tumor (T1– 4), regardless of whether lymph nodes are influenced (N0 or N1), and whether the growth has spread or metastasized (M0 or M1). In light of the TNM numbers, the specialist at that point works out the growth's general stage (I– IV) (Ficarra *et al*., 2007).

Stage 1: It is the most first phase of RCC in which tumor is of a width of 7 cm (approx. 2¾ inches) or littler, and restricted to the kidney. No lymph nodes is included or metastasis to other organ.

Stage 2: This is the second phase of renal disease and tumors of size more than 7 cm are found which are restricted to the limits of kidney. Lymph nodes inclusion and metastasis both are negative.

Stage 3: In this stage tumor of any size attacks the connecting lymph nodes yet no metastasis to far off organs. Tumors of this stage might be with or without spread to fatty tissue encompassing the kidney. Huge veins driving from kidney to heart might be included. In another situation tumor may spread to fatty tissue around the kidney as well as spread into the extensive veins driving from the kidney to the heart, yet without spreading to any lymph nodes or other nearby organs.

Stage 4: Tumor may spread specifically through the fatty tissue and the fascia ligament- like tissue that encompasses the kidney. There might be Inclusion of more than one lymph node close to the kidney or there might be inclusion of any lymph node not close to the kidney.

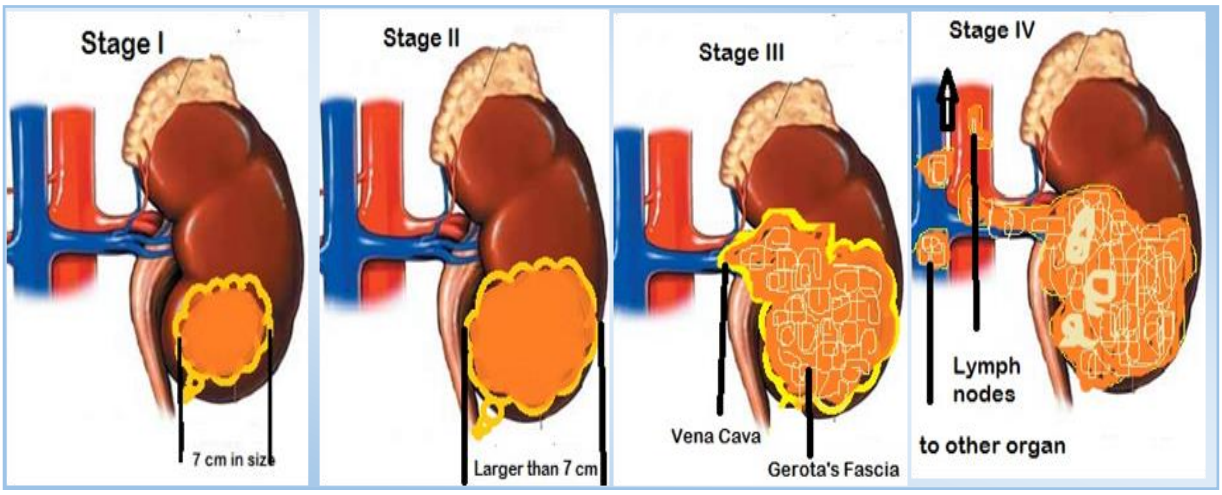


Figure 1.2 Different stages of development of tumors in Kidney

1.3 Antioxidants: Endogenous and Exogenous

Substance which interrupt or obstructs oxidative damage to target any moiety is knowns as antioxidants. An antioxidants moiety interact with single free radicals at a time and are able to counteracts these free radicals by offering their own electrons and stop the carbon-stealing reaction. A number of compounds fight against free radicals to nullify them and belong to exogenous and endogenous source (Valko *et al*., 2007). These are

i. Endogenous enzymatic antioxidants.

ii. Non enzymatic, metabolic and nutrient antioxidants.

iii. Metal binding proteins like ferritin, lactoferrin, albumin and ceruloplasmin.

iv. Phytoconstituents and phytonutrients

Human body generate various endogenous antioxidants to nullify these radicals and prevent the body from tissue damages. Exogenous antioxidants to human body are provided by external source which is present in the foods and prevent the body from diseases. Our body produces different types of endogenous antioxidant system and divided into two types: enzymatic antioxidant and non-enzymatic antioxidant.

The enzymatic antioxidants involves like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR). The non-enzymatic antioxidants

involves vitamin E, vitamin C and reduced glutathione (GSH). SOD is first line defence mechanism system against reactive free radicals and quenches superoxide radicle to hydrogen peroxide. GPx eliminates hydrogen peroxide by the method of coupling, reduce H2O2 to water, involve in oxidation of GSH and present in the cytoplasm of cells. GR is flavoprotein enzyme, which rejuvenates GSH from oxidized glutathione in the presence of NADPH. GSH also present in the cytosol of cells and major part of intracellular NADPH)(Matough *et al*., 2012)

Vitamin C and Vitamin E both are non-enzymatic antioxidant and present in the cells and interact with ROS to produce radicals themselves but less reactive than radicals and disrupt the radical chain reaction by duping peroxyl (El-Bahr, 2013).

Various mechanism involved to perform the antioxidant action such as overpowering the generation of reactive species by lowering hydroperoxides and hydrogen peroxide, removing of radical chain reaction by quenching reactive free radicals and by triggering Revamping and/or dissipating injury of cell. Thus, Antioxidants produced in the body or delivered by natural sources such as bioactive molecules shows a prominent role in the protection of the body from free radical instigated injury (Valko *et al*., 2007).

1.3.1 Chemoprevention

From the past, it was determined that human ailment were cured by ayurvedic medicine, recently it is known as alternative source of medicine It was observed from written and archaeological chronical, that 5000 years ago they were use a plant derived products and their parts for the treatment in pathological conditions. During 480 BC, Hippocrates specified history revealed that human illnesses were cured using traditional medicine, presently "Positive health requires knowledge of man's primary constitution and the powers of various foods, both those natural to him and those resulting from human skill "which means genetics factors and daily routine diet impact our health (Pai-Dhungat, 2015). So the chemoprevention is a novel term which was coined by Dr Micheal Sporn and also a Father of chemoprevention. Denotes

to avert or deferral the advancement of carcinogenesis by taking natural or synthetic compounds. The agents which are used in cancer is known as Chemopreventive agent (Steward and Brown, 2013). Characteristics of an ideal chemopreventive agent

1. Which reverse or supress the activity of cancer.
2. Should be economic or cost effective
3. Should have low side effects
4. Non Toxic in nature

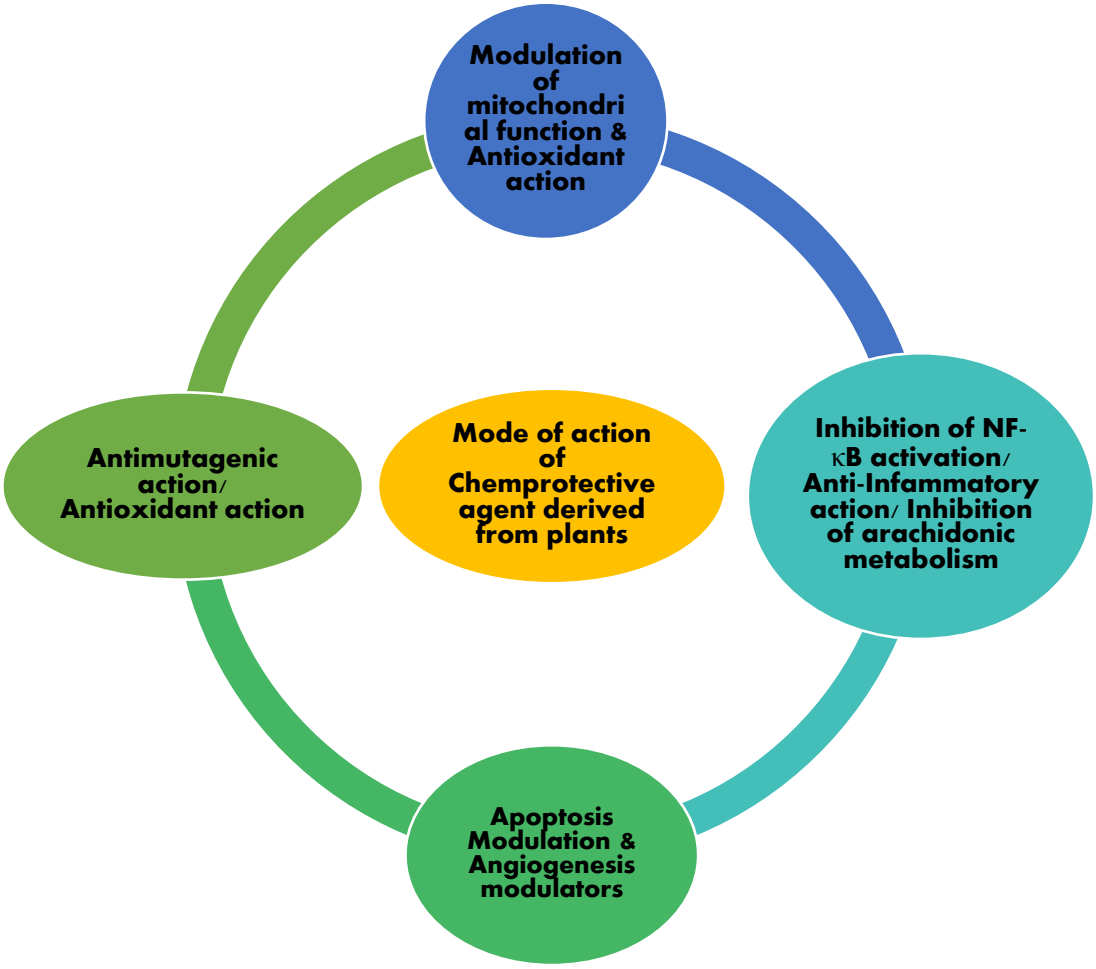


Figure 1.3 Possible mechanism of action of chemopreventive agents

1.3.2 Benefits of natural products in cancer

Since ancient time, Natural products plays an important role and used as a source for therapy of cancer. There are various agents which is derived from natural plants are used in clinical trials. A compound Paclitaxel which was isolated from the bark of plant *Taxus brevifolia.* A variety of chemotherapeutic agents isolated from natural source and only 70 % of compounds are approved for clinical practice (Veeresham, 2012). Plant based remedy that stimulate normal liver function are Taraxacum, Silybum and Cynara. Camptothecin a molecule, derived from Camptothecin acuminata impedes the activity of topoisomerase I, responsible for cell death. Podophyllotoxin a molecule isolated from podophyllum which has been chemically reformed into etoposide used in the treatment of lung and testes cancer (Oberlies and Kroll, 2004)

Some prominent molecules which was derived from plant source are vincristine, vinblastine, colchicine, ellipticine, flavopiridol and so on. Nutrition rich foods such as vegetable, fruits and legumes have an antioxidants in large quantity and prevent the body from the lethal effect of free radicals which is responsible for causing cancer. Synthetic drugs which is used in cancer treatment, only kill cells non-specifically whereas traditional drugs provide defensive and therapeutic effects to cancerous cells at low dose and are valuable in generating nutrient repletion.

Focussing on the approaches of natural products prominence, a research was accepted on some traditional plants for the effectiveness and management of cancer especially in hepatic cancer and renal cancer. Mileposts have been spanned in the research of cancer. There is a close linkage between the clinical practice and research work in the field of oncology which is a challenging task for the scientists and demanding teamwork. In a present days, there is only surgery, radioactive and chemotherapy is available which has side-effects and contradictions

due to lack of knowledge, lack of proper diagnosis and alteration in pathology (Prakash *et al*., 2013).

Traditional drugs and herbal therapy have been long used in the treatment of liver and renal disease. Now a day's clinical trials and pharmacological study have revealed them to be valuable as estimated by the liver and renal function tests. Scientists is continuously doing a research in the field of oncology to find out new molecule and formulate into novel drugs with better protocols .As we all are aware of synthetic anticancer drugs which kill the cancerous cell along with normal cells. It also increase the risks of many side-effects and intolerable hazards, so the population go back to natural sources which include folklore drugs (Feng *et al*., 2011). The current study was commenced to determine the efficacy of natural drugs against hepatic cancer and renal cancer. As per knowledge, any practical work in fighting against such frightful diseases is assured to be of chief importance

1.4 Nanoparticles

In the novel drug delivery system, there are various novel carriers which have advantage over conventional preparation (solution, suspension or emulsion) suffer from limitations like high dose and low availability, first pass effect, instability, and they exhibit fluctuations in plasma drug levels and do not provide sustained effect (Buzea *et al*., 2007). Novel drug delivery system is one of important tool expanding drug markets in pharmaceutical industry. This system can address issues by increasing efficacy, improving safety, patient compliance & product life (Roco and Bainbridge, 2005).

1.4.1 Nanoparticles as a targeted drug delivery system

The development of nanoparticles delivery systems for targeted drug delivery has been recently reviewed. Targeted drug deliveries can be actively or passively achieved. Active targeting requires the therapeutic agent to be achieved by conjugating the therapeutic agent or carrier system to a tissue or cell-specific ligand (Lamprecht *et al*., 2001). Passive targeting is

achieved by incorporating the therapeutic agent into a macromolecule or nanoparticles that passively reaches the target organ.

Nanoparticles (NP) are a type of sub-nanosized colloidal drug delivery system composed of synthetic and semi synthetic polymers with a size range from 10 to 1000 nm in diameter(Irving, 2007)[6]. The system shows different inner structure.

a) Nanospheres in matrix type system in which oliogmer of polymer unit is entangled throughout the particle matrix.

b) Nanocapsules in which oily core is surrounded by polymeric shell (reservoir type).

1.4.2 Advantages of nanoparticles

Nanoparticles has a number of advantages are

(1) Ease of preparation & scale up,

(2) Improved bioavailability,

(3) Increasing resistance time in the body,

(4) Targeting drug to specific location in the body (its site of action) and

(5) Incorporation of both hydrophilic and hydrophobic substances.

1.4.3 Metallic nanoparticle

In 9th century, the concept of nanotechnology was considered as a modern science. The artisans of Mesopotamia used the silver and gold nanoparticle to produce a glittering effect on pots. In 1950-1960 the scientists had focus towards the use of nanoparticle in the field of biomedical. Most pioneers and famous Professor Peter Paul Speiser and his research group first time developed a polyacrylic beads for oral administration and further leads to produce microcapsules. In Mid 19 century the first nanoparticles were developed for the drug delivery system and for vaccines (Sadowski, 2010).

Nanomaterial gaining an incredible importance in all phases of human life and development of new metallic nanoparticle day by day opened a several arm in field of NDDS. Due to unique properties of noble metal such as gold, silver, magnesium, zinc, platinum, iron, palladium, manganese used for the preparation of nanoparticle and their potential application are widely studied in uv irradiation, ultrasonic field, cancer, topical properties, aerosol technologies, chemical sensing, antimicrobial and etc. noble metal NPs have been considered as nontoxic carriers for drug and gene-delivery system. However, their usage in diverse field, it is most important to focus on clean, nontoxic, simple and eco-friendly method for the preparation of these nanoparticle (Arvizo *et al*., 2012).

Now a day's metal nanoparticle become a major research area by using a natural organism and plant extract to develop an eco-friendly formulation. The wide variety of biochemical composition present in plant extract act as a reducing & stabilizing agent for synthesis of metal nanoparticle (Kuppusamy *et al*., 2016).

Advantages of metal nanoparticles:

1. Due to reduced sizes and shape, its exhibit diverse optoelectronic properties.
2. Good biocompatibility with polymer.
3. Chemically stable
4. They are suitable for cellular uptake.
5. Good property of tenability.
6. Easily conjugate with antibodies, ligands and drugs.

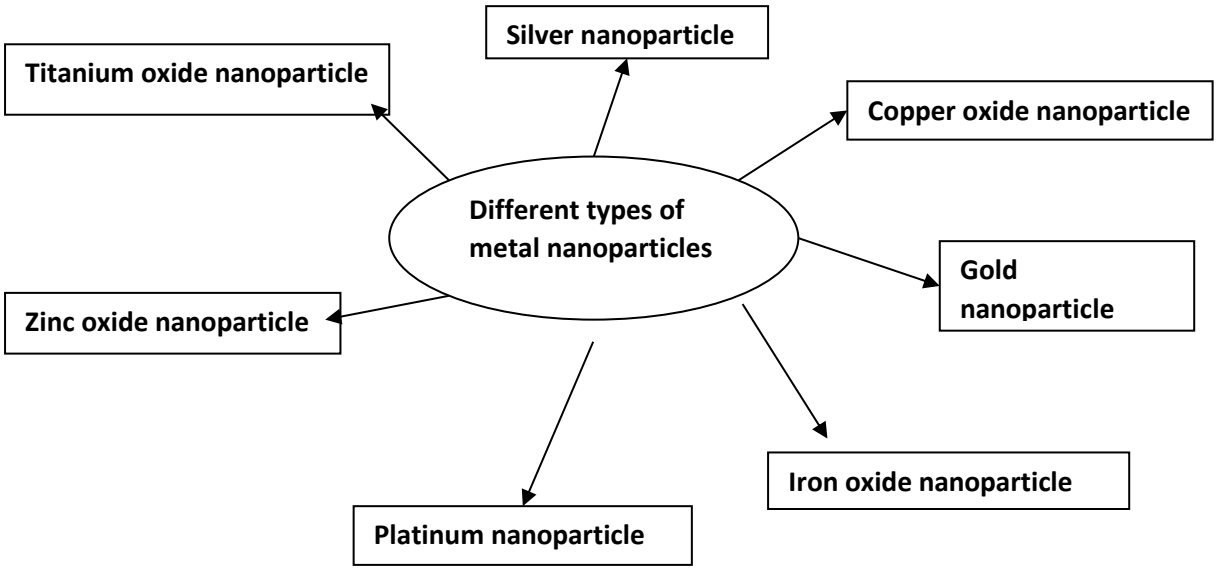


Figure 1.4. Different types of metal nanoparticles

1.4.4 Method of Preparation of metal Nanomaterial (C. Binns, 2008)

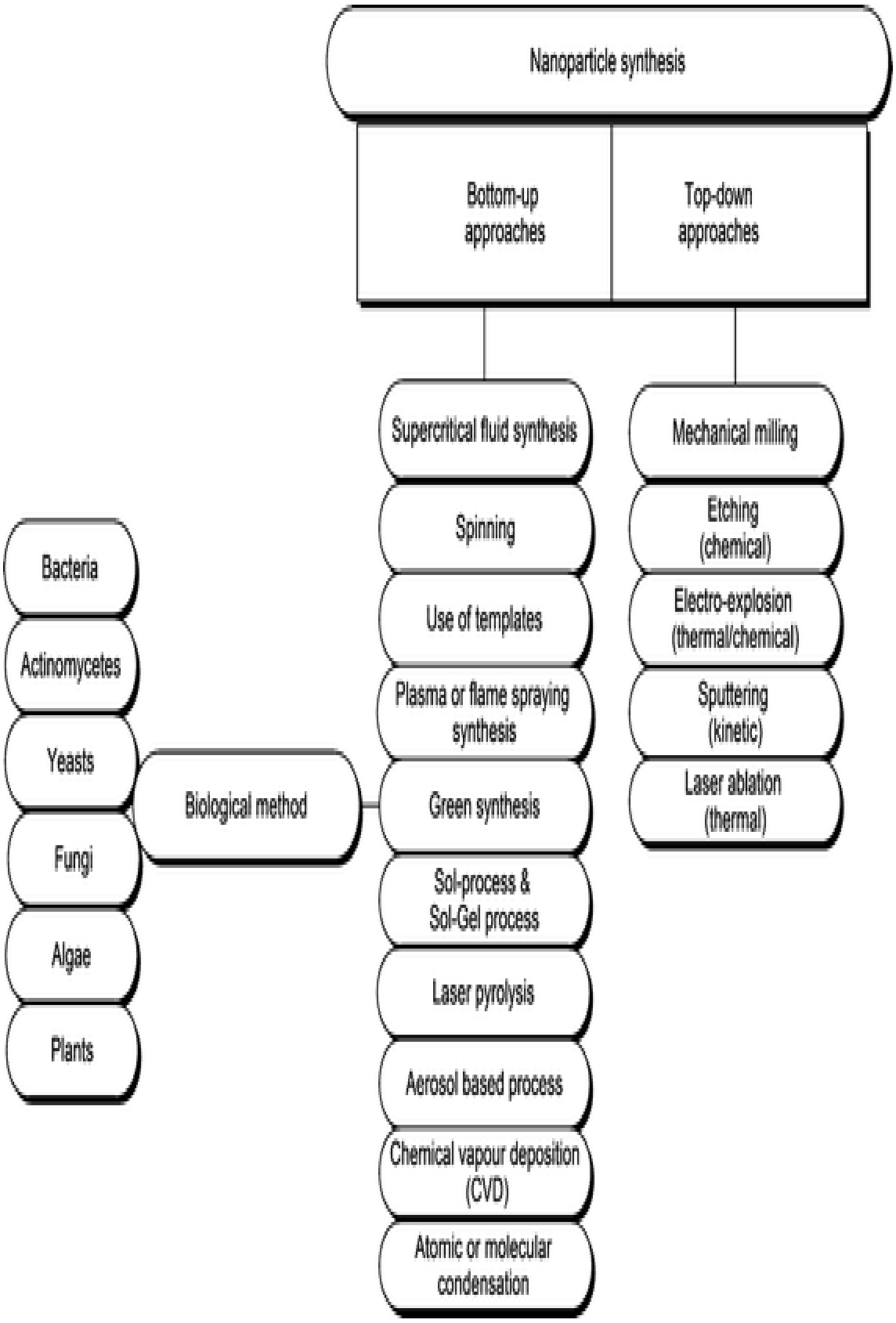


Figure 1.5 Different method of preparation of metal nanoparticles

1.4.5 Silver nanoparticle:

Silver and its related compound possess a low toxicity toward human cells but effective against bacteria and fungi. Due to recent advancement in the field of nanotechnology, silver ions also having a wide range of application in water purification, in wound, cut or burn, wood preservative and etc. Fabrication of silver nanoparticles develops a much interest in the scientific community. Due to unique property of silver nanoparticles, it enables researcher to focus on numerous investigations in field of drug delivery system (Ravindran *et al*., 2013).

As synthesis of silver nanoparticles are cheaper than gold nanoparticles so, they are concluded as important class of metal nanoparticle. Due to their physical, chemical and biological properties silver nanoparticles gaining a special interest as compared to other system(Ahmed *et al*., 2016a).

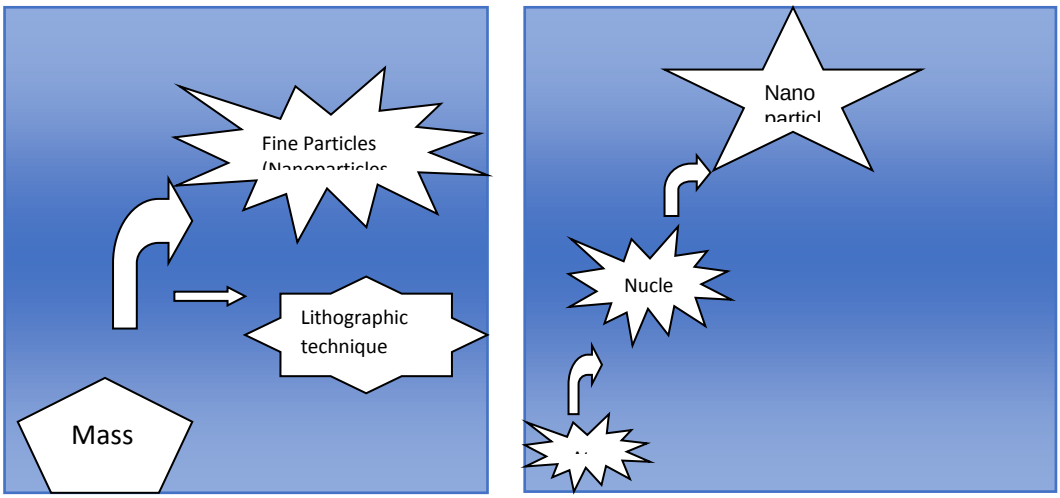
1.4.5.1 Physical & chemical properties of silver nanoparticles:

a) High electrical and thermal conductivity
b) Surface –enhanced Raman scattering
c) High chemical stability
d) Having a good catalytic activity
e) Non- linear optical behavior

Chemical and physical methods for preparation of nanoparticles are quite expensive and health hazards due to use of toxic chemicals. Biologically developed experimental processes overcome the drawback of synthesis of nanoparticle. There are two approaches which is involved in the production of silver nanoparticles which are as follows(Keat *et al*., 2015):

a) Top to bottom approach: Substance or material break down into fine particles by the process of size reduction.

b) Bottom to top approach: Using a biological & chemical method in which self-assembly of atoms take place to form a particle of nanosize.



a. Top to bottom b. Bottom to top

Figure 1.6 Different approach for the production of silver nanoparticles

1.4.5.2 Method of preparation of silver nanoparticles

There are three methods to prepare silver nanoparticles which are as follows (Natsuki *et al*., 2015):

a) Chemical method

- Chemical reduction: Various reducing agents like sodium citrate, polyols, $NaBH_4$, N_2H_4, sodium citrate and N, N-dimethylformamide are used. Different types of capping agent such as Sodium dodecyl sulphate (SDS), polyvinyl pyrrolidone (PVP), tri-sodium citrate are used to stabilize and to stop the agglomeration of particles. The developed system which is formed has high stability, low toxicity and reduced size with spherical shape.
- Photochemical method**:** instead of using a chemicals method, a variety of irradiation methods are used to fabricate silver nanoparticles. Microwave irradiation methods are used to synthesize 4 to 10 nm nanoparticles.
- Pyrolysis In this method, about size of 100 nm is synthesizing by spray pyrolysis by using silver nitrate. Stabilizer is used to prevent the aggregation of silver nanoparticles and produce pure Ag nanoparticles.

b) Physical method

- Physical vapor condensation: A target material are vaporized by means of heat source and condensed and finally get nanoparticles.
- Arc discharge method: This is one of novel procedure to synthesize silver nanoparticles without utilizing any surfactant and stabilizers. 1mm of silver wires is dunked in deionized water acts as electrodes. The DC system operates at a pulse voltage of 70–100 V for 2–3 mins. The surface layer of Ag wires evaporates during arc-discharge and condenses in the water. The solution turns into pale yellow colors and produced a silver suspension.

c) Biological method: Living organisms such as bacteria, fungi and plants have a potential for the development of metallic nanoparticles. Microorganisms have recently been investigated as a potential biohub for the preparation of metallic nanoparticles such as CdS, Ti/Ni, titanate, zirconia, gold and silver. The utilization of microorganisms in the blend of nanoparticles develops as an eco-accommodating and energizing methodology. Then again, researchers have swung to biological synthesis on account of the great control over size dissemination of nanoparticles.

1.4.5.3 Green biosynthesis of silver nanoparticle

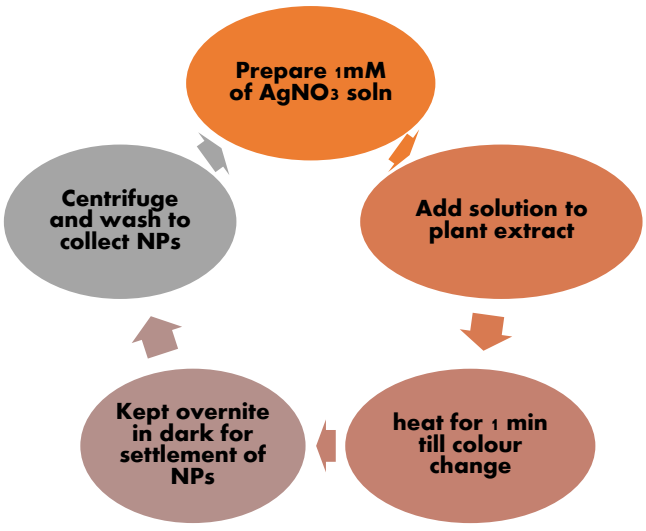


Figure 1.7 Biosynthesis of silver nanoparticles

1.4.5.4 Characterization of silver nanoparticles:

After biofabrication of nanoparticles, it is important to characterize the size, shape, surface area of formulation to understand and control the synthesis of NPs. various different parameter and technique are employed to characterize which are as follows: After green synthesis of nanoparticles, characterization is an important step to identify the nanoparticles by their shape, size, surface area and dispersity. To characterize nanomaterials various techniques are employed which are shown below (Alaqad and Saleh, 2016)

a) **UV-visible spectrophotometer**- they allow identification, characterization and analysis of metallic nanoparticles in the range of general 200-800 nm. It shows optical absorption peak due to surface plasmon resonance.
b) **Dynamic light scattering (DLS)** - to determine the surface charge and the size distribution and polydispersity index of nanoparticles.
c) **Scanning electron microscopy (SEM), and Transmission electron microscopy (TEM)** – Both are used to determine the morphological character of prepared metallic nanoparticles. Only the difference is that TEM provide exact size and shape of Nanoparticles at a higher resolution and SEM can provide morphological information on the submicron scale.
d) **Fourier transforms infrared spectroscopy (FTIR)** - FTIR is used to determine the presence of functional group which are adhered to the surface of nanoparticles.
e) **Energy dispersive spectroscopy (EDS)** – EDS provides an information on the presence of elemental composition of silver nanoparticles.
f) **X-ray Diffractometry:** X-ray diffraction (XRD) is a one of the technique used to determine the crystal structure and atomic spacing.

1.4.5.5 Application of silver nanoparticles (Abou El-Nour *et al*., 2010):

a) **Antibacterial Activity of AgNPs**: It was observed that Silver nanoparticles have a tendency to overcome the bacterial resistance and potent antibacterial agents. It inhibits and kills the gram positive and gram negative bacteria. It is one of promising tool for antibacterial agents having a large surface volume and crystallographic structure.

b) Antifungal Activity of AgNPs: Fungal infection occurs when patients is supposed to be immunosuppressed and limited number of drugs for fungus are available in market. So there is requirement of biocompatible, non-toxic, lesser side effects and eco-friendly antifungal agents. AgNPs have a potential to battle against fungi.

c) Antiviral Activity of AgNPs: Viral intervened diseases are more common and are normal and become more noticeable worldwide, so it is necessary to focus on such type of formulation which is effective against viral mediated diseases. The mechanism of the antiviral action of AgNPs is a critical viewpoint in antiviral treatment. Based on the size and shape of AgNPs, it is prominent therapy against viral diseases.

d) Anti-Inflammatory Activity of AgNPs: It also plays an important role in treating inflammation and various plant extract fabricated silver nanoparticles reduces the inflammation.

e) Anti-Angiogenic Activity of AgNPs: Obsessive Angiogenesis is related to cancer and inflammation. Various scientist inspired by finding novel molecule to combat angiogenic related ailment. There are few engineered molecules having an angiogenic property. Currently, a few researches gave supporting confirmation utilizing both in vitro and in vivo models demonstrating that AgNPs have both against angiogenic and anticancer property.

f) Anticancer Activity of AgNPs: Numerous scientists are involved in developing silver nanoparticles as a prominent tool to target tumor cells particularly. A few research labs

have utilized different cell lines to develop a new formulation to fight against cancer. Due to small size and shape AgNPs is effective against cancerous cell in vitro and in vivo models.

1.4.5.6 Medicinal plant extract used to biosynthesized silver nanoparticles

The usage of plants extract as the fabrication assembly of silver nanoparticles has drawn attention, because of its fast, ecological, non-pathogenic, cost-effective and providing a single step processes. The capped and reduction of silver ions by blend of various biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already recognized in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures. A large number of plants are stated to assist silver nanoparticles syntheses are mentioned in table 1.1.

Table 1.1 Green synthesis of silver nanoparticles using plant extracts

S.No.	Botanical name	Family	Parts used	Activity
1.	*Tectona grandis Linn*	Lamiaceae	leaves	Antibacterial *(Devadiga et al.*, 2015)
2.	*Euphorbia prostrata Ait*	(Euphorbiaceae)	leaves	Antiparisitic (Zahir and Rahuman, 2012)
3.	*Sesuvium portulacastrum*	Aizoaceae	Callus, leaves	Antimicrobial (Nabikhan *et al*., 2010)
4.	*Emblica Officinalis*		Fruit extract	Hepatoprotective (Ramesh *et al*., 2015)
5.	*Plukenetia volubilis L.*	Euphorbiaceae	leaves	Anticancer (Kumar *et al.*, 2014)

6.	*Ficus sycomorus*	Moraceae	Leatex, leaves	Antimicrobial (Salem *et al*., 2014)
7.	*Maple leaf (Acer pseudoplatanus)*	Sapindaceae	leaves	Photocatalyst(Vivekanandhan *et al*., 2014)
8.	*Mentha piperita (Lamiaceae).*	Lamiaceae	leaves	Antibacterial (MubarakAli *et al*., 2011)
9.	*Azadirachta indica*	Meliaceae	leaves	Antibacterial (Ahmed *et al*., 2016b)
10.	*Morinda citrifolia;*	Rubiaceae	root	Cytotoxic (Suman *et al.*, 2013)
11.	*Nelumbo nucifera*	Nelumbonaceae	root	Antioxidant, protein binding, cytotoxic (Sreekanth *et al*., 2014)
12.	*Sapindus emarginatus*	Sapindaceae	pericarp	Antibacterial (Swarnavalli *et al*., 2017)
13.	*Ficus panda*	Moraceae	leaf	Catalytic (Tripathi *et al*., 2013)
14.	*olive*	Oleaceae	leaf	Antibacterial (Khalil *et al*., 2013)
15.	*Melia azedarach*	Meliaceae	leaf	Antibacterial (Mehmood *et al*., 2013)
16.	*Abelmoschus esculentus*	Malvaceae	pulp	Anticancer, antimicrobial(Mollick *et al*., 2015)
17.	*Eucalyptus camaldulensis*	Myrtaceae	leaf	Anticancer, antimicrobial (Mohammed, 2015)

18.	*Svensonia hyderobadensis*	Verbenaceae	stem	Antimicrobial (Rao and Savithramma, 2012)
19.	*Ocimum sanctum*	Lamiaceae	leaves	Antibacterial (Singhal *et al*., 2011)
20.	*Boswellia ovalifoliolata*	Burseraceae	Stem barks	Antifungal, antibacterial (Savithramma *et al*., 2011)
21.	*Shorea tumbuggaia*	Dipterocarpaceae	sTem bark	Antifungal, antibacterial (Ankanna and Savithramma, 2011)
22.	*Solanum trilobatum*	Solanaceae	leaf	Antibacterial (Ramar *et al*., 2015)
23.	*Artemisia annua*	Asteraceae		Antibacterial (Khatoon e*t al*., 2015)
24.	*Malus domestica*	Rosaceae		Cytotoxic, antimicrobial (Lokina *et al*., 2014)
25.	*Alternanthera sessilis*	Amaranthaceae)		Antimicrobial , antioxidant (Niraimathi *et al*., 2013)
26.	*Solanum torvum*	Solanaceae	fruit	free radical scavenging, antibacterial (Govindaraju *et al*., 2010)
27.	*Ficus religiosa*	Moraceae	leaf	Antitumor (Antony *et al*., 2013)
28.	*Moringa oleifera*	Moringaceae	Stem bark	Anticancer (Vasanth *et al*., 2014)

29.	*Withania somnifera Linn*	Solanaceae	leaf	Antibacterial, antifungal (Raut *et al*., 2014)
30.	*Ipomoea carnea*	Convolvulaceae	Leaf, stem, root	Organic pollution *(Ganaie et al*., 2014)
31.	*Ficus racemosa*	Moraceae	bark	Antimalarial *(Velayutham et al.*, 2013)
32.	*Alpinia katsumadai*,	Zingiberaceae	seed	Cytotoxic, antioxidant, antibacterial (He *et al*., 2017)
33.	*Alysicarpus monilifer*	Fabaceae	leaf	Antibacterial (HIV infections) (Kasithevar *et al*., 2017)
34.	*Elephantopus scaber*	Compositae	leaf	Cytotoxic, antimicrobial (Kharat and Mendhulkar, 2016)
35.	*Protium serratum*	Burseraceae	leaf	Antibacterial *(Mohanta et al*., 2017)

1.5 Plant Profile

1.5.1 Amla

Name *Phyllanthus emblica*

Synonyms *Emblica officinalis*

Family Phyllanthaceae

Class Dicotyledonae

Genus Phyllanthus

Vernacular names: Emblic, emblic myrobalan, myrobalan, Indian gooseberry, Malacca tree,

Habitat:

Grown on sloppy hills in dry deciduous forests and found in Indian subcontinent, south and Southeast Asia.

Plant morphology:

It is a large deciduous tree, 8-18 m in height with a spreading branches and thin light grey bark. Leaves are simple, sub sessile, pinnate, light green in colour and found in branchlets. Flowers are greenish yellow, unisexual and numerous on short slender pedicels. Fruits are spherical, greenish yellow and found along with branchlets of leaves appear hard with furrows all over fruits (Singh *et al*., 2012).

Chemical Constituents:

Fruit contains quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, vitamin C and various polyphenolic compounds.

Root contains ellagic acid and lupeol, phyllaemblic acid, a novel highly oxygenated norbisabolane, oneohesperiodoside

Bark contain leucodelphinidin.

Seeds contains following fatty acids: linolenic (8.8%), linoleic (44.0%), oleic (28.4%), stearic (2.15%), palmitic (3.0%) and myristic (1.0%)

Leaves apigenin7-O-(6”-butyryl-beta)-glucopyranoside, along with four known compounds gallic acid, methyl gallate, 1,2,3,4,6-penta-Ogalloylglucose and luteolin-4' (Ur-Rehman *et al*., 2007).

Uses:

Medicinal and traditional plant having a wide range of medicinal property. As mention in ayurveda it balances all the three doshas. It was reported that fruit possess an analgesic, rejuvenative property, reduce fever, asthma, and stimulate hair growth, effective in liver diseases. Bark is used in diarrhoea and dysentery and effective against hepatic diseases. Leaves are used in cancer, atherosclerosis, diabetes, peptic ulcer, anaemia, liver, hear diseases. It is main ingredient of polyherbal formulation knowns as triphala, chavanprash (Vasant *et al*., 2013).

Figure 1.8 Leaves of *Phyllanthus emblica*

Figure 1.9 Tree of *Phyllanthus emblica*

1.5.2 Mahua

Name *Madhuca longifolia*

Synonyms Madhuca latifolia Macb., Bassia latifolia Roxb.,

Order Ericales

Family Sapotaceae

Genus Madhuca

Vernacular name Honey tree, butter tree, mahua, mi, illuppai

Habitat:

Multipurpose tree grow in marginal areas of dry tropical and subtropical forest of south Asia (India, Srilanka, Nepal, Myanmar) for the flowers and its seed oils.

Plant Morphology

M. Longifolia attains a height of 70 ft and bearing at 9-14 years and remains (fruits) upto 60 years. Leaves are long, lanceolate, narrowed at both ends, subsessile, thick and leathery. Flowers are small in size and have pale white colour. Fruits looks ovoid fleshy and when ripe have greenish yellow colour. It is evergreen tree have an economic importance (Devi and Sangeetha, 2016).

Chemical Constituents:

Flower Vitamins A and C

Bark ethylcinnamate, 3β-monocaprylic ester of eythrodiol and 3β-capryloxy oleanolic acid.

Fruits α-and β-amyrin acetates

Seeds arachidic, linolelic, oleic, myrisic, palmitic and stearic acids, α-alanine, acid, , myricetin, quercetin, Mi-saponin A & B.

Leaves β-carotene and xanthophylls; erthrodiol, palmitic acid, myricetin and its 3-O-arabinoside and 3-O-L-rhamnoside, quercetin and its 3-galactoside; 3β-caproxy and 3β-

palmitoxy-olean-12-en-28-ol, oleanolic acid, β-sitosterol and its 3-O-β-Dglucoside, stigmasterol, β-sitosterol-β-Dglucoside, n-hexacosanol, 3β-caproxyolca n-12-en-28-ol, β -carotene, n- octacosanol, sitosterol, quercetin (Akshatha *et al*., 2013).

Medicinal Uses:

It is a traditional and medicinal Plant and having a wide range of properties. Leaves of *M. Longifolia* are edible and used as a vegetable in India. *Madhuca longifolia* leaves are expectorant and also used for chronic bronchitis, liver diseases and cushing's disease. The leaves are applied as a poultice to relieve eczema. Stem bark is used to cure skin disease, hydrocoele and skin disease. Mahua oil is used to make soaps a, candle and is an treatment against pest infestations in seeds (Ramadan *et al*., 2016)..

Figure 1.10 Leaves of *Madhuca longifolia*

Figure 1.11 Tree of *Madhuca longifolia*

1.5.3 Karonda

Name *Carissa carandas*

Synonyms *Carissa salicina* Lam

Order Gentianales

Family Apocynaceae

Genus Carissa

Vernacular names: avighan, avighna, karanda

Habitat

Grows naturally in the Siwalik Hills, the western ghat and Afghanistan. In India, it is grown in Rajasthan, Gujarat, Bihar, West Bengal and Uttar Pradesh.

Plant morphology

It is a deciduous small shrub having a height of 24 m tall. Stem contain a white latex and branches have a sharp spike. Fruits contain a seeds and is ovoid in shape and formed in bunch of 4-8 number. Fruits are pink in colour at young age and become red to brown when ripe. Bark is thick and soft. Leaves are conical, oblong, green colour on top and brown colour below. Flowering occurs in month of January and February. Flowers are white in colour and small in size (Hasmah *et al*., 2013).

Chemical Constituents

Seeds contains palmitic (66.42%), stearic (9.36%), Oleic (2.04%) and linoleic (0.99%) acids

Roots contains 2- acetyl phenol, Lignan , carinol sesquiterpenes , namely carissone and carindone

Leaves contains triterpenoid constitutes well as tannins, and a new isomer of urosolic acid namely carissic acid

Fruits 2 - phenly ethanol, linalool, β - caryophylline, isoamyl alcohol, benzyl acetate and a novel triterpenic alcohol, carissol (Singh and Uppal, 2015).

Uses

Fruit and seeds is used in rheumatoid arthritis, anorexia, colic disease anxiolytic, anthelmintic, anti-parasitic and estrogenic properties. Roots helpful in diabetics, ulcer, pruritus, stomach disorder, cardiotonic effect. Plant is also effective against blood pressure. It is used for alleviating vata and pitta disorders (Bhalodia and Shukla, 2011).

Figure 1.12 Leaves of *Carissa carandas*

Figure 1.13 Tree of *Carissa carandas*

1.6 Justification of study

- The commonly used anticancer drugs such as Fluorouracil (5-FU), Gemcitabine (Gemzar), Doxorubicin (Adriamycin), Cyclophosphamide, and Carboplatin have been reduced, now a days due to their side effects such as nausea, vomiting, hair loss, confusion, kidney damage, diarrhea etc. So the population returning back to the herbal formulation.
- The potential application of the plants i.e. *Phyllanthus emblica, Madhuca longifolia and Carissa carandas* have been proved through its reported pharmacological studies in the therapy of analgesic, antibacterial, antifungal, anthelmintic, antidiabetic, antipyretic, wound healing, anticancer, hepatoprotective and etc.
- Silver nanoparticles using herbal drugs reduced the side-effects, cost effective and eco-friendly in nature.
- Biosynthesized silver nanoparticles of plant extract have a tremendous potential and considerable work have done to adventure the pharmacological application of these plant, enormous possibilities remain in new area of biological activities.
- On the basis of previous literature, there are possibilities to explore photosynthesized silver nanoparticles of all the plants for hepatic and renal cancer on animal model.

1.7 Objectives

- To evaluate pharmacognostical studies of the leaves of *Phyllanthus emblica, Madhuca longifolia* and *Carissa carandas*
- To perform preliminary phytochemical screening of aqueous extract of *Phyllanthus emblica*, *Madhuca longifolia* and *Carissa carandas*
- To biofabricate silver nanoparticles of the plants
- To characterize silver nanoparticles of the plants by UV spectroscopy, FTIR, XRD, FESEM with EDX and TEM.
- To perform *in vitro* cytotoxic studies of biosynthesize silver nanoparticles of plants.
- To evaluate *in vivo* anticancer activity of the biosynthesize silver nanoparticles of the plants.

Chapter-2

Review of Literature

2. Review of literature

2.1 Literature review of *Phyllanthus emblica*

Chaphalkar *et al.*, (2017) reported the hepatoprotective activity of *Phyllanthus emblica* bark extract against ethanol-induced hepatic damage in rat models. PEE arbitrated hepatoprotection because of its free radical scavenging and antioxidant activity. Antioxidants activity may be due to its antioxidant components, namely, ellagic acid and gallic acid.

Zeng *et al.*, (2017) isolated a novel water-soluble polysaccharide named PEPW80-1, from the pulp tissues of *Phyllanthus emblica*, and cleaned by sephadex G-100 column and sephacryl S-300 HR chromatography. This polysaccharide was evaluated for in vitro antioxidant and immunomodulatory activity. In vitro immunomodulatory assays reveals that PEPW80-1 could downregulated the multiplication of mouse splenocytes. They suggested that PEPW80-1 might be industrialized as a promising value-added product with the activities of immunomodulator and free-radical inhibitors.

Tahir *et al.*, (2016) presented the antioxidant activity and in vivo pulmonary fibrosis of methanolic extract of *Phyllanthus emblica* leaves. They also screened out the phytoconstituents present in leaves by HPLC-DAD. The conclusions of this study establishes the presence of polyphenolics and phytoconstituents in PELE which play a momentous role in refurbishing the pulmonary injuries initiated by CCl_4.

Uddin *et al.*, (2016) investigated the ethanolic extracts of *Phyllanthus emblica* (EEPE) ripe (EEPEr) and EEPE unripe (EEPEu) fruits on Cognitive Performance, Brain Antioxidant Markers and Acetylcholinesterase Activity in Rats. They explored EEPE fruit retains an outstanding source for natural cognitive enhancer which could be developed in the treatment of AD and other neurodegenerative diseases. The effects of EEPEr and EEPEu fruits (i.e., 100 and 200 mg/kg b.w.) were examined in Swiss albino male rats for 12 days and its effect on

cognitive functions, brain antioxidant enzymes, and AChE activity determined. Learning and memory enhancing activity of EEPE fruit was examined by using passive avoidance test and rewarded alternation test.

Vadde *et al.*, (2016) investigated and evaluated the anti-cancer activity of *Emblica officinalis* and pro-apoptotic mechanisms on human colon cancer stem cells (HCCSC). They also observed that amla suppressed proliferation and induced apoptosis independent of p53, a tumour suppressor gene, via effectively targeting Wnt/β-catenin signaling pathway in HCCSCs.

Zhao *et al.*, (2015) reported in-vitro and in-vivo immune regulating activities in animal models. They observed that fruits and fruits extract of *Phyllanthus emblica* have potential to inhibit the growth of cancerous cells by lowering DNA damage which is induced by chemicals and radiation. The anticancer effects in the extract is due to presence of polyphenols, especially tannins and flavonoids.

Ramesh *et al.*, (2015) investigated a biofabrication and antimicrobial activity of silver nanoparticles using fruit extract of *Emblica officinalis* which acts as a reducing and capping agent. The AgNPs were characterized by various instrument such as SEM, FT-IR, and XRD. The synthesized AgNPs revealed inhibition and had important antibacterial against both gram-positive and gram-negative bacterial strains.

Golecha *et al.*, (2014) determined the anti-inflammatory activity of hydroalcoholic extract of *Emblica officinalis* against carrageenan induced inflammation in Swiss rats. The consequences substantial inhibited the paw edema in rats. It was observed that HAEEO substantial upregulated glutathione, superoxide dismutase, and catalase activity and consequently down-regulated the lipid peroxidation evinced by reduced malondialdehyde. So, the results directed

that HAEEO obsessed potent anti-inflammatory activity and it may hold beneficial in the controlling of acute and chronic inflammatory conditions.

Zhu *et al.*, (2013) reported the impacts of polyphenol extract on cervical cancer cells and its mechanism. Polyphenol extract of *Phyllanthus emblica* (PEEP) have potential to inhibit the HeLa cell multiplication by supressing cell cycle arrest at G2/M phase and apoptosis. It also produce significant effect in the activation of Fas and FasL and caspase-8 cleavage.

De *et al.*, (2013) explained anticancer property of *Phyllanthus emblica* L by inducing cancer cell apoptosis by inhibiting cell proliferation, angiogenesis, and growth of mouse xenograft Tumors. Amla Extract hinders ovarian cancer cell growth both *in vitro* possibly through inhibition of angiogenesis and triggering of autophagy. Therefore, Amla extract may be used as adjunct therapeutic medicine in the treatment of ovarian cancer.

Rosarina *et al.*, (2013) investigated the biofabrication of silver nanoparticles by using amla extract, and evaluate its activity against cytotoxic, oxidative stress and apoptotic effect on Hep2 cell line (laryngeal carcinoma cells) *in vitro*. They also compare the results of extract with synthesized AgNPs and standard drug fluorouracil.

Vaithiyanathan *and* **Sankaran (2013)** showed the chemoprotective effects of fruit juice of plant *Phyllanthus emblica* in 7,12-dimethylbenz(a)anthracene (0.25 mg/kg/bwt)-induced mammary cancer female Sprague-Dawley. They determined the antioxidant effect, levels of lipid profile and status of hormone estrogen/ progesterone receptor. So the research specified that active constituent present in fruit juice of amla could overwhelm the mammary tumour.

Srirama *et al.*, (2012) investigated the hepatoprotective and antioxidants property of Phyllanthus species (*P. amarus Schumach., P. urinaria L., P. debilis Klein ex Willd, P. tenellus Roxb., P. virgatus G. Forst., P. maderaspatensis L., P. reticulatus Poir., P. polyphyllus Willd.,*

P. emblica L., P. indofischerii Bennet. and P. acidus (L.) Skeels.). They used the stem and leaves of each plant and extracted in methanol and water. The aqueous and methanolic extract were found to be effective against HepG2 cells.

Chen *et al.,* (2011) evaluated N-nitrosodiethylamine-induced inflammation in hepatic tissue by appraising reactive oxygen species (ROS) responses in the liver. Amla expressively conserved MnSOD and catalase expressions and diminished iNOS and CYP2E1 protein expressions in N-nitrosodiethylamine-treated livers. Amla declined N-nitrosodiethylamine-enhanced liver apoptosis and autophagy presences through lowering Bax/Bcl-2 ratio and Beclin-1 expression. Therefore Amla administration neutralizes N-nitrosodiethylamine-induced hepatic injury via its antioxidant, anti-inflammation, anti-apoptosis, and anti-autophagy properties.

Ngamkitidechakul *et al.,* (2010) described the therapeutic anticancer effects of aqueous extract of *Phyllanthus emblica* L (PE) in various cancer cell lines, *in vitro* apoptosis, mouse skin tumourigenesis and *in vitro* invasiveness. The aqueous extract of PE considerably repressed the growth of cell in six human cancer cell lines A549 (lung), HepG2 (liver), HeLa (cervical), MDA-MB-231 (breast), SK-OV3 (ovarian) and SW620 (colorectal). These outcomes suggest *P. emblica* extract have anticancer activity on selected cancer cells, and certify further work as a promising chemopreventive and antiinvasive agent.

Sharma *et al.,* (2009) explained the modulatory activity of *Emblica Officinalis* fruit extract in Swiss albino mice against Arsenic induced oxidative stress. Histopathology demonstrated the reduced karyolysis, necrosis and cytoplasmic vacuolization and recovered by emblica extract. It is proved that the pre- and pro supplementation of *Emblica officinalis* fruit extract substantial lowering the oxidative stress in liver.

Penolazzi *et al.*, (2008) investigated that extracts of *Emblica officinalis* fruits were found effective in the treatment of rheumatoid arthritis by triggering programmed cell death of human primary osteoclasts. They validated that extracts specifically contend with the binding of transcription factor NF-kB to its specific target DNA sequences. Due to this property, the extracts of *Emblica officinalis* shows effects on the expression levels of interleukin-6, which is a NF-kB specific target gene.

Sultana *et al.*, (2008) investigated the anticancer activity of *Emblica Officinalis* against diethylnitrosamine (DEN) (200 mg/kg body wt., i.p.) followed 2-acetylaminoflourine for 6 weeks in swiss albino wistar rats. They revealed that the methanolic extract of fruit of *E. officinalis* attenuate the carcinogenic effect and revert back the histopathological changes and down regulate the number of γ-GT-positive foci in hepatoma bearing rats.

2.2 Literature review of *Madhuca longifolia*

Shrirao *et al.*, (2017) investigated aqueous extract of *Madhuca longifolia* bark and evaluated it its hypocholesterolaemic and hypotriglyceridaemic activities using Triton WR-1339 induced hyperlipemic rats as experimental model. This study proved the intragastric administration of ML 750mg/kg/day has a beneficial effect in treating dyslipidemia with decrease in oxidative stress.

Ramadan *et al.*, (2016) investigated composition, nutritional value, biological activities and antioxidative properties of mahua butter which was prepared from *Madhuca longifolia* fruit-seeds. *Madhuca longifolia* fruit-seeds showed both application of food and non-food of mahua butter cups.

Sunita *et al.*, (2016) reported the cardioprotective effect of *Madhuca longifolia* (Koenig) against isoproterenol-induced myocardial infarction in rats. Outcomes exhibited that *Madhuca longifolia* endangered heart against cardiotoxic effects of isoproterenol by antioxidant activity with preserving structural integrity of cardiac.

Verma *et al.*, (2016) investigated the biologically synthesized silver nanoparticles of *Endophytic Fungus Pestalotia Sp. Isolated from Madhuca Longifolia. They also evaluated the antibacterial and antioxidant property of silver nanoparticles.* Hence, environment-friendly fabricated antibacterial and antioxidant silver nanoparticles are emerging application in nanoparticles mediated drug delivery and therapy of disease.

Roy *et al.*, (2015) reported the hepatoprotective activity of ethanolic extract of *Madhuca longifolia* bark (EEMLB) against D-galactosamine (d-GalN) induced hepatotoxicity in rats. Ethanolic extract of *Madhuca longifolia* bark has shown a significant hepatoprotective activity against D-GalN induced hepatotoxicity in rats.

Bhaumik *et al.*, (2014) explained that the bioactive compounds which is derived from fruits – seeds of *Madhuca longifolia* possess anticancer activity. They performed in vitro anticancer studies of extract by Methanol (E1), Ethanol (E2), Acetone (E3), chloroform (E4)from fruit-seeds of *Madhuca longifolia* against human cancer cell line (HeLa) by MTT assay.

Annalakshmi *et al.,* (2013) reported the phytochemical constituents present in the ethanolic extracts leaves of *Madhuca longifolia* (Koenig) Linn by using GC-MS and HPTLC analysis. The chemical compounds found were effective against various disease.

Triveni *et al.*, (2012) isolated the) and a compound a triterpene, derivative of madhucic acid (dMA) from The methanolic extract of leaves of *M. longifolia* (MLME) and explored their potential neuropharmacological activities in mice using phenobarbitone induced sleeping time, spontaneous motor activity, marble burying test and Eddy's hot plate method Both MLME and dMA also showed significant antinociceptive activity in experimental mice. The consequences recommend that both MLME and dMA have CNS depressant activity in animals.

Agarwal ***et al.,*** (2011) investigated the comparative antioxidant activity of methanolic extracts of *Terminalia paniculata* and *Madhuca longifolia* by using models ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), Nitric oxide, hydroxyl radical and hydrogen peroxide scavenging. Butylated hydroxyanisole, butylated hydroxytoluene. The antioxidant activity of these plants propose their possible use to lessen the oxidative stress and use as therapeutic products in diabetes, liver problems, inflammatory conditions or cancer.

Fyaz *et al.*, (2011) reported the biofabrication of anisotropic gold nanoparticles using aqueous extract of *Madhuca longifolia* and characterized by using instruments UV-Vis spectrophotometer, FTIR, TEM and HR-TEM. They verified the gold nanoparticles in infrared

absorption. It was found that biosynthesized gold nanotriangles can be easily coated in the glass windows and effective in absorbing IR radiations.

Umadevi *et al.*, (2011) reported hepatoprotective activity of methanolic extract of flowers of *Madhuca longifolia* using paracetamol-induced liver damage in Wistar albino rats. Two doses of methanolic extract of *Madhuca longifolia* (100 and 200 mg/kg) demonstrated significant hepatoprotective effect on the animal model by lowering the serum levels of various biochemical profile.

Verma *et al.*, (2010) reported the antibacterial activity of flower of *Madhuca longifolia* against *Bacillus subtilis* and *Klebsiella pneumonia*. Aqueous and methanolic extract of flowers were used for analysis. Aqueous extract showed more activity than methanolic extract for both bacteria.

Dahake *et al.*, 2010) studied the antihyperglycemic activity of bark of *Madhuca longifolia* in alloxan -induced diabetic rats. It was reported that, its methanolic extract is a potential antidiabetic agent.

Prashanth *et al.*, 2010) studied the antihyperglycemic activity of bark of *Madhuca longifolia*. It was reported, its ethanolic extract has potential antidiabetic and antioxidant properties.

Gaikwad *et al.*, (**2009)** studied the anti-inflammatory activity of seeds of *Madhuca longifolia.* It was reported that Madhuca longifolia seed saponin mixture may exhibit a significant anti-inflammatory activity in cotton pellet granuloma.

Ghosh et al (2007) investigated the cloning of a stearoyl/oleoyl specific fatty acyl-acyl carrier protein thioesterase from the seeds of *Madhuca longifolia* (latifolia). They also characterized the stearoyl/oleoyl specific fatty acyl-acyl carrier protein thioesterase. It also designated that SO-Fat indeed is the product of the MlFatB gene existing in the maturing seeds of *M. latifolia* in nature.

Yoshikawa *et al.*, (2000) investigated and isolated the four new oleanane-type triterpene glycosides, madlongisides A-D (1-4 from the seeds of *Madhuca longifolia*. They also elucidated the structure by extensive NMR and chemical methods. They also investigated known compounds mimusopside A, Mi-saponins A, B, and C, and 3-O-beta-D-glucopyranosyl protobassic acid.

2.3 Literature review of *Carissa carandas*

Khatun *et al.*, (2017) reported antioxidant, cytotoxic and anticancer effects methanol extract of *Carissa carandas* leaves (MELC). They investigated In vitro cytotoxic activity of MELC against colonic adenocarcinoma cell lines (SW-480 and SW-48) and in vivo antineoplastic property against Ehrlich ascites carcinoma (EAC). So the research favours the utilization of *Carissa carandas* in ayurvedic medicine as well as highpoints the necessity to further explore the efficacy of MELC as an antineoplastic agent.

Bhusan *et al.*, (2017) isolated, purified and characterized antioxidative steroid derivative from methanolic extract of leaves of *Carissa carandas* (L.). They also evaluated the antioxidative and erythrocyte membrane stabilizing potential of methanol extract of *Carissa carandas* leaves Cc(L)M and its purified fraction CM 1. Phytochemicals Cc(L)M revealed presence of alkaloid, steroids, saponins and tannins. The FT-IR and GC-HRMS analysis of CM 1 reveal the presence of steroid derivative 20-hydroxypregnan 18-oic acid.

Madhumita and Anupama (2017) investigated the bio-friendly synthesis of silver nanoparticles using dried fruit extract of *C. carandas*. The biosynthesized AgNPs were characterized by using instrumentation UV-Vis analysis, FTIR, XRD, SEM, and TEM with histogram. They also studied catalytic degradation on crystal violet dye in the presence of *C. carandas* dried fruit extract and its biosynthesized AgNPs.

Bhadane and Patil (2017) evaluated the antioxidant and erythrocyte membrane stabilizing activity of methanol extract of *Carissa carandas* leaves. They also isolated, purified and characterized the steroid derivative 20-hydroxypregnan 18-oic acid from the leaves of *Carissa carandas* by FT-IR and GC-HRMS.

Verma *et al.*, (2015) evaluated the antioxidant activity and in vitro DNA damage inhibition efficacy of methanolic extract of *Carissa carandas* leaves. The methanolic extract provide

protection from free radical-mediated oxidative stress by preventing pBR322 plasmid DNA in a DNA damage inhibition assay. The antioxidant and DNA damage inhibition properties of *C. carandas* can be accredited to a high amount of phenolic compounds, determined by the Folin–Ciocalteu assay. The high antioxidant and DNA damage inhibiting property of *C. carandas* could be utilized to develop antioxidant compounds for therapeutic applications.

Galipalli *et al.*, (2015) reported anti-inflammatory activity of the extracts, fractions and compounds isolated from methanol extract of *Carissa carandas* (L.) roots. All the isolated compounds stigmasterol (1), lupeol (2), oleanolic acid (3), carissone (4) and scopoletin (5) displayed the anti-inflammatory effects of *Carissa carandas* (L.) roots, partially mediated by inhibition of TNF-α, IL-1β and NO.

Dhodi *et al.*, (2015) elucidated the mode of action of methanolic extract of *Carissa carandas* fruits (MCCF) via down regulated diabetic nephropathy against gentamicin induced nephrotoxicity model. Methanolic Extract of fruit attenuated oxidative stress which was generated after the introduction of gentamicin, which is one of the mechanisms for its protecting the diabetic nephropathy against gentamicin toxicity.

Gupta *et al.*, (2014) reported the anticancer and antidiabetic effect of seven Indian tropical fruit residues against cervical cancer cells (HeLa), breast cancer cells (MCF-7), hepatocellular carcinoma cells (HepG-2) and bone sarcoma cells (MG-63) and alpha amylase inhibition assay was used for antidiabetic activity. Consequences presented that *Carissa carandas* possessed best activity with IC_{50} value as 29.66 mg/mL followed by other residues in methanol extract and these fruit residues reveal favourable antidiabetic and anticancer activity that proven its ethno medicinal use and may provide new molecules for the treatment of these diseases.

Anupama *et al.*, (2014) reported the anti-inflammatory activity of methanolic extract of dried fruits of *Carissa carandas* against carrageenan-induced hind paw edema in rats. The 11 phytochemical constituents present in the methanolic extract of dried fruits of *Carissa carandas* were analysed by GC-MS. The anti-inflammatory effects of the methanol extract of the dried fruits reveals that the presence of phytoconstituents in the extract may offer backing in the process of drug discovery.

*Arif **et al.,** (2013) investigated the adaptogenic effects of triterpenoid compound lanostane which was isolated from Carissa carandas fruit and evaluated against* cyclophosphamide induced immunosuppression model in mice. These work exhibit that extract and isolated compound displayed significant adaptogenic activity. Triterpenoid isolated Lanostane structure was confirmed by Mass spectra and ^{13}C NMR.

Garg *et al.*, (2012) reported the analgesic and anti-pyretic activity of aqueous extract of leaves of *Carissa carandas* using hot plate, acetic acid induced writhing and yeast induced hyperthermia method is traditionally used to treat painful and inflammatory conditions.

*Itankar **et al.,** (2011) investigated the antidiabetic potential of methanol extract of unripe fruits of Carissa carandas and its fractions against alloxan induced diabetic rats. The highest antidiabetic activity of* ethyl acetate fraction when compare to methanol extract was observed. The polyphenolic, flavonoid and flavanone contents of methanolic extract of fruits were also estimated and interrelated with its antidiabetic potential.

Bhaskar and Balakrishnan (2009) investigated uses the folklore plant *Pergularia daemia* (Asclepiadaceae) and *Carissa carandas* (Apocynaceae). The traditional plants were evaluated for analgesic, anti-inflammatory and antipyretic activities. The ethanol and aqueous extract of

roots both plants displayed significant ($p<0.01$) analgesic, anti-inflammatory and antipyretic activities in rodents models.

Sharma *et al.*, (2007) reported the anti-inflammatory and analgesic activities of ethanolic (50% v/v) extracts of *Carissa carandas* (fruits) (Apocynaceae) and *Microstylis wallichii* (tubers) (Orchidaceae) experimental animals.

Chapter-3

Material & Methods

3.1 Collection and Authentication of Selected plants

Leaves of *Phyllanthus emblica, and Carissa carandas* were collected from the local area of Mirzapur, Uttar Pradesh and leaves of *Madhuca longifolia* was collected from the natural habitat of Jhunsi region, Uttar Pradesh which was authenticated by the Dr. R.M. Kadam, Director, Aerobiology Research centre, Ahmedpur, latur, India. Voucher specimen given to the sheets number and submitted at the Institute.

Table 3.1 Authentication of leaves medicinal plants

S.No.	**V. No.**	**Plant name**	**Family**	**Part used**
1.	DI 11	*Phyllanthus emblica* L.	Phyllanthaceae	Leaves
2.	DI 12	*Madhuca Longifolia* J.F. Macbr.	Sapotaceae	Leaves
3	DI 13	*Carissa Carandas* L.	Apocynaceae	leaves

3.2 Pharmacognostical Study

As per the demand of the people for medicinal preparation of crude drugs, the advancement of pharmacognostical study has been essential for getting remedies which have potent therapeutic effect and prepared from traditional and medicinal plants. The pharmacognosists have a genuine obligation, to take the activity not just in effectively finding the plant already mentioned in old books but pharmacopoeias yet additionally making them accessible to researchers in different specialization to evaluate the activity for which the plant are proclaimed. These studies were done on the fresh and dried leaves of plant samples. The transverse section of plant leaves was cut to determine the microscopic character. Dried plant sample of coarse size was pulverized by using electric blender to study the powder characteristics. The dried plant leaves of *Phyllanthus emblica, Madhuca longifolia and Carissa*

carandas about 100 g were taken and allowed to macerate in double distilled water to obtain an aqueous extract.

3.2.2 Morphological or Organoleptic Evaluation

The macroscopic study of the selected plants were evaluated by visual and organoleptic observation by colour, odour, taste, size, shape and special features like touch and texture. These studies were depending on the morphological and sensory profile of drugs. The leaves were kept on the white plate to determine the organoleptic feature of selected plants.

3.2.3 Microscopy evaluation

Microscopy evaluation is done to identify the histological features of selected plants. This method is used for the qualitative evaluation of powdered drugs and organised drugs. The histological details of selected plants were inspected by the microscope.

3.2.3.1 Equipment used for the microscopic evaluation

- Microscopes with a wide range of magnification of 4 X, 10X, and 40 X and colour filter of ground glass.
- Polarizing filter sets
- A stage and ocular micrometer
- Slide and cover glasses
- Botanical dissecting instruments

3.2.3.2 Preparation of sample

3.2.3.2.1 Section cutting

Selected plant material parts being examined, can be sectioned with the use of razor and cut into the desired length. Before cutting the plant material section and razor, both must be kept wet. The plant materials were soaked in water prior to cut and prepared cross or transverse section was cut for the microscopic study. The section was placed in 70 % ethanol which was used to hold and store sections. The sectioned were mounted on the slide, stained

with phloroglucinol in alcohol (1% w/v) and excess of the alcohol was subjected to remove by using filter paper. The slide was covered with a cover slip with the help of glycerine.

3.2.3.3 Quantitative microscopy - Determination of leaf constants:

The leaves surfaces were used for the study of leaf constant. In this, the epidermal surface of leaf were scrapped, peeled and washed with a chloral hydrate to determine characteristics of leaf constant such as Stomatal Number, Stomatal Index, Vein-islet number, Vein termination number.

Stomatal number: defined as an average number of stomata per square mm of the epidermis of the leaf.

Upper and lower epidermal layer of leaves were cut by means of forceps, placed on a slide and mounted in a glycerine water. Detect the epidermis cell and stomata of a leaf and count the number of stomata present in the area of 1 sq. mm. include the cell if at least half of its area lies within the square by the means of camera lucida and drawing board. Record the result for each of the ten fields and calculate the average number of stomata per sq. mm.

Stomatal index: it is defined as the percentage in which number of stomata per unit area to the total number of epidermal cells in the same unit area. In this each stoma consider as single cell. Stomatal number is calculate as

$$S.I. = \frac{S}{E + S} \; X \; 100$$

S. I = Stomatal Index,

S = No. of stomata per unit area,

E = No. of epidermal cells in the same unit area

Procedure:

Boil the piece of leaf with chloral hydrate solution to clear it. Peele and scrape the upper and lower layer of leaf with the means of forceps. Place the leaf layer on slide and pot glycerine

water on it. With the help of camera lucida and drawing board detect the epidermis cell and stomata. Count the number of stomata and epidermal cell for upper and lower surface separately in each field.

3.2.3.4 Vein termination number & vein islet number

Vein-islets number is defined as the small area of the green tissue covers by the vein-islets of the central part between midrib and its margin of the leaf.

Procedure

Boil the piece of leaf with chloral hydrate solution to clear it. Peele and scrape the upper and lower layer of leaf with the means of forceps. Place the leaf layer on slide and pot glycerine water on it. With the help of camera lucida and drawing board, detect the veins in the square which is drawn on drawing board and count the number of vein islets. Calculate the average number of vein islets from the four adjoining squares, to sq. mm.

Veinlet termination number is defined as the small area of green tissue covers by the vein termination of the central part between midrib and its margin of the leaf.

Immerse the Piece of leaf in chloral hydrate solution and boil to clear it. Peele and scrape the upper and lower layer of leaf with the means of forceps. Place the leaf layer (epidermis leaf) on slide and pot glycerine water on it. With the help of camera lucida and drawing board, detect the veins in the square which is drawn on drawing board and count the number of Veinlet termination. Calculate the average number of Veinlet termination from the four adjoining squares, to sq. mm.

Powder microscopic characteristics

On a glass slide, placed one drop of water and glycerol/ethanol on a slide and wet the tip of needle with the help of water. Powder of the selected plants was placed carefully on the slide with the help of needle tip which contain a drop of fluid. The slide were covered with

cover slide, pressed lightly so that it should be freed from air bubbles and excess fluid were removed by the help of filter paper (Evans, 2009).

3.2.3.5 Photomicrographs

Microscopic observation of tissue is done with micrograph and Images were captured at different magnification with sony digital camera. Magnification of figures was indicated by scale –bars.

3.2.4 Physio-chemical Evaluation

The standardization of traditional plants was done in order to achieve therapeutic efficacy. Standardization is done to control the quality or to evaluate the crude drugs. WHO suggested various parameters to estimate the standardization of crude drugs. Physio-chemical evaluation of crude drugs is done in manner to ensure the identity, quality, purity and strength. Physico-chemical study of drugs were done with special reference to ash value, total ash, acid insoluble ash, extractive value, alcohol soluble extractive , water soluble extractive and loss of drying.

3.2.4.1 Determination of Foreign organic matter

The other materials than plant parts or its organ are defined as the foreign organic matter. Its maximum limit for is mentioned in the monograph of crude drugs. If the limits of foreign organic matter exceeds, quality of the crude drugs is found to be deterioted.

About 100 gm of leaf powder was spread in a thin layer and the foreign matter was sorted by the visual inspection or by using magnifying lens. Remaining sample was sieved through sieve no. 250. The percentage of foreign matter was calculated by the given formula

$$Percentage\ of\ foreign\ content\ = \frac{weight\ \ of\ sample - weight\ of\ foreign\ matter}{weight\ of\ sample} X\ 100$$

3.2.4.2 Determination of moisture content by loss on drying

The amount of chemical constituents present in crude drugs is determined on air –dried basis. If moisture content of drugs not controlled, it will lead to decomposition of crude drugs and development of fungal and bacterial growth.

10 gm of powders was weighed, placed in a crucible and kept in a hot air oven at a temperature of 100-110°C, overnight to remove moisture. When weight gets constant, the dried samples were collected and weighed after cooling. The loss of weight were considered as a moisture content of crude drugs and calculated by the

$$Percentage\ of\ moisture\ content = \frac{Weight\ of\ sample - weight\ of\ dried\ sample}{weight\ of\ sample} X100$$

3.2.4.3 Determination of ash value

The residue remaining after complete incineration of the crude drug materials, which is inorganic salt may be adhere to crude drug or naturally present inside the drugs or present in form of adulterant. The ash value estimated by total ash, acid-insoluble ash and water soluble

3.2.4.3.1 Determination of Total ash:

Weighed accurately one gram of powdered drug, transfer to crucible and charred by increasing the temperature to 550–600ºC for 2 hours and cooled in a desiccator. This process is repeated till constant weight was observed. % of total ash was calculated on the basis of air dried drug.

$$Percentage\ of\ ash = \frac{Weight\ of\ ashed\ sample}{Weight\ of\ sample\ taken}\ X\ 100$$

3.2.4.3.2 Procedure for Acid insoluble ash:

After incinerate, the ash value obtained was used for the determination of acid insoluble ash. The ash was digested with 25 ml of 2 N HCL in a crucible for 5 minutes in a boiling water bath. The insoluble ash filtered by using ash less filter paper. The collected matter was washed

with hot water, incinerates and weighed. The percentage of acid insoluble ash was calculated as:

$$Percentage\ of\ soluble\ ash = \frac{\text{Weight of acid insoluble ash}}{\text{Total weight of ash}} \text{X } 100$$

3.2.4.3.3 Procedure for acid soluble ash

The soluble ash was estimated by using 2 N HCl. The ash obtained was boiled in 25 ml of 2N HCL for 20 minutes in a crucible in a boiling water bath. The content were subjected to filter by using Whatman filter paper No: 42 and residue was carefully removed, dried in a hot air oven, incinerated in a furnace for 1 hour and weighed (Mukherjee, 2002).

The percentage of acid soluble ash was calculated as:

$$Percentage\ of\ soluble\ ash = \frac{\text{Weight of acid soluble ash}}{\text{Total weight of ash}} \text{X } 100$$

3.3. Extraction

The dried plant leaves of *Phyllanthus emblica, Madhuca longifolia and Carissa carandas* were shade dried, pulverized into coarse powder and passed through sieve#40. All plant materials leaves about 200 g were taken, allowed to macerate or were progressively separated in double distilled to obtain an aqueous extract and concentrated with the help of water bath.

3.3.1 Preparation of Aqueous Plant Extract:

3.3.1.1 *Phyllanthus emblica* aqueous leaf extract:

The *Phyllanthus emblica* leaves were collected locally and authenticated by the India. The collected Leaves were thoroughly washed with tap water and rinsed with double distilled water to remove debris and dust. The leaves were then shade dried at room temperature and crushed in an electrical blender to obtain a fine powder. The fine powder was stored in a brown colour bottle for further study.10 g of powder was taken in an Erlenmeyer flask and mixed in 100 ml of double distill water and kept in boiling water bath for 30 min at 70°C. The extracts

were filtered through Whatman filter paper No 1 and then stored in a refrigerator at 4 °C for further use (Ponarulselvam *et al.*,).

3.3.1.2 *Madhuca longifolia* aqueous leaf Extract:

The aqueous extract was prepared by using the leaves of Madhuca Longifolia by the procedure as mentioned above in section 3.3.1.1

3.3.1.3 *Cassia carandas* aqueous leaf extract:

The aqueous extract was prepared by using the leaves of Cassia carandas by the procedure as mentioned above in section 3.3.1.1.

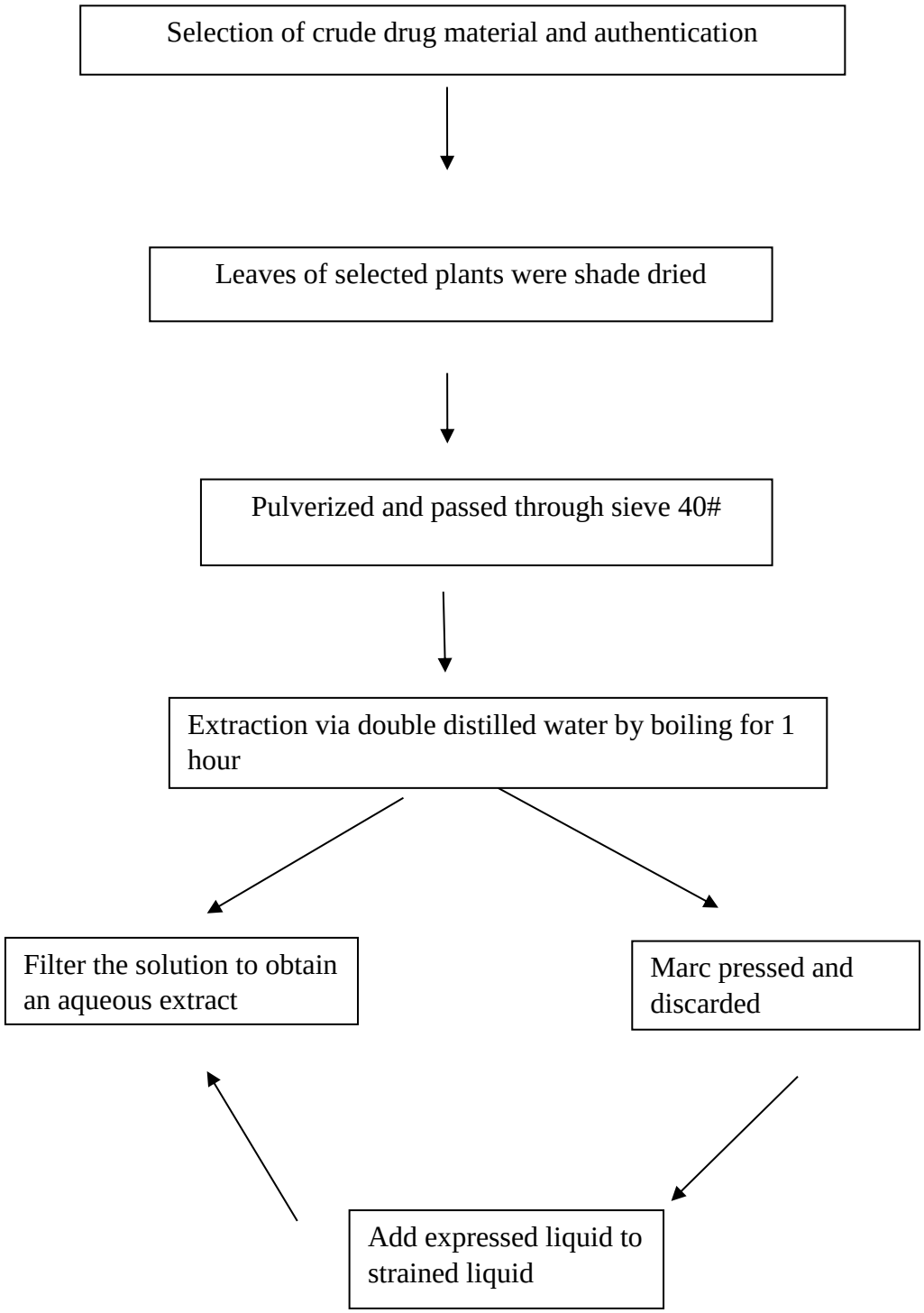


Fig. 3.1 Diagrammatic representation of Extraction process

3.4 Preliminary phytochemical analysis

Due to versatile application of plant derived substances, pharmaceutical industries focused on the plant for the medicament. A Medicinal plant contains a richest source of secondary metabolites which are used in nutraceutical, traditional medicines, food supplement and synthetic drugs. Medicinal plants show their therapeutic potency through simple extracts to isolated compounds (chemical constituents.) In modern times, European Scientific methods investigated the active constituents and therapeutic actions of medicinal plants. The plant itself knows as a biosynthetic laboratory not only for active compounds like carbohydrates, proteins and lipids but also have an abundance of compounds such as glycosides, alkaloids, volatile oils, tannins, etc. Secondary metabolites are those compounds which exert a medicinal property. To isolate the active constitute from the crude drugs and the technique involved is known as extraction. The choice of solvents and plant material for extraction depends on the requirement of compounds to be isolated.

The phytochemical evaluation composed of chemical test and chemical assays. The isolation, purification and identification of chemical constituents may be evaluated by chemical methods. To determine the chemical profile of crude drugs, it can be done by phytochemical evaluation and also cover phytochemical screening.

The extracts were subjected to various qualitative chemical test for the determination of active constituents present inside it.

1. **Test for alkaloids**

i. Mayer's Reagent: 1 ml of Mayer's reagent (Potassium mercuric iodide solution) was added to 1 ml of extract, the formation of white or cream coloured precipitate confirmed the presence of alkaloids.

ii. Dragendorff's Reagent: 1 ml of Dragendroff's reagent (solution of potassium bismuth iodide) were added to 1 ml of extract, the formation of orange yellow precipitate confirmed the presence of alkaloids.

iii. Hager's Reagent: 1 ml of Hager's reagent (saturated aqueous solution of picric acid) was added to 1 ml of extract the formation of yellow precipitate confirmed the presence of alkaloids.

iv. Wagner's Reagent: Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to 1 ml of extract the formation of reddish brown precipitate confirmed the presence of alkaloids.

2. Test for glycosides

i. Legal test: plant extract was dissolved in pyridine and sodium nitroprusside was added to make the solution alkaline. Pink Red colour to red colour indicates the presence of glycosides.

ii. Keller-Kiliani test: To plant extract, 1 ml of glacial acetic acid which contains traces of ferric chloride and 1 ml of concentrated sulphuric acid was added, A reddish brown colour present at the junction of the liquid which indicates the presence of glycosides.

iii. Borntrager test: 1ml of when 1 ml of benzene and 0.5 ml of dilute ammonia solution were added to plant extracts and Appearance of pink colour indicates the presence of glycosides.,

3**. Test for flavonoids**

i. Ammonia test: The extract was dissolved in water and few ml of ammonia was added. The filter paper strip was dipped in ammoniated solution and the formation of fluorescence confirms the presence of flavonoids.

ii. Shinoda/Pew test for flavonoids: The extract was dissolved in water and a few pieces of metallic magnesium or zincwas added into it. 1 ml of concentrated HCl was added to the solution and gives reddish brown colour which confirms the presence of flavonoids.

4. Test for Sterols

i. Liebermann-Burchard test: to 1 gm of plant extract was dissolved in few drops of in chloroform and 3 ml of acetic anhydride were added. In this, a few drops of concentrated sulphuric acid was added along the sides of the tube. Formation of blue to blood red color confirms the presence of sterols in the extract.

ii. Salkowski reaction: 1 gm of extract was dissolved in few drops of chloroform. In this 2 ml of concentrated sulphuric acid was added and appearance of a yellow ring at the represent the presence of sterols in the extract.

5. Test for Carbohydrates

i. Molish test: to 2 ml of plant extract, 1 ml of naphthol (20% in ethyl alcohol) was added and concentrated sulphuric acid was added through the sides of the tubes. Appearance of reddish violet ring at the junction of two layers was seen, which confirms the presence of carbohydrates

ii. Fehling's test: to 1ml of extract, 1 ml of Fehling's Solution A and B (copper sulphate in alkaline conditions) was added and heated on water bath. Formation of brick red precipitates indicates the presence of sugars.

6. Test for Saponins

i. Foam test: To 1 ml of the extract, few drops of water were added and. Appearance of foam show positive test for the presence of saponins.

ii. Sodium Bicarbonate test: To 1 ml of extract, few drops of sodium bicarbonate were added and solution was shaken well shaken well. Appearance of foam indicates honey comb like frothing ishow positive test for saponins.

7. Test for Phenolic Compounds and Tannins

i. Ferric Chloride test: to 1 ml of extract, ferric chloride solution (5%) was added and blue color was appeared which shows positive test for phenolic compounds.

ii. Lead Acetate test: 1 ml lead acetate solution (5%) was added to the aqueous extract which gives a yellow/white precipitate, shows the presence of phenolic compounds/tannins in extract.

8. Test for Proteins and Free Amino Acids

i. Million's test: In a 2 ml of the aqueous extract, 5-6 drops of Million's reagent (solution of mercury nitrate and nitrous acid) were added and formation of red precipitate indicates the presence of proteins and free amino acids.

ii. Biuret test: to 1 ml of test solution 1 ml of 40 % sodium hydroxide solution and 2drops of 1% Copper sulphate solution was added till a blue colour was formed. Appearance of a red/violet color confirms the presence of proteins in extract.

9. Test for Coumarins

i. Small quantity of sample was placed in a test tube and it was covered with filter paper which was already soaked in a dilute NaOH solution. This test tube was subjected to boiling on water bath. After sometime the filter paper was removed and exposed to UV light, and it give s green fluorescence which confirms the presence of Coumarins (Kokate, 2000).

3.5 Preparation of the solution and plant extract:

3.5.1 Preparation of metal precursor solution:

Silver nitrate solution (1mM): 0.017 g of silver nitrate was dissolved in 100 mL of distilled water.

3.5.2 Synthesis of Silver nanoparticles

3.5.2.1 Silver nanoparticles of *Phyllanthus emblica* leaf extract:

The Silver NPs were prepared by using 1mM aqueous solution of silver nitrate. 10 ml of plant extract (*Phyllanthus emblica*) is mixed with 90 ml of $AgNO_3$ solution under vigorous stirring in Erlenmeyer flask and incubated overnight at RT for the reduction into silver ions. Then the solutions are centrifuged at 6000 rpm for 15 min to remove the unwanted organic matter and washed twice with deionized water resuspended in a distill water and dried in an oven to get the pellet and further used for experimental studies (Jeyachandran *et al.*,2014).

3.5.2.2 Silver nanoparticles of *Cassia Carandas* leaf extract:

The silver NPs were prepared by using 1mM aqueous solution of silver nitrate with *Cassia Carandas* leaf extract by the procedure as mentioned above in section 3.5.2.1

3.5.2.3 Silver nanoparticles of *Madhuca longifolia* leaf extract:

The silver NPs were prepared by using 1mM aqueous solution of silver nitrate with *Madhuca longifolia* leaf extract by the procedure as mentioned above in section 3.5.2.1.

3.6. Characterization techniques:

Synthesized silver nanoparticles of *Phyllanthus emblica, Cassia carandas* and *Madhuca longifolia* were characterized by different techniques like Ultraviolet-visible spectroscopy, FTIR, TEM, FESEM with EDX, XRD.

3.6.1 Ultraviolet–Visible spectroscopy

Ultraviolet–visible spectroscopy or **ultraviolet-visible spectrophotometry** (**UV-Vis** or **UV/Vis**) is also known as absorption of reflectance spectroscopy. Molecules which have π-

electrons or non-bonding electrons (n-electrons) excite these electrons to higher antibonding molecular orbitals after absorbing the energy in the form of ultraviolet or visible light. The longer the wavelength of light depends on

The synthesized silver nanoparticles of *Phyllanthus emblica, Cassia carandas* and *Madhuca longifolia* were characterized by UV vis spectroscopy using a Shimadzu UV–visible spectrophotometer and the absorption spectra were recorded at a room temperature in quartz cells in the wavelength region of 400-700nm.

3.6.2 Fourier Transform Infrared spectroscopy

FTIR stands for Fourier transform infrared, and the infrared portion is divided into three regions Near IR, mid IR, far IR with relation to visible spectrum.

Near IR approximately 14000-4000 cm−1 (0.8-2.5 μm wavelength)

Mid-infrared, approximately 4000-400 cm−1 (2.5-25 μm)

The far-infrared, approximately 400-10 cm−1 (25-1000 μm)

Some Radiation is absorbed by sample and some radiation transmitted, When IR radiation is passed through this sample and resulting signal is the spectrum which shows a fingerprint of the sample. They are useful in the identification of an unknown compound, Quantitative information and in analytical tool to know the purity of the sample.

To know the presence of different functional groups, the synthesized silver nanoparticles of *Phyllanthus emblica, Cassia Carandas* and *Madhuca longifolia* were analyzed by FTIR spectroscopy. The dried Silver nanoparticles were mixed by KBR pellet and kept into the thin disc and the spectra recorded in the wavelength range of 4000 to 400nm-1by using a Perkin Elmer FTIR spectrophotometer.

3.6.3 X-ray Diffractometry

X-ray diffraction (XRD) is a one of the technique used to determine the crystal structure and atomic spacing. These rays were generated by a cathode ray tube and directly spread toward the target material. The scattered X- rays with the sample produce constructive interference. The diffraction rays by crystals is condition satisfy Bragg's Law, ($n\lambda=2d \sin\theta$). They are used to determine or identify and characterize the crystalline nature of compounds.

Crystalline phase and size of the reduced AgNPS of *Phyllanthus emblica*, *Cassia carandus* and *Madhuca longifolia* were determined by XRD analysis. The films of the respective synthesized AgNPs solution drops were spreaded on to the glass substrate on a XRD Instrument (Panalytical. SX. Pert Pro) at a voltage of 30 KV and a cuttern of 30 mA with Cu – K alpha radiation at in the angle range of 20-90 degree.

3.6.4 FESEM with EDX

FE-SEM stands for Field Emission Scanning Electron Microscope is a type of electron microscope and scan the image at high beam of electrons and used for the quantitative analysis, digital imaging and X-ray imaging. Provide a resolution upto arrange of nanometer and performance well at low kV for surface imaging.

Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS), sometimes called energy dispersive X-ray analysis (EDXA) or energy dispersive X-ray microanalysis (EDXMA), is an analytical technique used for the elemental analysis or chemical characterization of a sample excellent performance both at low kV for surface imaging and at high kV for EDS analysis. They are used to analyses the microstructure and major and minor elemental analysis. FESEM with EDX (Zeiss) is used to examine the elemental composition and surface morphology of the reduced AgNPS.

3.6.5 TEM

Transmission electron microscopy (**TEM**) is a very powerful microscopy technique for the material science. A high energy beam of electrons is allowed to pass through an ultra-thin sample and interaction between with the electrons and specimen to give featured images. TEM (Hitachi) specify the finest internal structure of specimen and they are used to examine the quality, shape and size of the sample.

The surface morphology of the prepared AgNPs was determined by TEM. Thin drops of respective samples were placed over coated copper grids and solvent is allowed to evaporate and operated at voltage of 200 Kv.

3.7 *In vitro* cytotoxicity study of silver nanoparticles

3.7.1 Preparation of cell culture

Human Hepatocellular carcinoma (HUH-7) cell lines were acquired from National centre for Cell Science (NCCS), Pune, India. Cells were kept up in DMEM (Dulbecco's Altered Bird Medium) and 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 mg/ml streptomycin, 0.14% sodium bicarbonate and 0.1 mM sodium pyruvate. Cells were developed in CO_2 incubator in 5% CO_2 air at room temperature and 95% humidity.

3.7.2 Study of anticancer property

MTT reagents were utilized to measure the *in vitro* anticancer effect of bioactive silver nanoparticles of leaves of *Phyllanthus emblica*, *Madhuca longifolia* and *Carissa carandas* on human hepatocellular carcinoma (HUH-7) cell line. In this method, MTT lessens to blue formazan by mitochondrial dehydrogenase, which demonstrates mitochondrial capacities and subsequently cell growth. In short, $3X10^3$ cells per well were seeded in 96 well plates in 100 mL cell culture medium and allowed to incubate for 24 h. After 24 h cells were treated with different dilution of the biofabricated AgNPS. Afterwards, 5 mg/mL of MTT reagent were mixed with a fresh medium into well and again subjected to an incubator for 4 hours. MTT was

dissolved in DMSO and formed formazon products. The absorbance of a mixture was recorded at 540 nm against blank (Patel *et al.*, 2009).

It was estimated as

$$\% \text{ of cell inhibition} = \left(Y - \frac{X}{Y}\right) X100$$

Where Y is absorbance of the control (untreated cells)

X is the mean absorbance of treated cells with AgNPs.

3.8 *In vivo* hepatic cancer and renal cancer activity

3.8.1 Animals

The wistar albino rats of either sex (175-210 g) were selected for the study. The animals were kept at the animal house of SIHAS, SHUATS, Allahabad under standard condition of temperature (25 ± 2°C) and relative humidity of (40-60%) with photoperiod of 12:12 dark & light hour. They were fed with commercial available standard pellet and water given *ad libitum.* All the protocols were reviewed by SHUATS university animal ethics committee (approval no IEAC/SHIATS/PA16III/SDSAV08) guidelines and CSCPEA regulations.

3.8.2 Chemicals

Diethylnitrosamine (DEN) was obtained from M/s. Sigma Chemcial Company, St. Louis, MO, USA. Other chemicals used in this study were obtained from Himedia Laboratories, Mumbai, India, and were of analytical grade.

3.8.3 LD_{50} determination by acute toxicity study

The acute oral toxicity studies and LD_{50} was performed according to Organization for Economic Co-operation and Development (OECD-423) guidelines. The dose selection for plant extract of *Phyllanthus emblica* was 250mg/kg body weight based on the previous literature available on toxicity study. PEAgNPs were administered to experimental animals in dose dependent manner ranging upto 75mg/kg body weight. The animals received test drug by oral route and observed for mortality and behavioural changes for 48 hrs.

3.8.4. Study design

Diethylnitrosamine (DEN)-induced hepatocarcinogenesis and renal carcinogenesis in animal model

Wistar albino rats were divided into 18 groups with six rats in each. Doses of silver nanoparticles were fixed after performing acute toxicity studies.

3.8.4.1 Treatment regimen for *Phyllanthus emblica*

All the animals were randomly divided into six groups and were subjected to following treatments and depicted in Figure 2

Group I: Animals were treated with a vehicle (0.9 w/v % of NaCl solution) and served as normal control.

Group II: All animals were treated with single intraperitoneally injection of diethylnitrosamine (200 mg/kg). This group served as a negative control without any drug treatment.

Group III: DEN+ *PE* at a dose of 250 mg/kg throughout the experimental period.

Group IV: DEN+ PEAgNPs (10 mg/kg, B.W.) throughout the experimental period.

Group V: DEN+ PEAgNPs (20 mg/kg, B.W.) throughout the experimental period.

Group VI: DEN+ Silymarin (30 mg/kg, B.W.) throughout the experimental period.

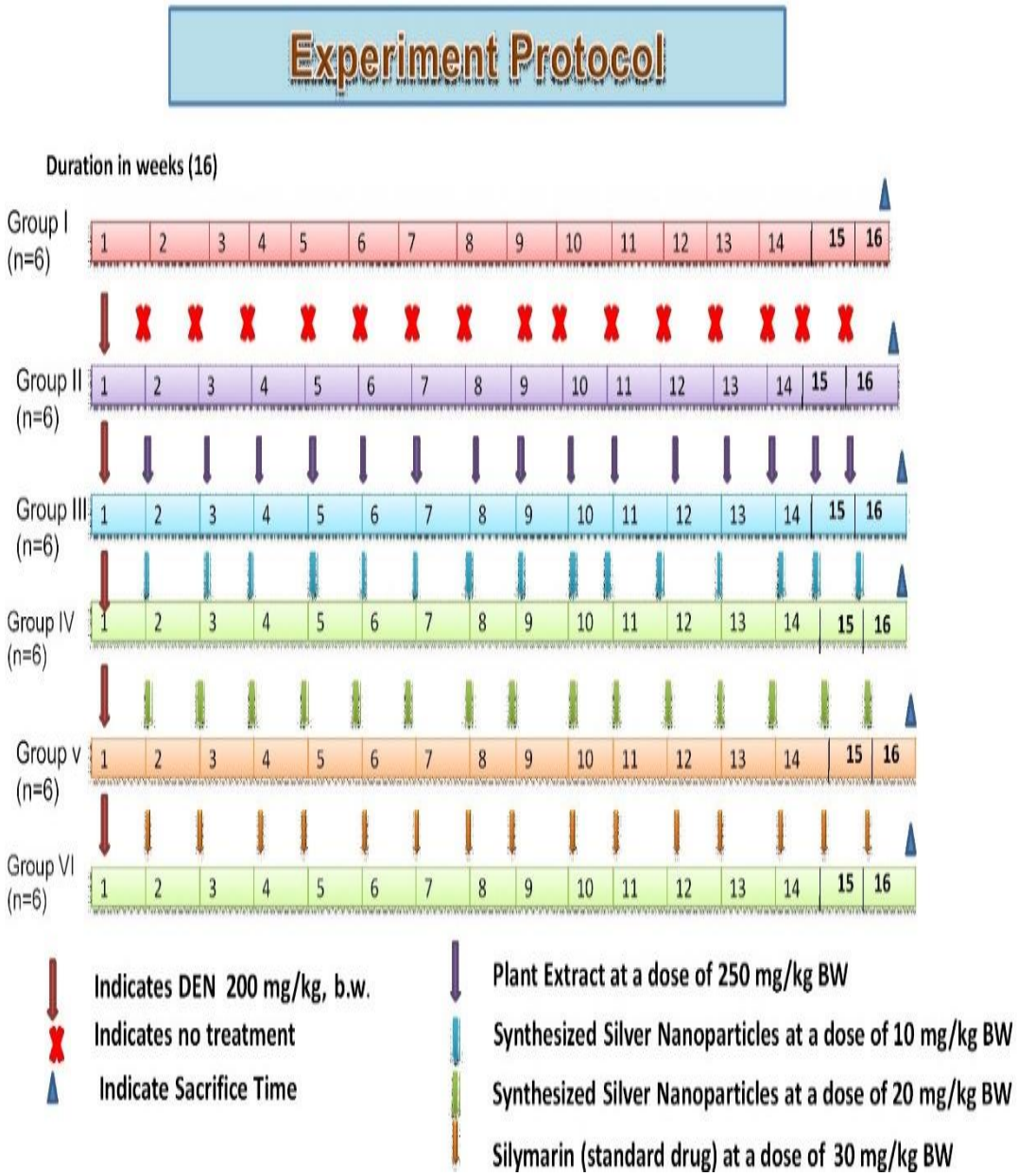


Figure 3.2 Treatment regimen for *Phyllanthus emblica*

3.8.4.2 Treatment regimen for *Madhuca Longifolia*

Six groups of rats were used and each group contain six animals to study the impact of the MLAgNPs on DEN induced cancer and represented in figure 2.

Group I- received normal saline (0.9%) daily for 16 weeks.

Group (II to VIII) - Liver cancer induced with single intraperitoneal injection of a DEN (200 mg/kg BW) in a saline solution.

Group II- received DEN solution only.

Group III- treated orally with MLE (300mg/kg BW) upto successive 16 weeks.

Group IV- treated orally with MLAgNPs (20mg/kg BW) upto successive 16 weeks.

Group V- treated orally with MLAgNPs (30 mg/Kg BW) upto successive 16 weeks.

Group VI- treated orally with Silymarin (30mg/kg BW) upto successive 16 weeks.

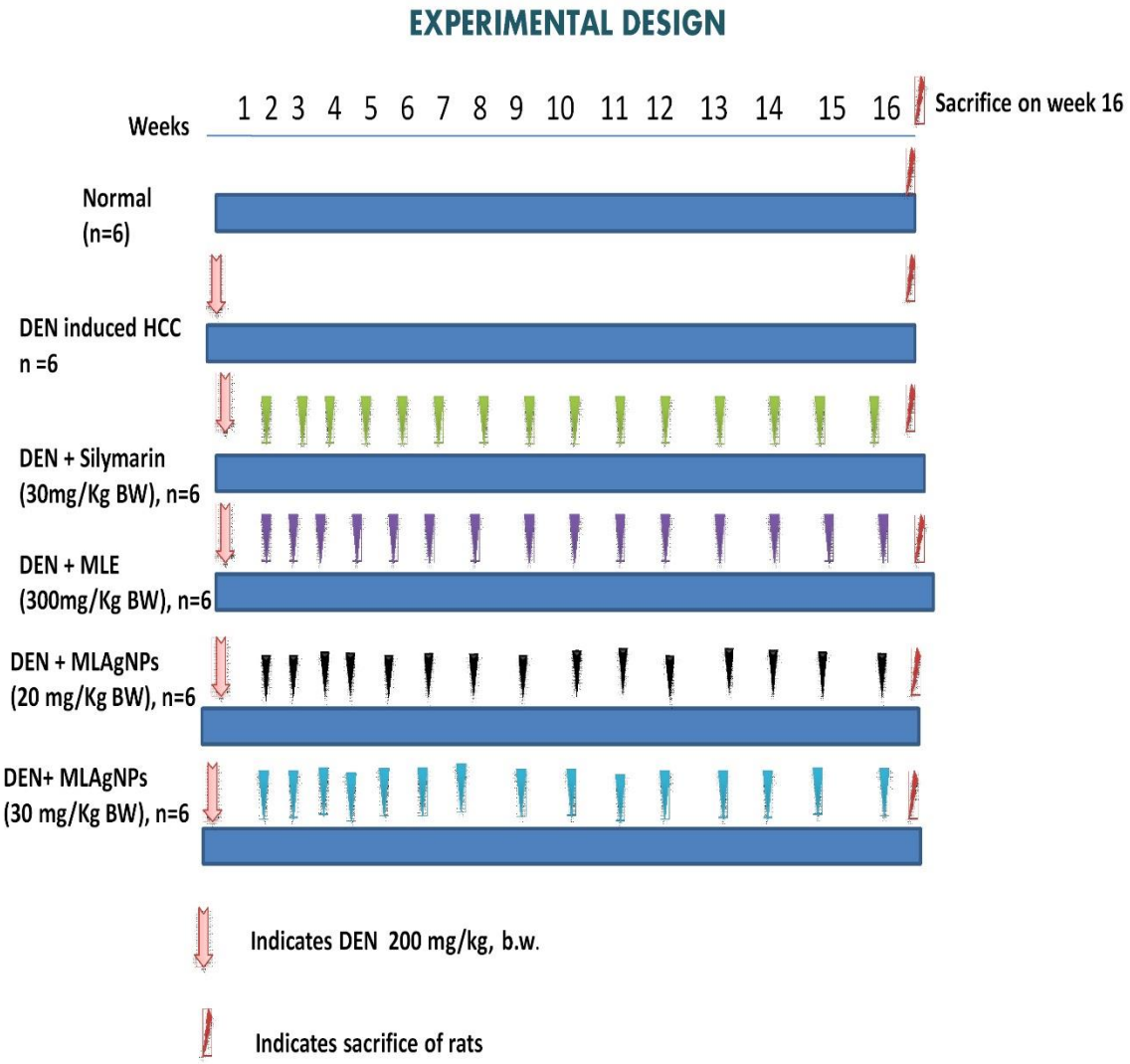


Figure 3.3 Treatment regimen for *Madhuca Longifolia*

3.8.4.3 Treatment regimen for *Carissa carandas*

All rats were categorized in six groups and received a cancer-causing agent (DEN) and tested drug portrayed below and depicted in figure:

Group I: Normal control

Group II: received DEN only

Group III: DEN + Silymarin (30 mg/kg)

Group IV: DEN + CCE (300 mg/kg)

Group V: DEN + CCAgNPs (20 mg/kg)

Group V: DEN + CCAgNPs (30 mg/kg)

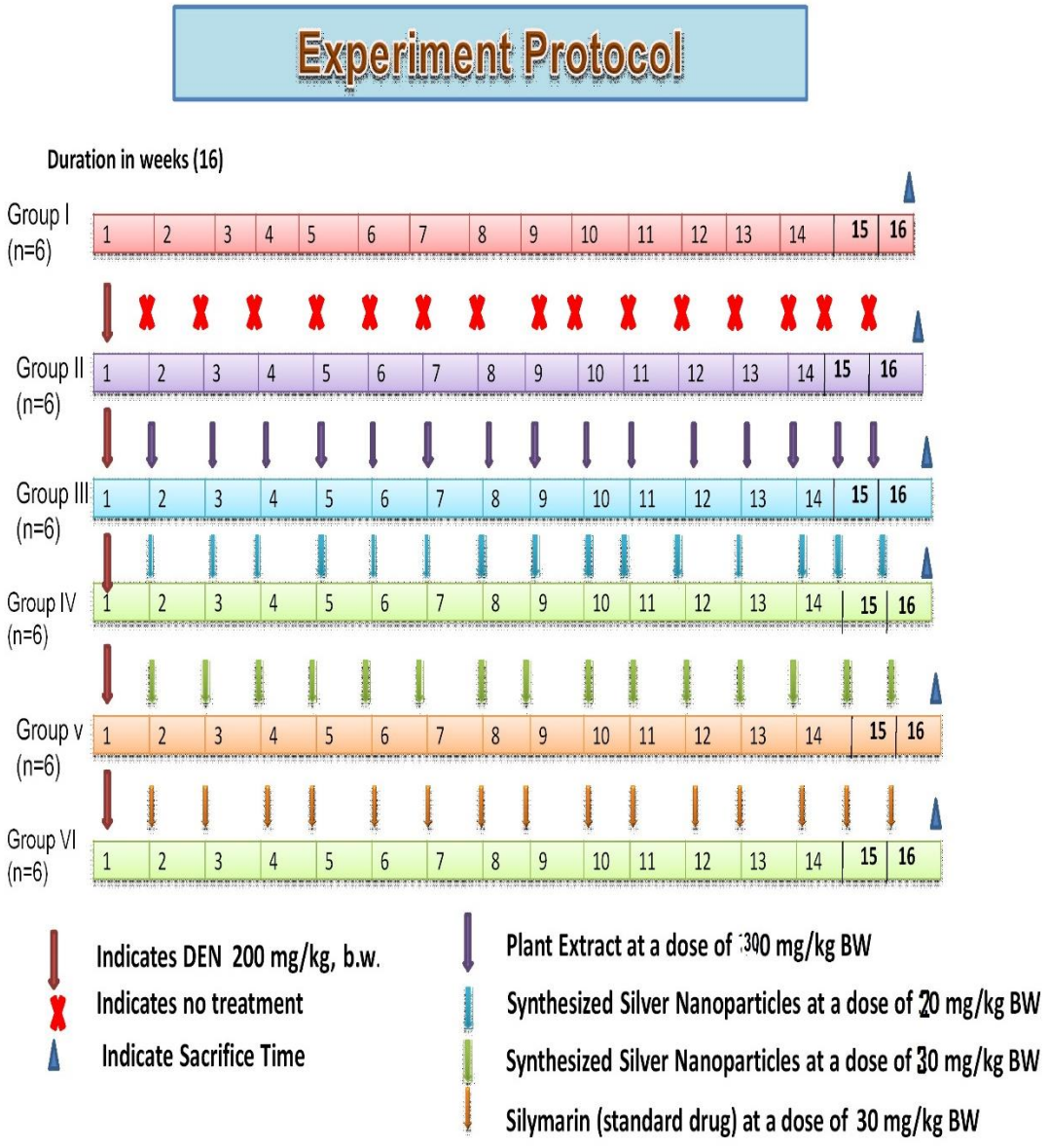


Figure 3.4 Treatment regimen for *Carissa carandas*

The test drug will be given orally daily for 4 weeks respectively. After 24 h of last dose, the rats from each group were sacrificed by cervical dislocation under mild anaesthesia. The blood was collected from rats of each group from retro-orbital sinus plexus and collected in labelled centrifuging tube stand and allowed to clot 30 min at room temperature. Serum will be separated by centrifugation at 10,000 rpm for 15 min.

3.8.5. Preparation of liver homogenate

The liver was removed from rat and perfuse immediately in ice-cold saline (0.9% NaCl) solution. Then the liver tissue for dissected stored at -90 degree for histopathological investigation. Liver tissue was homogenizer by using a homogenizer in chilled Tris-HCl buffer solution (0.025 M, pH 7.4). The homogenate was centrifuged at 10, 000 rpm for 5 min and supernatant was collected for analysis.

3.8.6. Biochemical analysis from serum

The absorbance of all the biochemical parameters was measured in a UV–VIS Spectrophotometer - 1601 (Shimadzu, Tokyo, Japan).

3.8.6.1 Estimation of aspartate aminotransferase (AST) activity

The serum aspartate aminotransferase was estimated by the method of **Reitman and Frankel (1957)** using AST test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered aspartate - α-KG substrate, pH 7.4

Reagent II: DNPH (2,4- Dinitrophenyl hydrazine) colour reagent

Reagent III: Sodium hydroxide, 4 N

Reagent IV: Working pyruvate standard, 2 mM

Solution I: Dilute 1 ml of Reagent III up to 10 ml with purified water.

Procedure

0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 60 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes mixed well and allowed to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505 nm.

3.8.6.2 Estimation of alanine aminotransferase (ALT) activity

The serum alanine aminotransferase was estimated by the method of **Reitman and Frankel, 1957** using ALT test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered alanine - α-KG substrate, pH 7.4

Reagent II: DNPH (2,4- Dinitrophenyl hydrazine) colour reagent

Reagent III: Sodium hydroxide, 4 N

Reagent IV: Working Pyruvate Standard, 2 mM

Solution I: Dilute 1 ml of Reagent III up to 10 ml with purified water.

Procedure

0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in the standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 30 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed it to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505 nm.

3.8.6.3 Estimation of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was estimated by the method of **King (1965)** using ALP test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered substrate, pH 10.0

Reagent II: Chromogen reagent

Reagent III: Phenol standard, 10 mg%

Working solution: Reconstitute one vial of reagent I, buffered substrate with 2.2 ml of purified water.

Procedure

All the test tubes were marked properly as blank (B), standard (S), control (C), and test (T). 0.5 ml of working buffered substrate was added in clean tubes. 1.5 ml of purified water was added in all the tubes. They were mixed well and incubated at 37°C for 3 min. 0.05 ml of serum was added in test (T), 0.05 ml of reagent III (Phenol standard) was added in standard (S) and 0.05 ml of purified water was added in blank (B) tubes. All the tubes were mixed well and incubated at 37°C for 15 min. 1 ml of reagent II was added in all the tubes. 0.05 ml of serum was added in control (C). All the tubes were mixed well and absorbance was read at 510 nm. Serum alkaline phosphatase activity is expressed as KA units.

3.8.6.4 Estimation of AFP (Alpha feto protein)

Alpha-fetoprotein (AFP) is measured quantitatively by solid phase enzyme linked immunosorbent assay (ELISA).

Reagents

1. Microwell strips Anti-AFP antibodies coated -wells.

2. Enzyme conjugate: Anti-AFP antibodies conjugated to horse-radish peroxidase.

3. Sample diluent or zero standard.

4. Reference standard set: Calibrated to 0, 37 10, 25, 100 and 200 IU/ml

5. Solution A: Buffer solution containing hydrogen peroxide.

6. Solution B : Tetramethyl benzidine.

7. Well holder for securing wells.

8. Microwell reader.

9. 1N H_2SO_4

10. Pippetor with tip for 10 µl and 100 µl.

Procedure

The collected blood was allowed to clot and serum was separated. All the reagents and samples were brought to room temperature and mixed. Then 25 µl of serum sample, control and standards were dispensed in to the assigned wells. Then 100 µl of 0 IU/ml of AFP standard solution was dispensed, immediately in to those assigned wells and incubated for 30 minutes at room temperature. The incubation mixture was removed and the wells were rinsed 5 times with running water. Then 100 µl of enzyme conjugate was dispensed in to each well and again incubated for 30 minutes at room temperature. 100 µl of solution A and 100 µl of solution B were added into each well, and incubated for 10 minutes at room temperature. The reaction was stopped by the addition of 1 N H_2SO_4 to each well and optical density at 450 nm was read with microwell reader. The AFP content is expressed in IU/ml**.**

3.8.6.5 Estimation of total protein content

The serum total protein was estimated by modified Biuret method (Yatzidis, 1977) using the total protein test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Biuret Reagent (Copper sulphate - 7 mM/L; sodium hydroxide - 200mM/L; sodium potassium tartrate - 20 mM/L)

Reagent II: Protein standard (BSA - 6.5 g/dL)

Procedure

3.0 ml of Reagent I was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at 37ºC for 5 minutes. The absorbance was read at 578 nm.

3.8.6.6 Estimation of albumin content

The serum albumin was estimated by the method given by **Corcoran and Durnan (1977)** using albumin test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Albumin reagent (Succinic acid - 37 mM/L; bromocresol green - 0.15 mM/L; sodium hydroxide - 1 mM/L; buffer pH - 3.68).

Reagent II: Albumin standard (BSA - 4 g/dL).

Procedure

3.0 ml of albumin reagent (Reagent I) was added to all the test tubes. Thereafter, 0.03ml serum was added for the test and 0.03 ml Reagent II was added for the standard while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at room temperature for 1 min. The absorbance was read at 630 nm.

3.8.6.7 Determination of serum bilirubin

The bilirubin level in serum was determined by **Dangerfield and Finlayson, (1953)**. Sulfanilic acid reacts with sodium nitrite to produce deoxidized sulfanilic acid. Total bilirubin couples with deoxidized sulfanilic acid in the presence of methylsulfoxide to produce azobilirubin which may be measured at 532-536 nm. In the absence of methyl sulfoxide, only direct (conjugated) bilirubin forms azobilirubin complex.

Reagents

1. Total bilirubin reagent, Sulfanilic acid, Dimethyl sulfoxide, Stabilizer.

2. Direct bilirubin reagent, Sulfanilic acid, Preservative.

3. Activator, Sodium nitrile.

4. Artificial standard – 10 mg/dl.

Procedure

To 1.0 ml total bilirubin reagent, 0.02 ml of activator and 0.1 ml of serum were added, mixed well and incubated for exactly 5 minutes at room temperature. Sample blank was prepared by mixing 1.0 ml total bilirubin reagent with 0.1 ml of distilled water, mixed well and incubated for exactly 5 minutes at room temperature. The absorbance of each sample blank and test were measured at 532-546 nm against distilled water blank. Total bilirubin and direct bilirubin level in serum was expressed as mg/dl.

3.8.6.8 Evaluation of cytokines/inflammatory mediators

The levels of proinflammatory cytokines and inflammatory mediators (IL-6, IL-1b, TNF-a, PGE2, and NF-κB) in the renal tissue of normal control and treated animals were analyzed by specific ELISA kits as per manufacturer instructions. Absorbance of proinflammatory cytokines was calculated at 450 nm spectrophotometrically [29]. Calibration curve was plotted using standard cytokines and concentrations of unknown samples were evaluated from standard curve. NF-κB was correlated with the triggering of NF-κB pathway. The kit was used to measure the NF-κB in the renal tissue homogenate as per the instruction provided by manufacturer.

3.8.7 Determination of lipid peroxidation

This study was determined by measuring MDA as explained by Ohkawa et al **(Ohkawa, 1979)**. The level of malondialdehyde (MDA) acts as a biomarker of lipid peroxidation. 1.5 ml of 20 % acetic acid, 0.2 ml of SDS and 1 ml of 0.67% thiobarbituric acid were added to 0.2 ml of tissue homogenates, and volume made up to 4 ml with distilled water,

heated for 30 minutes and allowed to cool. Then 4 ml of n-butanol- pyridine mixture was added and centrifuge it. The organic layer was aspirated, and absorbance was taken at 532 nm.

The MDA level was expressed as nmol of MDA formed/min/ mg of protein.

3.8.8 Determination of enzymatic and non-enzymatic antioxidant parameter

3.8.8.1 Estimation of Catalase enzyme activity

To 0.05 ml of liver homogenate, 1.2 ml of phosphate buffer and 1.0 ml of hydrogen peroxide was added. The absorbance was taken at 240 nm.

It was expressed as n moles of H_2O_2 consumed per minute per mg protein (Li and Schellhorn, 2007).

3.8.8.2 Estimation of Superoxide dismutase enzyme activity

1.5 ml of buffer (0.1 M carbonate – bicarbonate buffer pH 10.2, containing 57 mg EDTA/dl) was added to 0.05 ml of liver tissue homogenate. The reaction was started by the addition of 0.4 ml of epinephrine and absorbance was taken at 480 nm in a Shimadzu spectrophotometer. One unit of SOD activity is expressed as an amount of protein required to produce 50% of inhibition of epinephrine auto-oxidation.

It was expressed as units /ml of enzyme /mg of protein (Fridovich, 1972).

3.8.8.3 Estimation of Glutathione peroxidase enzyme activity

The Glutathione Peroxidase enzyme activity was determined by the method of Tapel et al. To a liver homogenate, 50mM potassium- phosphate buffer (pH 7.0), 1 mM EDTA, 1 rnM NaN_3, 0.2 mM NADPH, 1 IU/ml glutathione reductase, 1 mM GSH, 1.5 mM cumene hydroperoxide or 0.25 mM H_20_2 and an appropriate amount of the enzyme was added in a total volume of 1 ml.

The absorbance of the system was recorded at 340 nm and enzyme activity was defined as micro mol NADPH oxidized per min per mg of protein (Tappel, 1978).

3.8.8.4 Measurement of Glutathione

GSH was measured by the method of Jollow et al. To the liver homogenate, 1 ml of 4% sulphosalicyclic acid was added, incubated and centrifuged for 15 min. 0. 4 ml of supernatant was taken; in this 2.2 ml of Phosphate buffer (pH 7.4) and 0.4 ml of dithio-bis-2-nitrobenzoic acid was added. The absorbance was recorded at 412nm.

GSH concentration was noted as nmol GSH conjugates/g tissue (Jollow DJ, 1974).

3.8.8.5 Estimation of Vitamin C

Vitamin C was estimated by the method of Omaye et al. Homogenate was treated with 5% ice cold tricarboxylic acid and allowed to centrifuge for 20 min at 6500 rpm. 0. 1 ml of a supernatant was mixed with 0.2 ml 2, 4 dinitrophenyl hydrazine: thiourea: copper sulphate and incubated for 3 hr at room temperature. In the reaction mixture, 0.1 ml of ice cold 65% H_2SO_4 was added, and solution was kept at room temperature for 30 min. Absorbance was recorded at 520 nm.

Ascorbic acid values are recorded as µg/mg protein (Omaye ST, 1979).

3.8.8.6 Measurement of glucose-6-phosphate dehydrogenase (G6PD) activity

Glucose-6-phosphate dehydrogenase enzyme activity was detected by the method of Zaheer et al. The reaction mixture comprised of 0.3 ml Tris–HCl buffer (0.05 M, pH 7.6), 0.1 ml NADP (0.1 mM), 0.1 ml glucose-6-phosphate (0.8 mM), 0.1 ml $MgCl_2$ (8 mM), 0.3 ml PMS (10%) and 2.1 ml distilled water and volume made upto 3ml.

Absorbance were noted at 340 nm and values are expressed as nmol NADP reduced/min/mg (Zaheer,).

3.8.8.7 Determination of Na/K+ ATPase

This activity was performed to know the amount of phosphorus liberated from the mixture which containing the liver homogenate, ATP and chloride salt of electrolytes. Na/K+ ATPase was determined according to the method of bonting (Bonting, 1970). The mixture

contain 1.0 ml of buffer, 0.2 ml of $MgSO_4$, KCl, NaCl, EDTA, ATP and enzyme and allowed to incubate at RT for 15 min. The reaction mixture was initiated by the addition of 10 ml of TCA, centrifuged and the supernatant was taken to measure the inorganic phosphorous.

This activity was expressed as micro moles of inorganic phosphorous liberated/min/mg protein.

3.8.8.8 Determination of Ca^{2+}ATPase

Ca^{2+}ATPase in the plasma membranes was measured under as explained by Lotersztajn (Lotersztajn *et al.*, 1981).

Reagents

1. Tris-HCI buffer: 125 mM pH 8.0: 1.514 g of Tris was dissolved in 100ml of deionised. H_2O and the pH was adjusted to 8.0 C HCL

2. $CaC1_2$ 50 mM : 273.6 mg of calcium chloride was dissolved in 25 ml of deionised H_2O.

3. ATP 10 mM: 33 mg of ATP was dissolved in 6.0 ml of deionised H_2O.

Procedure

The incubation mixture contained. 0.1 ml each of buffer, $CaCl_2$, ATP and tissue homogenate. The mixture was incubated at 37oC for 15 minutes. The reaction was arrested by the addition of 1.0 ml of 10% TCA.

The enzyme activity was expressed as μ moles of inorganic phosphorus liberated/min/mg protein.

3.8.9 Histological examination

The liver tissue was fixed in formalin solution to preserve them and was embedded in paraffin as per standard histological procedure. Sections of six micrometres were prepared and stained with eosin and haematoxylin. The stained slides were observed under a light microscope for the qualitative analysis of hepatic histopathology.

3.9.9 *In vivo* renal cancer

To determine the impact of plant extract and biosynthesized silver nanoparticles, the kidney of control group and experimental animals were taken.

The body weight of all rats was monitored regularly on weekly basis till accomplish of experimental study. All the rats of each group were killed by cervical dislocation 12 hour after the treatment with drugs. Blood was collected in centrifuged tube from retro-orbital sinus before killing the animals. Kidney tissue was excised, washed with ice-cold saline and separated out for the conformation of renal malignancy and kept at -90 degree. Kidney tissues were freely processed for the approximation of different biochemical parameters and histopathological study.

3.9.10 Post-mitochondrial supernatant (PMS) and microsomes preparation

Post-mitochondrial supernatant (PMS) and microsomes preparation was done by the reported method of S. Ansar & M. Iqbal. Briefly kidneys were abruptly removed, cleaned of foreign matter and perfused promptly with ice cold saline. The kidney was allowed to homogenize by homogenizer in 0.1 M phosphate buffer, pH 7.4 contain 1.17% w/v KCl. A muslin cloth was used to filter the homogenate and allowed to centrifuge at 800 g for 5 min at 4°C in an ultracentrifuge apparatus (model RC 4100 D) to remove the nuclear debris. Sample was centrifuged at 12000 rpm for 20 min at 4 °C to get Post-mitochondrial supernatant (PMS). A small portion of the PMS was again centrifuged at 12000 rpm for 1 hour at 4°C to obtain a pellet and was expected as microsomal fraction and was stored in 0.1 M phosphate buffer pH 7.4 (Iqbal and Ansal, 2013).

3.9.11 Assay of serum marker enzyme

3.9.11.1 Blood Urea Nitrogen

Diacetyl monoxime was used for the study of BUN and done by the method reported by Kanter. In the serum, equal amount of 10% TCA was added, mixture was centrifuged at

2000 rpm for 10 min and supernatant was taken which consider as Protein-free filtrate. 3.5 ml of distilled water, 0.8 ml diacetylmonoxime (2%) and 3.2 ml sulphuric acid–phosphoric acid reagent (reagent was prepared by mixing 150 ml 85% phosphoric acid with 140 ml water and 50 ml of concentrated sulphuric acid) were added to 0.5 ml of protein-free filtrate. The prepared mixture was kept on water bath for 30 min and cooled. The absorbance at 480 was noted down (Kanter, 1975).

3.9.11.2 Creatinine

Alkaline picrate was used for the estimation of Creatinine and determined by the following method of Hare. 1.0 ml sodium tungstate (5%), 1.0 ml sulfuric acid (0.6 N) and 1.0 ml distilled water were added to 1.0 ml of serum with uniform mixing. The sample was kept in centrifugation apparatus to centrifuge the mixture at 800× *g* for 5 min. Mixture of 1.0 ml picric acid (1.05%) and 1.0 ml sodium hydroxide (0.75 N) was added to a protein free filtrate. Absorbance was read after 20 min at 520 nm (Hare, 1950).

3.9.11.3 Uric acid

Estimation of uric acid was done by the method of Singh et al. In the PMS, 1·0 mL of 66·7 rnrnol/L phosphate buffer, pH 7·5, and 5 IJ.L of uricase solution (16 U/mL) was added and left out the solution for 15 min. In the reaction mixture, 50 IJ.L of HCl (2 rnol/L) was added and mixed well. The absorbance of mixture was taken at 410 nm against the blank (Singh *et al.,* 1984).

3.9.12 Estimation of tumour marker enzyme

3.9.12.1 Xanthine Oxidase Activity

Xanthine oxidase (XO) activity was determined by the method of Stripe and Della Corte. 0.2 ml PMS with 0.8 ml phosphate buffer (0.1 M, pH 7.4) were incubated for 5 min at 37°C. In the above reaction mixtures, 0.1 ml xanthine (9 mM) was added and stands for 20 min

at 37°C. The reaction was carried forward by the addition of 0.5 ml ice-cold perchloric acid (PCA) (10% v/v) and 2.4 ml of distill water was added after 10 min.

The reaction mixture was centrifuged at 4000 r.p.m. for 10 min and absorbance was read at 290 nm. Xanthine Oxidase Activity was expressed as μg uric acid formed per minute per mg protein (Della and Stripe, 1972).

3.9.12.2 Lactate dehydrogenase (LDH) activity

The activity of Lactate dehydrogenase (LDH) was assayed by following method of Korenberg. The reaction mixture contains 0.2 ml of serum, 0.1 ml of 0.02 M NADH, 1.1 ml of 0.1 M (pH 7.4) phosphate buffer, 0.1 ml of 0.01 M sodium pyruvate and 3 ml of distilled water.

Enzyme activity was expressed as nmol NADH oxidized/min/mg protein and read exactly at 340 nm (Kornberg, 1955).

3.9.13.3 γ-glutamyl transpeptidase Activity

The Activity of GGT was estimated according to the method of Orlowski and Meister. The mixture consists of 0.2 ml of serum with 0.8ml of the substrate which contains 4 mM Gammaglutamyl *p*-nitroanilide, 40 mM glycine and 11 mM MgCl2 in 185 mM Tris HCl buffer, pH 8.25 at RT. The reaction takes place by the addition of 1.0 ml of Trichloroacetic acid (TCA) (25%), sample was centrifuged and the upper layer was noted at 405 nm.

The activity of this enzyme was measured by using a molar extinction coefficient of *p*-nitroaniline as 1.74 × 103 M-1 cm-1 and as nmol p-nitroaniline formed min^{-1} $(mg\ protein)^{-1}$ (Orlowski , 1973).

3.9.13 Estimation of lipid peroxidation

The lipid peroxidation (LPO) assay was determined by the method of Ohkawa *et al*,. The reaction mixture consisted of 0.58 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml microsome, 0.2 ml of ascorbic acid (100 mM) and 0.02 ml of ferric chloride (100mM) in a total of 1 ml. It was incubated at 37°C on water bath for 60 min. The reaction was terminated by the

addition of 1 ml of TCA (10%).The tubes were placed on water bath for 20 min and cooled it. The tubes were transferred for centrifugation at 2500 × g for the period of10 min. The quantity of malondialdehyde (MDA) in each tube was calculated by taking absorbance at 535nm and measured as nmol MDA formed/h/g tissue at 37°C (Ohkawa *et al.*, 1979).

3.9.14 Estimation of enzymatic and non-enzymatic antioxidant profile

3.9.14.1 Catalase

The Catalase assay was determined by the method of Claiborne. Briefly, the 3 ml of total solution comprised of 0.05 ml of PMS, 1.0 ml of hydrogen peroxide (0.019M), 1.95 ml of phosphate buffer (0.1 M, pH 7.4). The absorbance was quantified at 240 nm.

It was expressed as nmol H_2O_2 consumed per min per mg of protein (Claiborne, 1985).

3.9.14.2 Measurement of superoxide dismutase (SOD) activity

The activity of SOD was assessed by the reported method of Marklund and Marklund. 3 ml of total solution contain a 2.875 ml Tris–HCl buffer (50 mM, pH 8.5), pyrogallol (24mM in 10mM HCl) and 100 µl of post mitrochondrial supernatant.

Absorbance was taken at the optical density of 420 nm and SOD activity was calculated units/mg protein **(**Marklund and Marklund, 1974).

3.9.14.3 Estimation of GSH

The activity of Glutathione was assessed by the reported method of Jollow *et al*. 1.0 ml of (4%) sulphosalicylic acid was mixed in PMS solution and incubated at 5°C for 60 min and mixture was centrifuge at 1500 rpm for 15 min. The solutions (3ml) contain a 0.4 ml of DTNB (4 mg/ml) and 2.2 ml of phosphate buffer (0.1 M, pH 7.4).

Reduction of reaction mixture develops a yellow colour and recorded at 412 nm and concentration was measured as nmol GSH conjugates/g tissue (Jollow *et al*., 1974).

3.9.14.4 Assay for glutathione peroxidase activity

Glutathione peroxidase (GPx), activity was measured via using the following method of Mohandas *et al.*. 0.1 ml of PMS solution was mixed with 1 ml of EDTA (1 mM), 0.1 ml of sodium azide (1 mM), 1.44 ml of phosphate buffer (0.1 M, pH 7.4), 0.05 ml of GR (1 IU/ml), 0.05 ml of GSH (1 mM), 0.1 ml of NADPH (0.2 mM) and 0.01 ml of H_2O_2 (0.25 mM). Optical density was taken at 340 nm by consumption of NADPH and activity read as nmol NADPH oxidized/min/mg protein (Mohandas *et al.,* 1984).

3.9.14.5 Estimation of glutathione reductase activity

The GR activity was assessed by the following method Kumar et al. with slight moderation. 3 ml of solution composed of the EDTA (0.1 ml, 0.5 mM), phosphate buffer (1.65 ml; 0.1 M, pH = 7.6), NADPH (0.1 ml; 0.1 mM), PMS (0.1 ml), and oxidized glutathione (0.05 ml; 1 mM) at 25 °C. It was determined by diminishing of NADPH at 340 nm and estimated as nmol NADPH oxidized per min per mg protein (Kumar *et al.,* 2013).

3.9.14.6 Glutathione –S- Transferase activity

The 3 ml reaction mixture composed of 0.2 ml GSH (1 mM), 0.2 ml CDNB (1 mM), 2.5 ml phosphate buffer (0.1 M, pH 6.5), in 0.1 ml of the cytosolic fraction (10%). Enzyme activity quantified at 340 nm and expressed as nmol CDNB conjugate formed per min per mg protein (Habig *et al.,* 1974).

3.9.14.7 H_2O_2 assay

2 ml of (10% w/v) microsomes were dispersed in the 1 ml of phenol red (0.28 nM), horseradish peroxidase (8.5 U), dextrose (5.5 nM) and phosphate buffer (0.05 M, pH 7.0)) and the reaction mixture was subjected to incubate at RT for 1 hour. The reaction was terminated after adding 0.01 ml NaOH (10 N) and supernatant was collected after centrifuge for 800 g for 5 min. Absorbance was noted down at 610 nm against a blank. H_2O_2 assay was calculated as nmol H_2O_2/h/g tissue (Pick, 1981).

3.9.15 Evaluation of cytokines/inflammatory mediators

The levels of proinflammatory cytokines and inflammatory mediators (IL-6, IL-1b, TNF-a, and NF-κB) in the renal tissue of normal control and treated animals were analyzed by specific ELISA kits as per manufacturer instructions. Absorbance of proinflammatory cytokines was calculated at 450 nm spectrophotometrically. Calibration curve was plotted using standard cytokines and concentrations of unknown samples were evaluated from standard curve. NF-κB was correlated with the triggering of NF-κB pathway. The kit was used to measure the NF-κB in the renal tissue homogenate as per the instruction provided by manufacturer.

3.9.16 Estimation of tumour promotion markers

3.9.16.1 Ornithine decarboxylase (ODC) activity

ODC activity was done by the method of O'Brien et al. It was estimated by using 0.4 ml of supernatant fraction per tube and quantified the $14CO_2$ amount release from DL-[14C] ornithine. In test tube, total volume of 0.495 ml contained 400µl enzyme and 0.095 ml co-factor mixture containing pyridoxal phosphate (0.32 mM), EDTA (0.4 mM), dithiothreitol (4.0 mM), ornithine (0.4 mM), Brig 35 (0.02%) and [14C] ornithine (0.05 /µCi). Then the all tubes were closed after the addition of 0.2 ml ethanolamine and methoxyethanol mixture with rubber stopper, kept in a boiling water bath and incubate for 1 hr. The activity was terminated by the addition of 1.0 ml citric acid solution (2.0 M) and again incubated for 1 hour for complete absorption of $^{14}CO_2$. In this, 2 ml ethanol and 10 ml toluene-based scintillation fluid was added and transferred to a vial. It was placed in a liquid scintillation counter to count the radioactivity and calculated as pmol $^{14}CO_2$ released/h mg protein (O'Brien *et al.,* 1975).

3.9.16.2 Renal DNA synthesis assay

The isolation of Renal DNA and measurement of incorporation of [^{3}H] thymidine into DNA was explained by Smart et al. The kidney tissue was homogenates and precipitate was incubated with cold PCA (10%) for overnight at 4 ºC. Further this mixture was allowed to

centrifuge and the precipitate thus obtained was suspended in warm PCA (10%). The mixture was incubated in water bath for 30 min and filtered it. In the filtrate, 10 ml toluene-based scintillation fluid was added, transferred to a vial and placed in a liquid scintillation counter to count the [^{3}H] counting (Smart *et al.*, 1986).

3.9.17 Histopathological analysis

Kidney was examined for histopathological changes. The renal tissue was settled in buffered formalin solution which was dehydrated in ethanol, afterward cleaned with xylene and implanted in paraffin. For the preparation of slides, section of 3-4 μm (thickness) of renal tissue were cut by a sledge microtome and hematoxylin-eosin stain was used to recolour. Histological analysis was done under light microscope at magnification of 40 X.

3.10 Statistical Analysis

For the estimation of statistical analysis, Graph pad Prism 5 software was used. All analysis was performed in triplicates. Results were expressed as the mean ± standard error (SEM) and comparisons were made by one-way ANOVA with Dunnett's post-test. Differences were considered statistically significant when P value <0.001.

Chapter-4

Result

Result of *Phyllanthus emblica*

4.1 Pharmacognostical character of leaf of *Phyllanthus emblica*

4.1.1 Macroscopical characters of leaf of *Phyllanthus emblica*

The leaves of *P.emblica* is simple, alternate and distichous. The arrangement of vein is pinnate, structure is oblong and glabrous. The leaves are 5cm to 10 cm long and 1 cm wide. Texture of leaves are soft in touch. Leaflets are oval in shape having acute apex and rounded base with entire margin and tiny stipules. Colour is light green on upper side and dark green on below side (figure 1.8).

4.1.2 Microscopic characters of leaf of *Phyllanthus emblica*

The transverse section of *P.emblica* leaf showed larger cells on upper epidermis with thick outer walls and smaller cell on lower epidermis. The layer of the collenchyma is present below the epidermis layer on adaxial surfaces and on the lower surface two layer collenchyma is present followed by parenchymatous cortex. The cuticle is thick and present on the both side of the cells. The cuticles showed papillae structure or a pattern of striation. Trichomes are absent on both side of surface of leaf. Mesophyll are consists of palisade and spongy tissue. Palisade comprises of large cells and spongy tissues of minor loosely arranged cells. A curve shaped vascular bundle present in the centre in which primary xylem facing upwards. The transverse section of leaf of *P.emblica* is shown in figure 4.1.

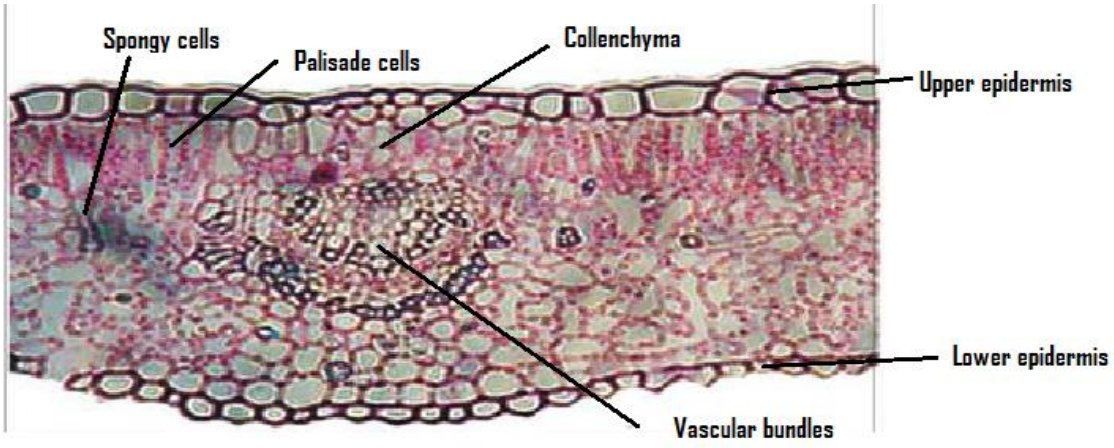


Figure 4.1 Transverse section of leaf of *P.emblica*

The results of leaf constant and physicochemical parameter is displayed in table no 4.1 and 4.2.

Table 4.1 Determination of leaf constant of *Phyllanthus emblica*

Leaf Constant	**Range**	**Average**
Stomatal Number		
Upper surface	10-18	14
Lower surface	20-29	34.5
Stomatal Index		
Upper Surface	22.3-32.7	38.65
Lower Surface	18.5-20.9	28.95
Vein islet number	8-15	11.5
Vein termination number	29-36	47

Table 4.2 Physico-chemical parameters of leaves of *P.emblica*

S. No.	**Parameter**	**Observation (% w/w)**
1	Ash value	
A	Total ash	5.6
B	Acid soluble ash	1.2
c	Acid insoluble ash	3.2
2.	Moisture content	7.68
3	Foreign matter	0.7

4.2 Extraction of Aqueous extract of *Phyllanthus emblica*

The leaves of *P.emblica* were subjected to double distilled water and the yield was obtained as 11.01% (w/w) which is depicted in table 4.3

Table 4.3 Percentage yield of extract of leaves of *Phyllanthus emblica*

Botanical name	**family**	**Part used**	**Solvent used for extraction**	**yield (Extract)**
Phyllanthus emblica	Phyllanthaceae	leaves	Water (aqueous)	11.01

4.3 Preliminary phytochemical studies of aqueous extract of *Phyllanthus emblica*

Phytochemical studies were performed to determine the presence of various chemical compounds present in the plant extract. The different chemical tests were performed on the aqueous extract of *P.emblica*. *P.emblica* revealed positive test for various chemicals constituents such as and presented in table 4.4

Table 4.4 Preliminary phytochemical screening of aqueous extract of *Phyllanthus emblica*

S. No.	Chemical Test	Aqueous extract
1.	**Alkaloids**	
	Mayers Reagent	+
	Dragendroff Reagent	+
	Hagner reagent	+
	Wagner reagent	-
2.	**Glycosides**	
	Legal test	+
	Keller Killani Test	+
	Borntragers Test	-
3.	**Flavonoids**	
	Ammonia Test	+
	Shinoda/ Paw test	+
4.	**Sterols**	
	Liebermann-Buchard test	-
	Salwoski Test	-

5.	**Phenolic compounds and tannins** Ferric chlorides test Lead acetate test	 - -
6.	**Proteins** Millions test Biuret test	 - -
7.	**Coumarins**	-

+ Positive - negative

4.4 Biosynthesis of silver nanoparticles of extract of *P.emblica*

Briefly, 10 ml of plant extract (*Phyllanthus Emblica*) was mixed with 90 ml of 1mM $AgNO_3$ solution under vigorous stirring in erlenmeyer flask and incubated overnight at RT for the reduction of silver ions. The solution turns from brown to dark-brown colour which is represented in figure 4.2. The solution was centrifuged at 8000 rpm for 15 min to remove the unwanted organic matter, washed twice with deionized water and dried in an oven.

Figure 4.2 Biosynthesis of silver nanoparticles of *P.emblica* aqueous extract

4.5 Characterization of silver nano-drugs of PEAgNPs

4.5.1 UV-Vis spectral analysis of PEAgNPs

The current study deals with the bio-fabrication of silver nanoparticles via using the aqueous extract of PE leaf and chemical constituents present in the leaf extracts and acts as reducing agent and capping agents to reduce the silver ion.

The strong intense peak was revealed at 418 nm, which indicated the presence of AgNPs as shown in figure 4.3. It might be happened due to excitation of surface plasmon absorption band (SPR) and suppressed effect of $AgNO_3$. Similar results already reported by different researchers that the phytochemical present in plants leaf extract acts as a reducing agent for the stabilization of silver nanoparticles.

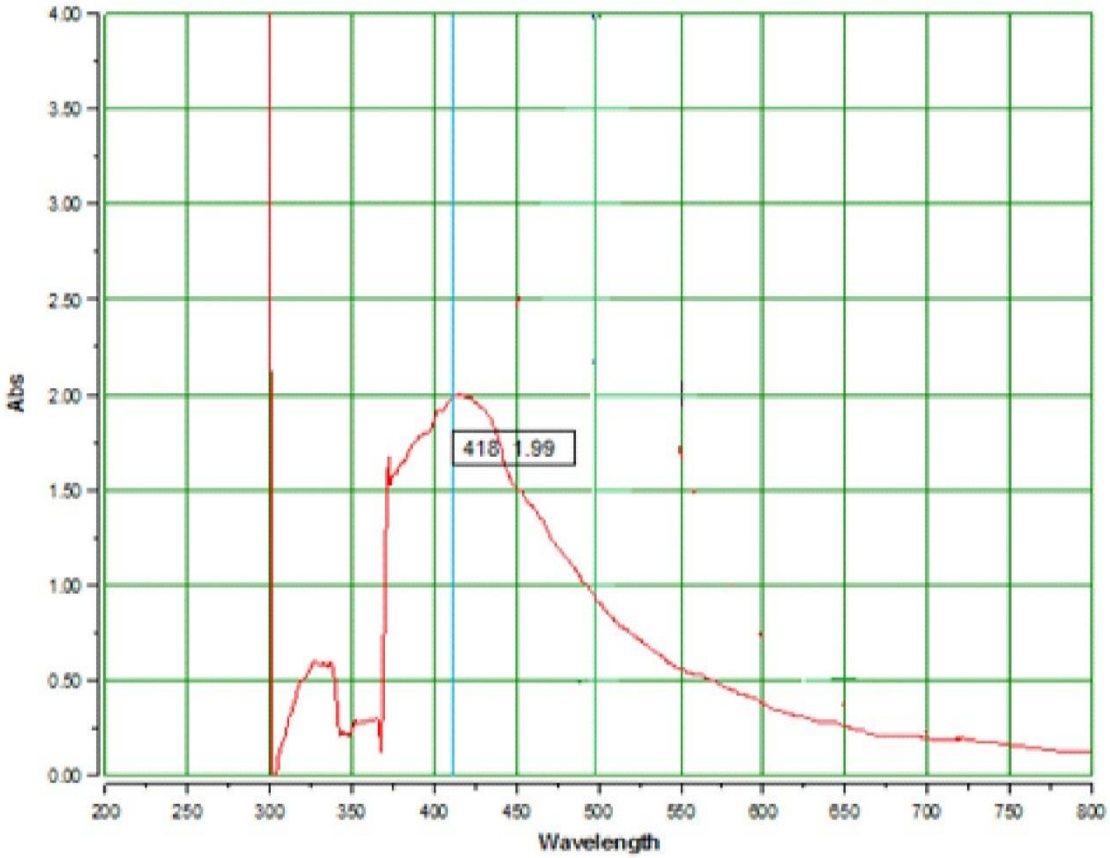


Figure 4.3 UV-Vis spectrum of PEAgNPs

4.5.2 FTIR spectral analysis of PEAgNPs

FTIR is a very important tool used to characterize the different functional groups present in the leaf extract and synthesized nanoparticles. It identifies the biomolecule which stabilized and capped the silver nanoparticles (figure 4.4 a & b). PE leaf extract and silver nanoparticles showed a broad peak at 3541cm^{-1} and 3727 cm^{-1} of which is corresponded to –OH stretching in alcohols and phenolic compounds. The band observed at 2881 cm^{-1} (Silver nanodrugs) and 2889 cm^{-1} was characteristics of C–H stretching vibrations of methoxy, methylene and methyl groups. A peak was observed 1600 cm^{-1} (C=O stretch of Aromatic), 1511 cm^{-1} (C=C stretch of aromatic), 1394 cm^{-1} (N-O stretch of Nitro), 1196 cm^{-1} (C-N stretch of Amine group), 1087 cm^{-1} (C-O stretch group of Alcohol), 923 cm^{-1} & 709 cm^{-1} (=C-H bending of Alkene), 578 cm^{-1} (C-Br stretch of Alkyl halide) were observed in biofabricated silver nanoparticles. The IR spectra of PE leaf extract shows peak at 1670 cm^{-1} (C=O stretch of anhydride), 1492 cm^{-1} (Aromatic C=C bending), 1371 cm^{-1} (N-O stretch of Nitro), 1061 cm^{-1} (C-O stretch group of Alcohol), 923 cm^{-1} & 751 cm^{-1} (=C-H bending of Alkene), 454 cm^{-1} (C-Br stretch of Alkyl halide). It was concluded that green synthesized silver nanoparticles were surrounded by proteins and attached with a different functional group which was responsible for the reduction of silver. It prevents agglomeration of particles and provides stability in the medium.

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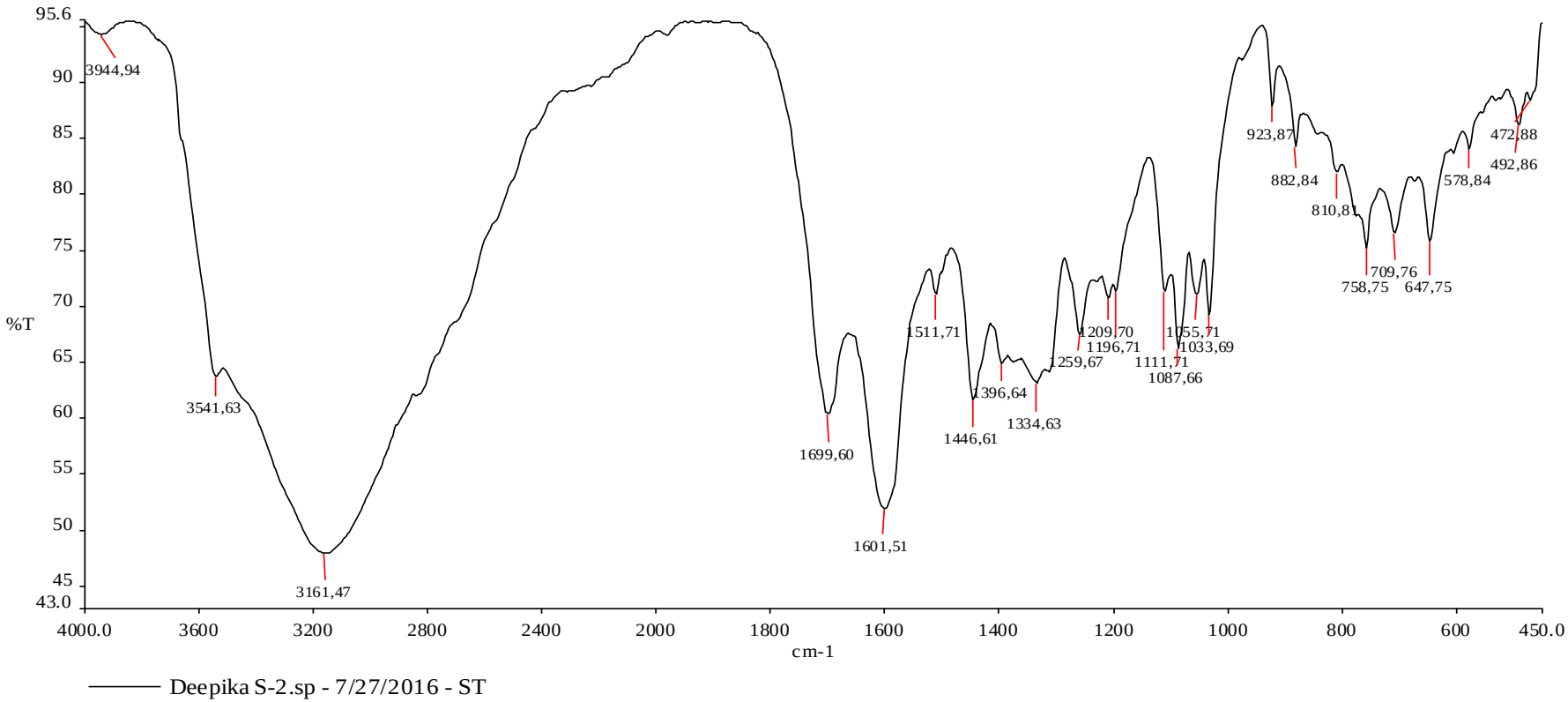


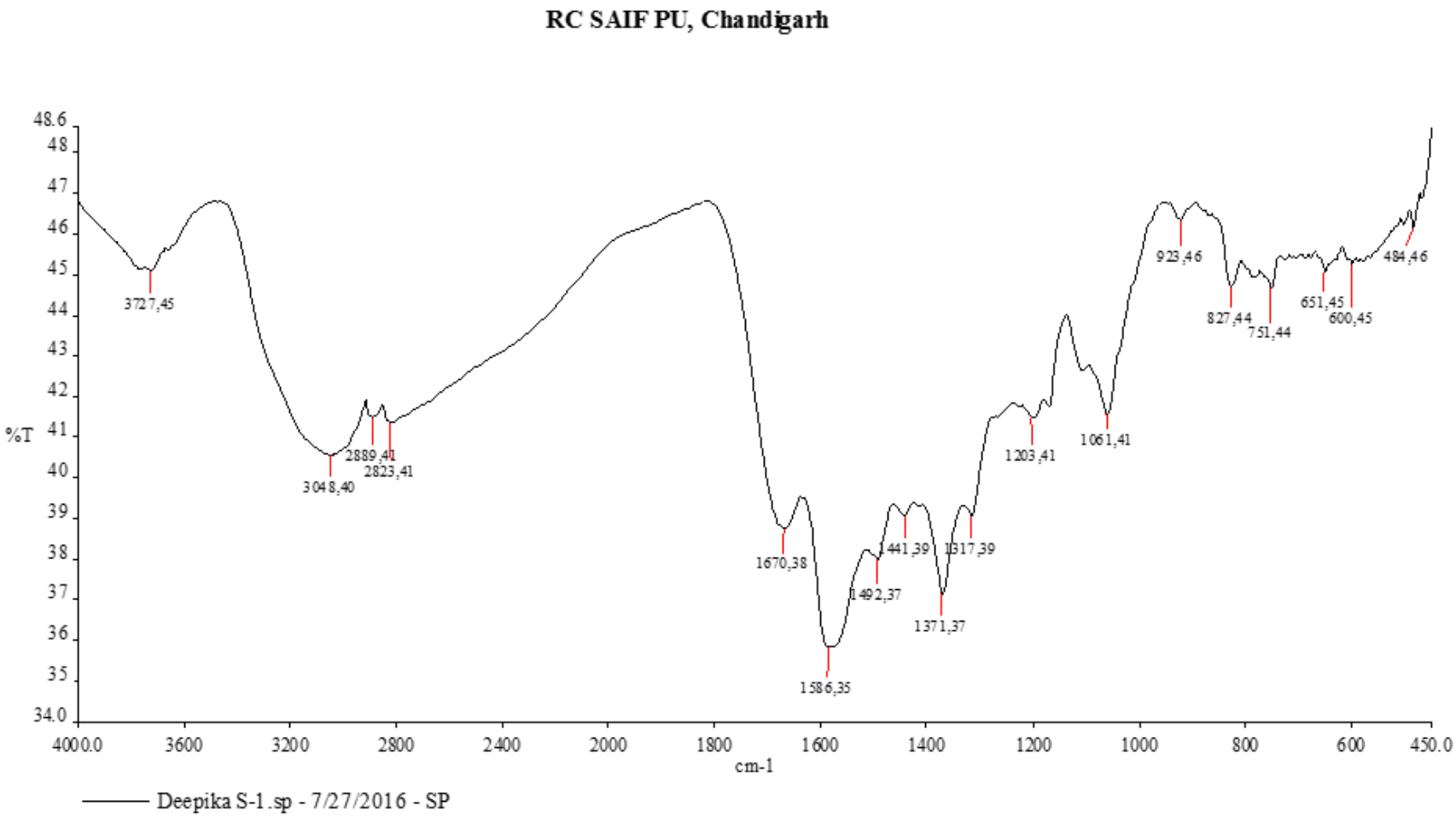


Figure 4.4. FTIR spectrum: a) PEE b) Biogenic silver nanoparticles of *P.emblica*

4.5.3 XRD analysis of PEAgNPs

Crystalline structure of synthesized AgNPs using aqueous extract of plant was obtained from X- ray diffraction patterns. Table 4.5 and figure 4.5 shows the diffraction peak characteristics of synthesized AgNPs. The XRD shows characteristics peaks at 38.13, 44.33, 64.51, 77.47 and 81.60 could be attributed to lattices planes 111, 200, 220, 311, and 222 respectively. The XRD spectrum shows the interplanar spacing values (2.771, 2.043, 1.6708. 1.444, 1.2736, 1.232 Å) which was in agreement with the standard silver XRD values and indicates that the structure is face- centered cubic (FCC) [24].

Table 4.5 XRD analysis of PEAgNPs

Pos. [°2θ]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2θ]
32.3060	0.3178	2.77112	100.00	440.13
38.1378	0.4015	2.35973	71.63	398.25
44.3397	0.4684	2.04301	15.61	101.23
46.3456	0.2342	1.95915	47.96	155.52
54.9540	0.3346	1.67089	11.96	55.40
57.4723	0.3011	1.60352	11.37	47.42
64.5182	0.2676	1.44438	14.71	54.53
67.6249	0.4684	1.38539	4.06	26.31
74.4989	0.5353	1.27367	1.97	14.63
76.7927	0.3346	1.24125	10.37	48.04
77.4750	0.3346	1.23201	14.15	65.55
81.6049	0.6691	1.17979	3.38	31.33
85.7922	0.6691	1.13261	3.92	36.28

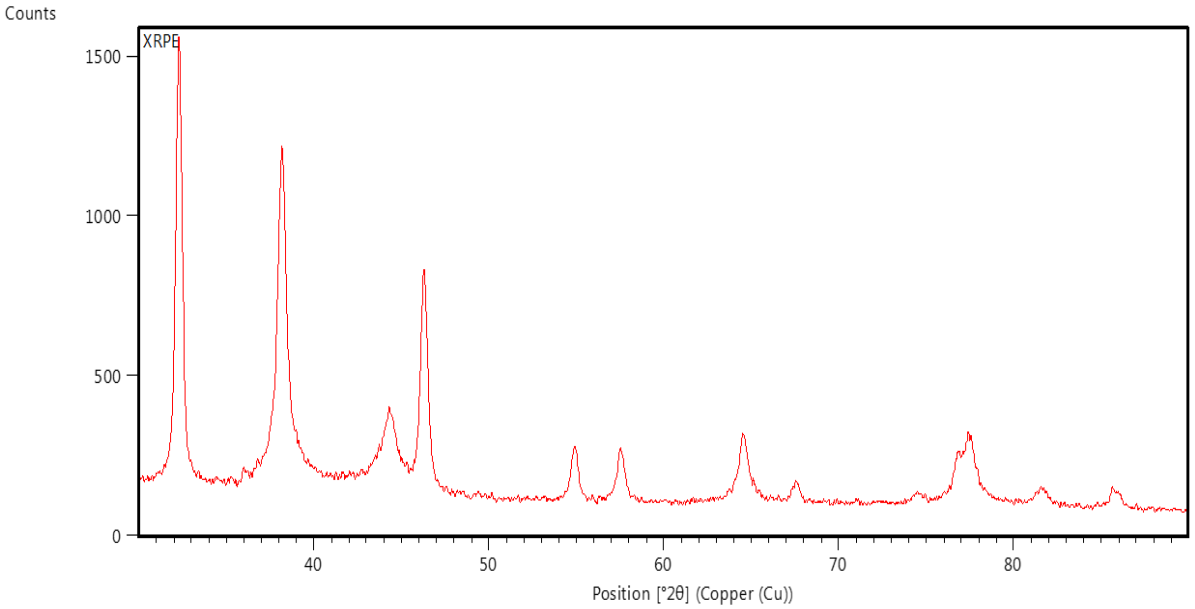
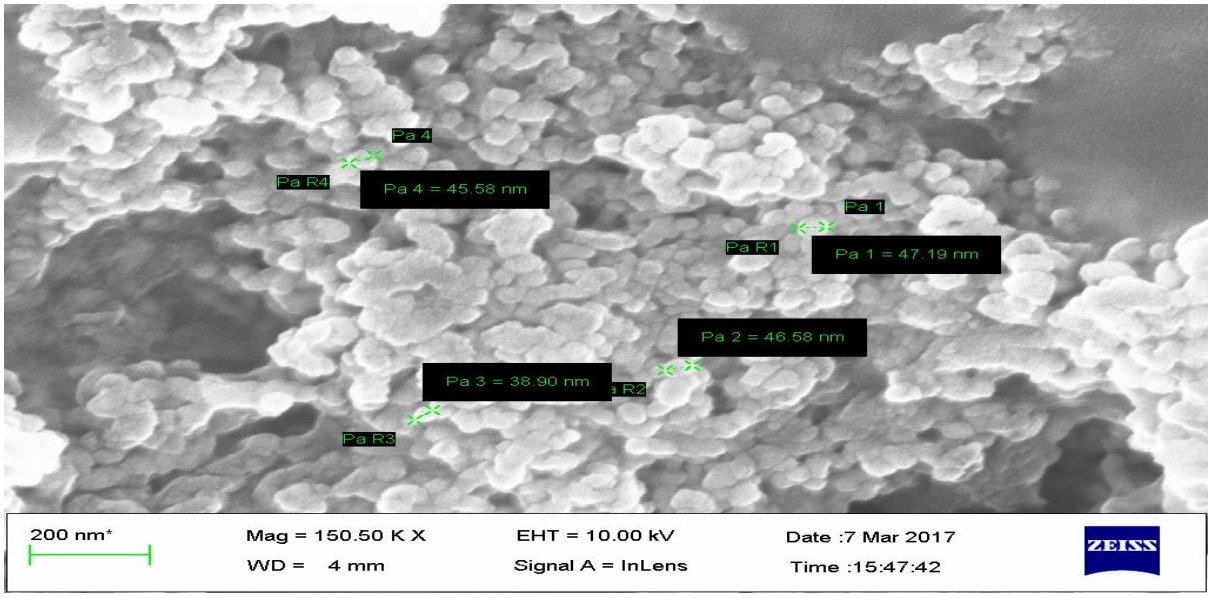


Figure 4.5 XRD spectrum of synthesized PEAgNPs

4.5.4 Field Emission Scanning Electron Microscope (FESEM) of PEAgNPs

FESEM is used to determine the surface morphology of silver nanoparticles and images are shown in figure 4.6. The surface morphology confirmed that the shape of silver nanoparticle looks like agglomerated spheres with rough surface and with a diameter of 38-50 nm. The result suggested that biomolecule present in leaf extract act as capping and reducing agents in the formation of silver nanoparticles.



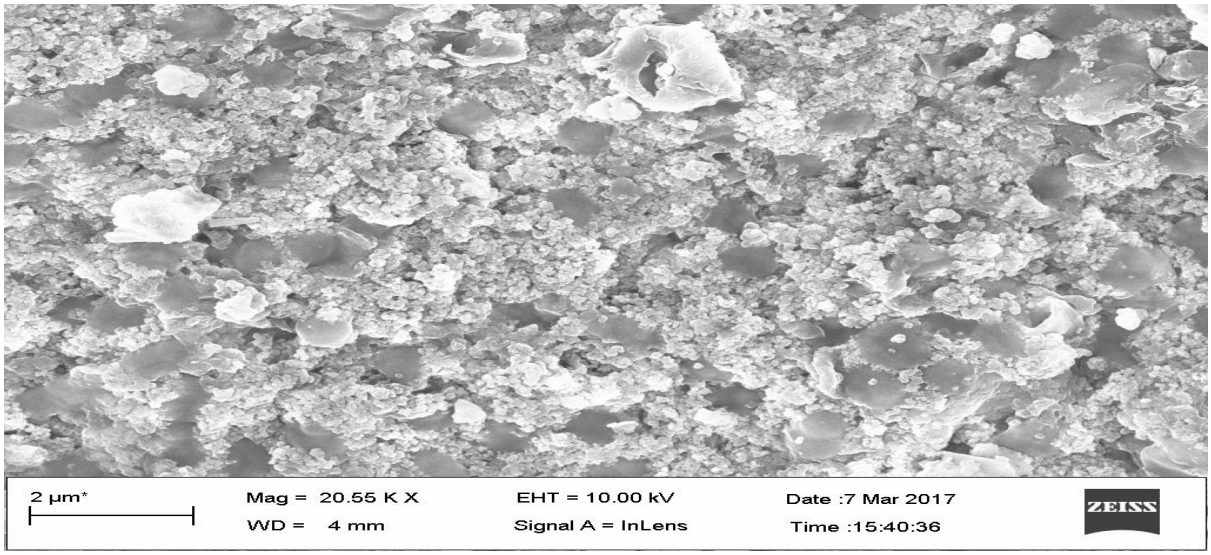


Figure 4.6 FESEM images of Synthesized PEAgNPs

4.5.5 Energy dispersive X-ray (EDX) analysis of PEAgNPs

Energy dispersive X-ray (EDX) spectroscopy suggests the existence of silver ion within the region. It reveals a strong peak for AG at 3 keV due to surface plasma resonance and confirm the production of metallic nanoparticles. It also shows peak of other elements like calcium, oxygen, carbon, which is due to biomolecules associated on the surface of silver nanoparticles. Figure 4.7 shows the quantitative analysis of biofabricated silver nanoparticles

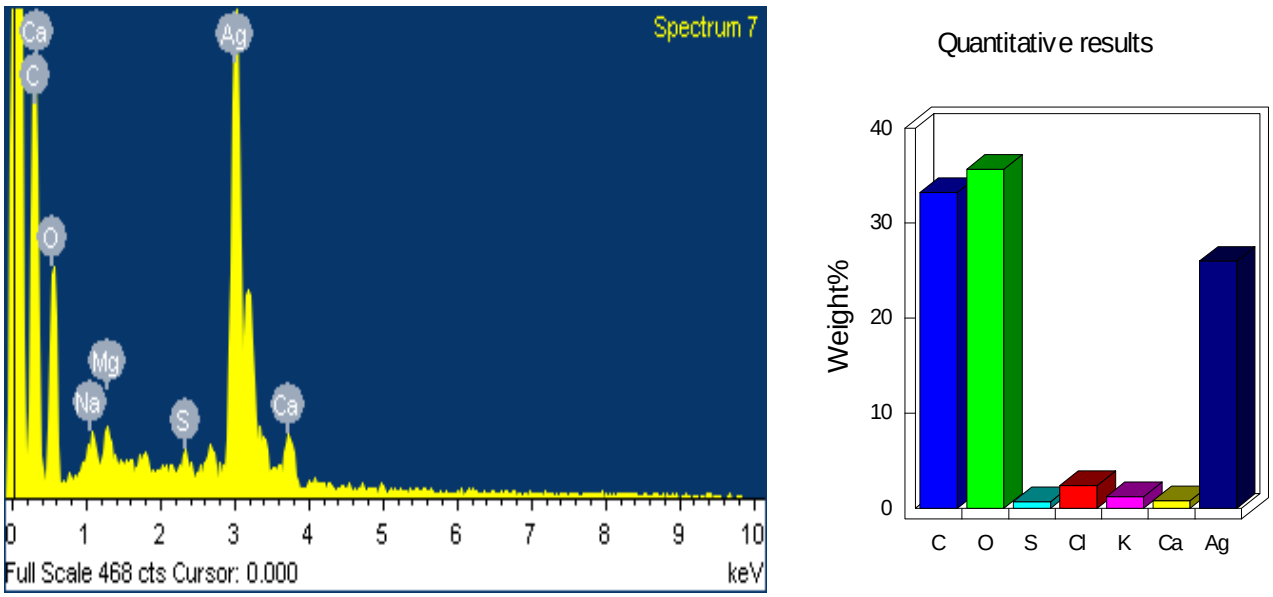


Figure 4.7 EDX spectrum of synthesized PEAgNPs

4.5.6 TEM of PEAgNPs:

TEM is used to elucidate the morphology, size and shape of silver nanoparticles. Synthesized silver nanoparticles predominates with quasi round, spherical triangle and decahedral structure and uniformly distributed. The particles sizes ranging from 15 to 30 nm as shown in figure 4.8. The different chemical constituent present in the leaf extract are responsible for the production of silver nanoparticles [27].

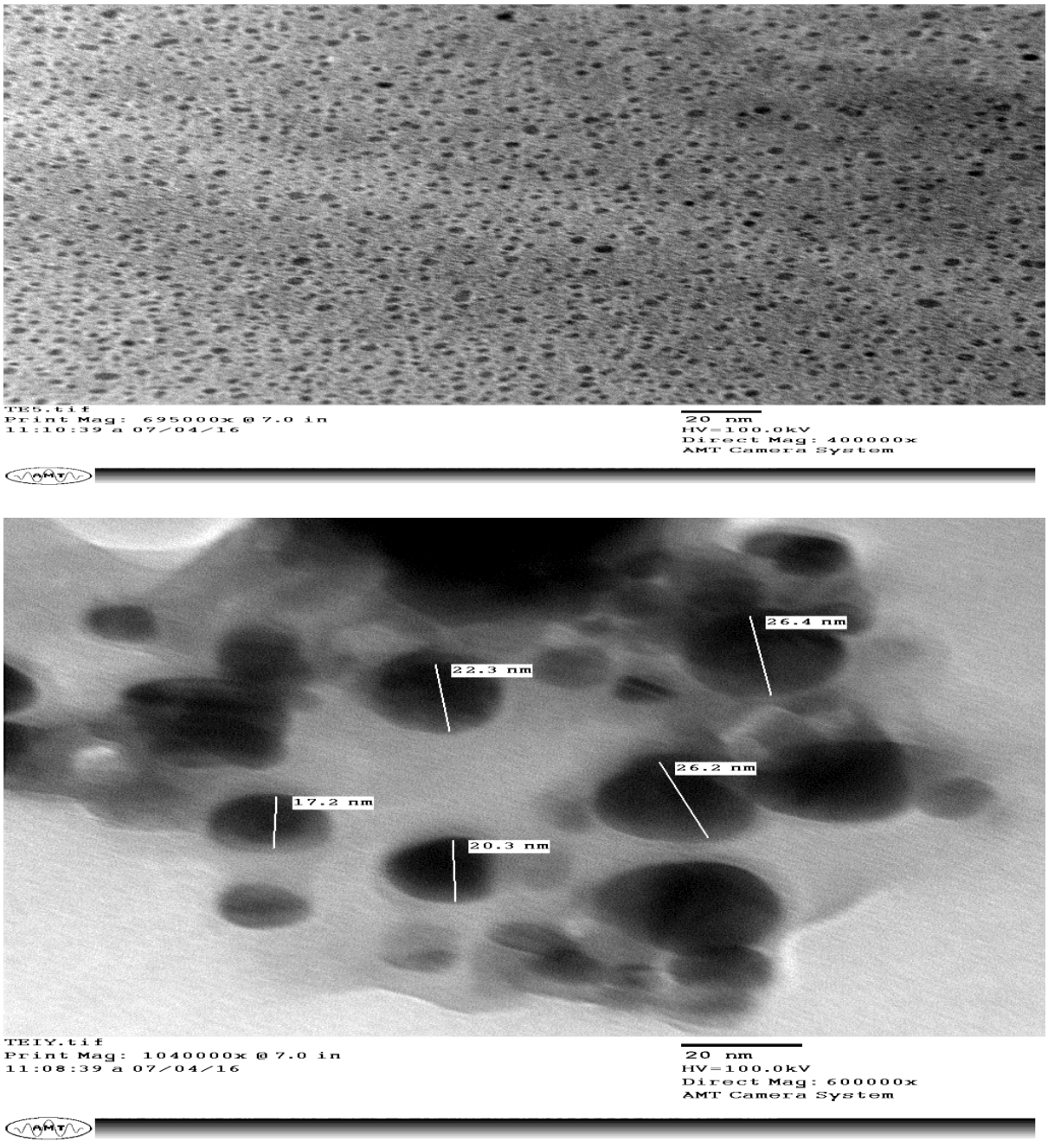


Figure 4.8 TEM images of synthesized PEAgN**Ps**

4.6 *In vitro* cytotoxic activity of PEAgNPs against HuH-7 Cell line

In vitro cytotoxic activity of PEAgNPs were performed on hepatic cancer cell line (HUH-7) by MTT assay. Table 4.6 and figure 4.9 showed the cytotoxic activity of biofabricated PEAgNPs at different dilutions. The IC_{50} cell inhibition of PEAgNPs against HuH-7 cell lines was found at 19.27 µg/ml. The result suggests that PEAgNPs treatment showed necrobiosis of cell lines at dose dependent manner.

Table 4.6 *in vitro* cytotoxic activity of PEAgNPs on hepatic cancer (HuH-7 cell line)

Log concentration	**% growth inhibition**
1.00	0.000
1.30	25.000
1.39	50.000
1.47	69.000
1.69	80.000

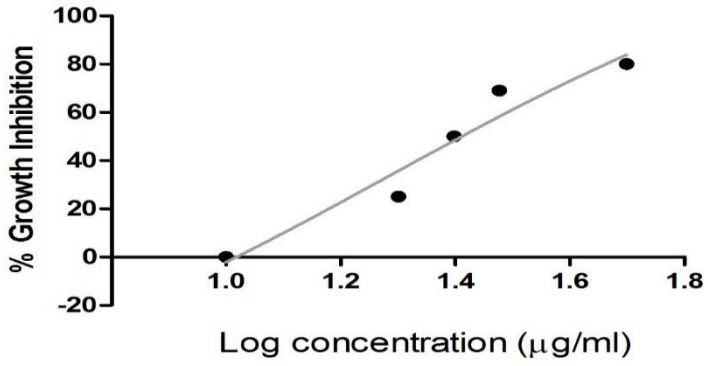


Figure 4.9 *in vitro* cytotoxic activity of PEAgNPs on hepatic cancer cell line

4.7 *In vivo* hepatic cancer Studies of PEAgNPs on rats

DEN induced hepatic cancer rats was used to study the anticancer activity of *PE* leaf extract synthesized silver nanoparticles. DEN itself a potent hepatocarcinogen to initiate proliferation of cell and cause cancer.

4.7.1 Effect of green Synthesized PEAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats

NC group rats demonstrated the underlying body weight was 164.54±1.10 to 312.75±1.27 with 1.51± 0.84 gm gain per day and DEN induced groups rats treated with PEAgNPs (10 mg/kg) showed the increased body weight 165.24±0.73 to 303.86±1.21 with 1.41± 0.63 gm growth gain rate as depicted in table 4.7. Reduction in the body weight of DEN control animals was observed with respect to NC and PEAgNPs control treated rats. The relative liver weight and liver weight were also assessed in all group rats. However, the DEN induced groups shows the expanded liver weight (17.41±0.06) and relative liver weight (6.46 ± 1.78), which were elevated than the normal control rat. PEAgNPs-treated group demonstrated the down-regulation in liver weight (14.01±0.20, 10.44±0.03 and 12.54±0.17) and relative liver weight (4.68 ±1.34, 3.45 ±0.67and 4.12 ±0.48) when contrasted with DEN Control group.

4.7.2 Effect of PEAgNPs on the development of liver nodules in control and DEN- induced HCC rats

Table 4.8 delineates the macroscopically character of liver in all group animals. NC affirmed the unaltered hepatic morphological character. DEN induced group rats affirmed the development of HCC via expansion of hepatic knobs; generation of patches on the liver surface which were appeared as rough surface and affirms the development of disease. DEN induced groups rats treated with PEAgNPs (10 mg/kg) showed the less hepatic knobs as contrasted with DEN induced hepatocarcinogenesis rats. None or less hepatic knobs were found in DEN-treated group with the PEAgNPs (20 mg/kg); these groups demonstrated white patches only on the external surface of the liver and show very smooth surface when contrasted with the DEN control group rats [28].

Table 4.7 Effect of green Synthesized PEAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats

Treatment	**Initial body weight**	**Final Body weight (g)**	**Liver weight (g)**	**Relative liver weight**
Control	164.54±1.10	312.75±1.27	9.53± 0.14	3.04 ± 0.13
DEN	166.74±1.73	269.37±1.87$^{###}$	17.41±0.06	6.46 ± 0.21$^{###}$
DEN+PEE	165.85±1.59	291.81±1.94***	15.64±0.11	5.35 ±0.16 ***
DEN+ PEAgNPs 10	165.24±0.73	303.86±1.21***	12.54±0.17	4.12 ±0.15 ***
DEN+ PEAgNPs20	166.60±0.89	302.56±1.21***	10.44±0.03	3.45 ±0.07 ***
DEN+ Sily	162.93±0.085	299.98±1.69***	10.03±0.01	3.34 ±0.11 ***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

Table 4.8 Effect of PEAgNPs on the development of liver nodules in control and DEN- induced HCC rats

Treatment	**Total no. of nodules**	**Tumour incidence (%)**	**Average number of nodules**
Control	0	0	0
DEN	89	100	$42.33\pm2.10^{\#\#}$
DEN+PEE	70	81.11	25.04 ± 1.26^{ns}
DEN+ PEAgNPs 10	35	35.56	$8.15\pm1.05^{*}$
DEN+ PEAgNPs20	22	32.22	$7.01\pm1.19^{**}$
DEN+ Sily	18	27.78	$5.32\pm1.42^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

4.7.3 Effect of PEAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

Table 4.9 and figure 4.10 revealed that the DEN group rats showed the up-regulation of AFP content and which was significantly (P<0.001) down-regulated by the PEAgNPs at different dose level.

Comparable result was observed in the AST, ALT and ALP level, which was significantly (P<0.001) increased in the DEN group and similar reduced trend was found in the PEAgNPs treated group.

Table 4.9. Effect of PEAgNPs on the activities of marker enzymes in the serum of control and DEN induced HCC in rats.

Treatment	ALT	AST	ALP	AFP
Normal Control	49.67±3.92	56.67±4.40	43.21±2.30	133.81±3.47
DEN	244.33±3.48$^{###}$	226.00±5.29$^{###}$	221.09±7.21$^{###}$	307.33±8.96$^{###}$
DEN+PEE	170.66±3.84***	182.33±4.05***	191.33±4.09**	216.33±3.48***
DEN+PEAgNPs10	148.00±3.21***	174.66±2.60***	161.00±4.01***	170.33±3.18***
DEN+ PEAgNPs20	134.66±2.60***	120.33±3.18***	153.33±4.25***	167.33±3.38***
DEN+Sily	55.33±2.02***	68.66±2.72***	69.00±5.57***	160.00±2.88***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

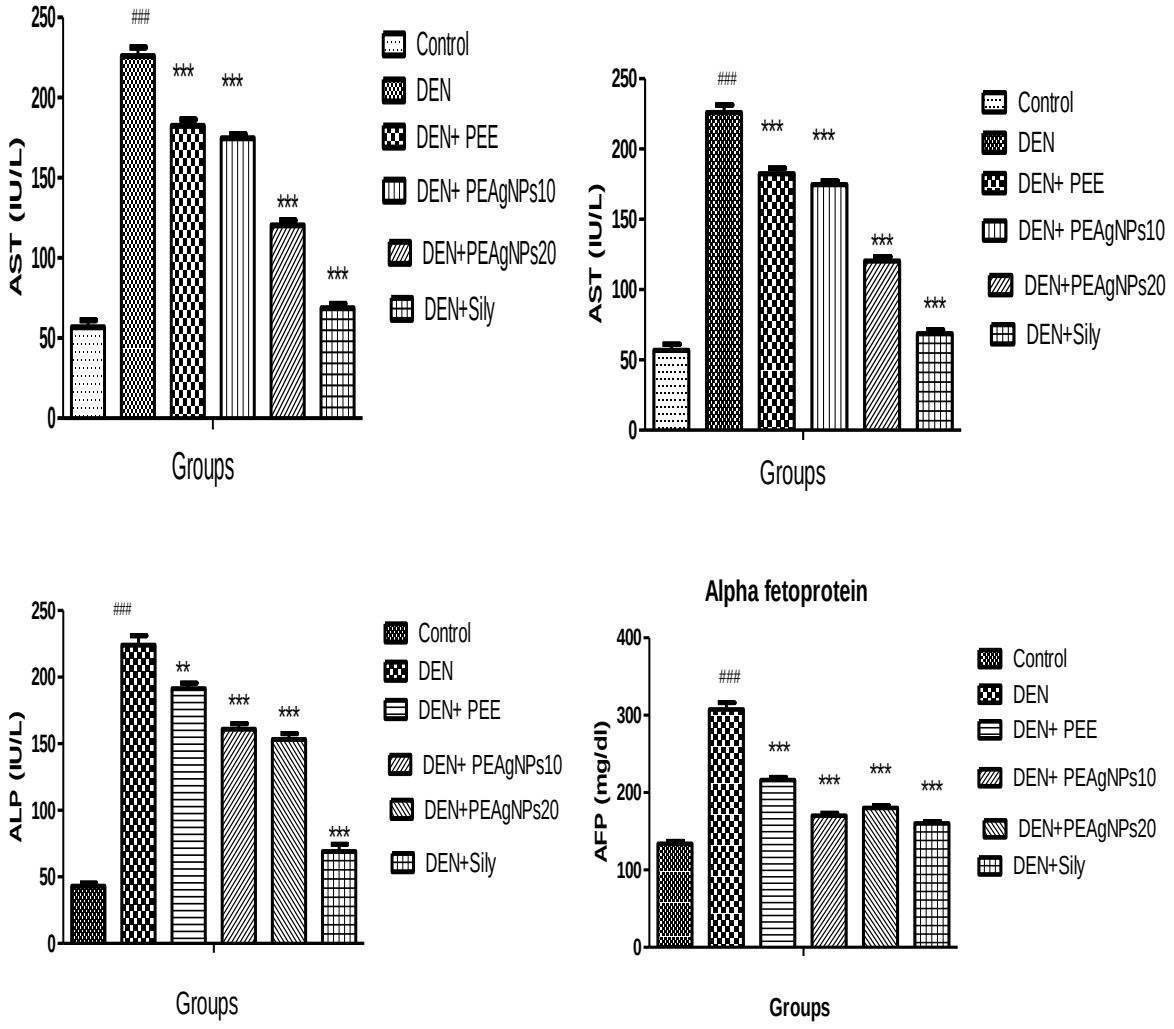


Figure 4.10 Effect of PEAgNPs on serum marker enzyme of hepatic parameter

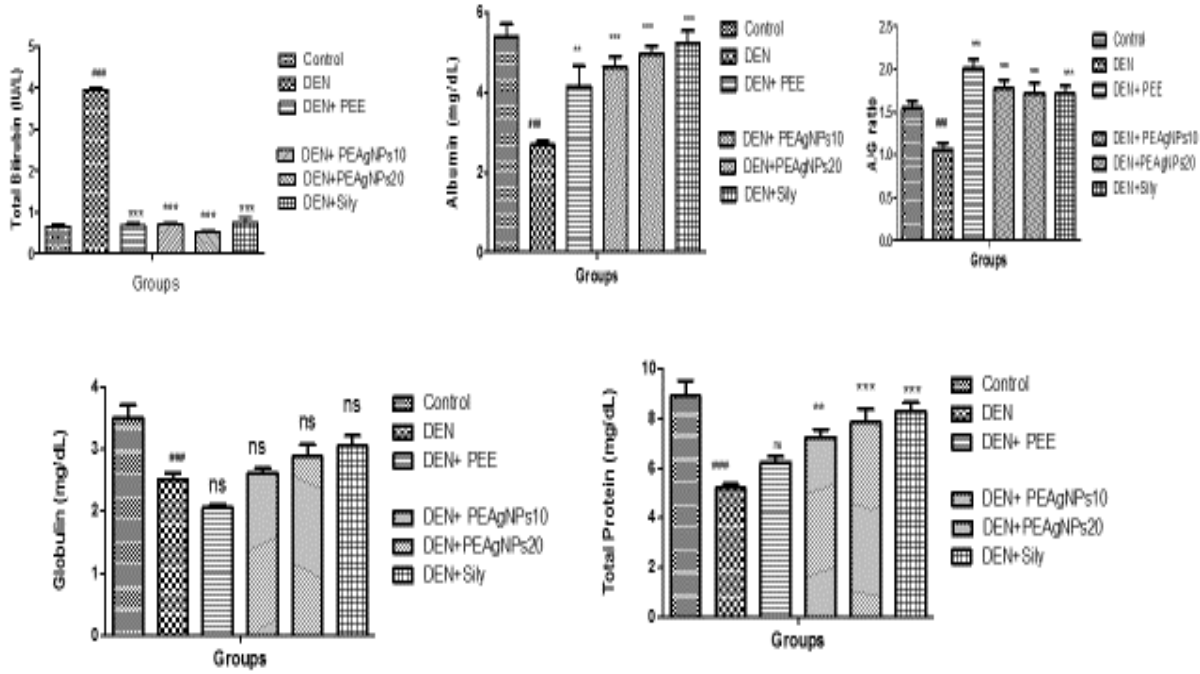
4.7.4 Effect of PEAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

The non-hepatic parameters viz. total bilirubin, globulins, albumin, total proteins and A/G ratio is shown in table 4.10 and figure 4.11. It was observed that that the level of albumin, globulin, and total protein were significantly ($P<0.001$) decreased and level of total bilirubin was increased in DEN group when compared to normal control. There is a concomitant decrease in the level of A/G ratio of DEN induced liver cancer groups. It causes defective protein synthesis in liver which in turn damage hepatocellular cells. However, the PEAgNPs significantly restored all the level of non-hepatic parameters.

Table 4.10 Effect of PEAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN induced HCC rats

Treatment	TB	TP	Albumins	Globulins	A/G ratio
Normal Control	0.65±0.08	8.90±0.62	5.40±0.31	3.50±0.21	1.54±0.09
DEN	3.94±0.06$^{###}$	5.20±0.15$^{###}$	2.68±0.10$^{###}$	2.52±0.10$^{###}$	1.06±0.16$^{##}$
DEN+PEE	0.67±0.05***	6.21±0.26^{ns}	4.15±0.51**	2.06±0.05*	2.01±0.09***
DEN+ PEAgNPs 10	0.7±0.03***	7.24±0.32**	4.63±0.26***	2.61±0.09^{ns}	1.77±0.03***
DEN+ PEAgNPs20	0.51±0.04***	7.84±0.53***	4.96±0.21***	2.88±0.05^{ns}	1.72±0.02***
DEN+Sily	0.75±0.11***	8.29±0.34***	5.24±0.31***	3.05±0.08**	1.71±0.07***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant



Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

Figure 4.11 Effect of PEAgNPs on serum marker enzyme of non-hepatic parameter

4.7.5 Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

Table 4.11 and figure 4.12 demonstrated the effect of pro-inflammatory cytokines and inflammatory mediators on the different group rats. Upregulation of inflammatory cytokines were observed in the DEN induced group rats and significantly (P<0.001) reduced by the PEAgNPs at both the dose level, in turn result suggested the anti-inflammatory effect of fabricated silver nanoparticles. DEN induced group afforded the raised level of NF-κB as compared with normal control rats. It was evident from the table that higher doses of PEAgNPs were found to be effective against HCC.

Table 4.11. Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

Treatment	**TNF-α**	**NF-κB**	**IL-6**	**IL-1β**
Normal Control	48.45±2.59	154.96±7.00	96.81±6.07	22.82±2.89
DEN	166.97±5.85$^{###}$	278.29±11.56$^{###}$	298.15±5.49$^{###}$	86.92±3.58$^{###}$
DEN+PEE	144.08±5.77*	235.89±7.80*	246.78±4.91***	61.92±3.46***
DEN+PEAgNPs10	98.02±6.35***	180.12±11.54***	187.34±3.85***	56.25±0.37***
DEN PEAgNPs20	90.15±5.77***	173.29±7.26***	178.11±3.16***	48.26±1.89***
DEN+Sily	63.78±2.73***	161.84±6.40***	101.53±3.57***	37.96±0.60***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

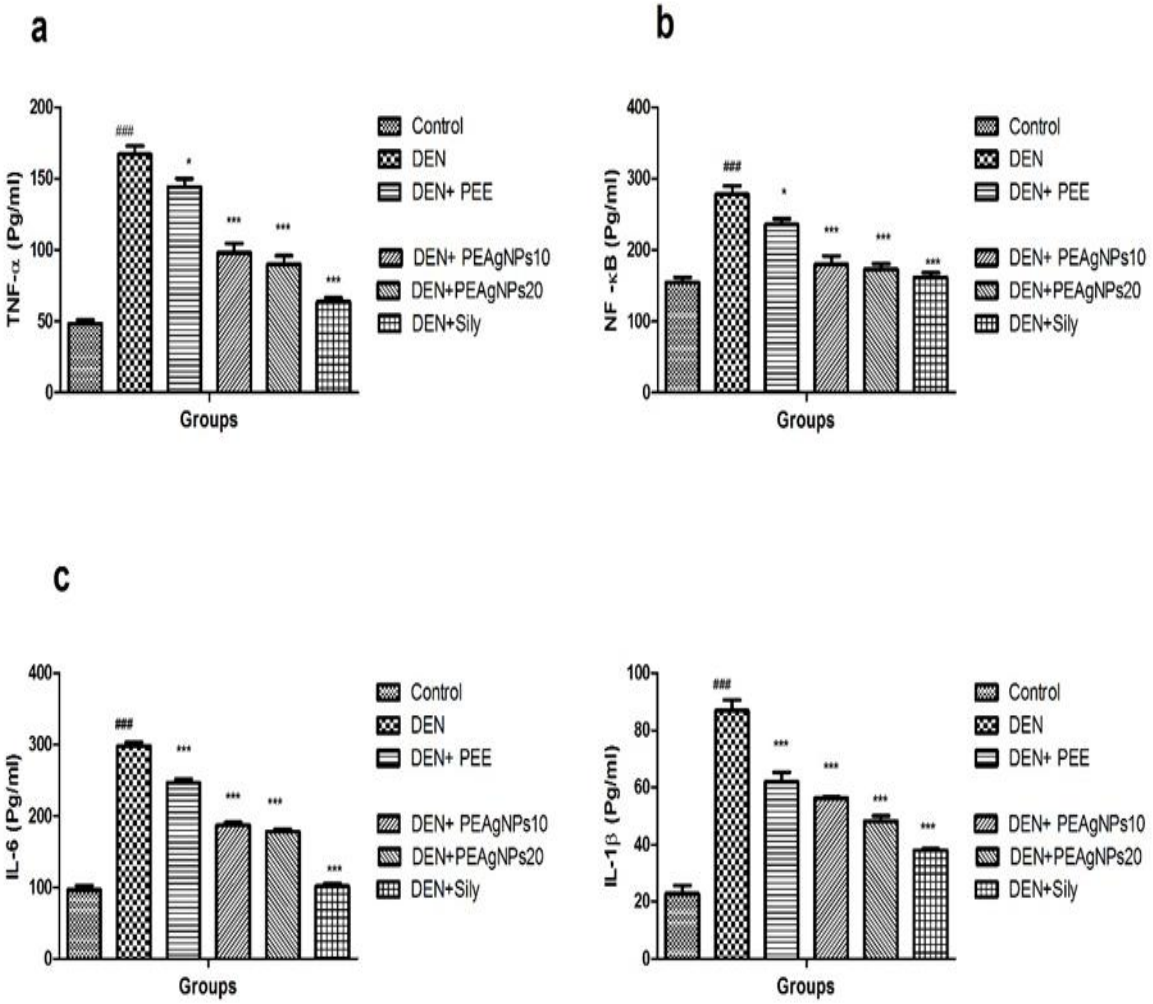


Figure 4.12 Effect of PEAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

4.7.6 Effect of PEAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats

The level of MDA (an indicator of LPO) were significantly increased in the DEN induced liver cancer group rats, suggested the peroxidise chain reaction and induction of tissue damage. DEN induced group when treated with PEAgNPs significantly ($P<0.001$) reduced the LPO level in term of inhibition of MDA content as shown in table 4.12 and figure 4.13. Similar result of reduction in the level of MDA was also observed in DEN + Sily group animals.

Table 4.12 Effect of PEAgNPs on lipid peroxidation activity in liver of control and DEN-induced HCC rats

Treatment	MDA
Normal Control	17.36±1.76
DEN	40.00±2.88$^{###}$
DEN+PEE	24.96±1.73**
DEN+ PEAgNPs 10	21.15±2.31***
DEN+ PEAgNPs20	20.22±1.74***
DEN+Sily	19.58±2.02***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

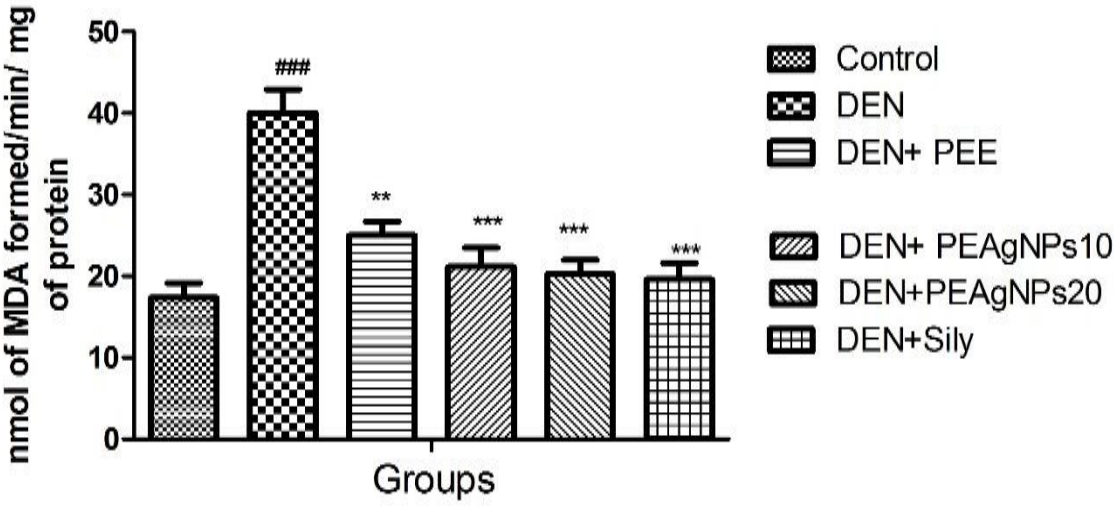


Figure 4.13 Effect of PEAgNPs on lipid peroxidation activity in liver of control and DEN-induced HCC rats

4.7.7 Effect of PEAgNPs on the levels of antioxidant enzymes

DEN group showed reduction in the content of enzymatic and non-enzymatic antioxidant viz. SOD, Catalase, GPx, GSH, G6PD and vitamin C as shown in table 4.13 and figure 4.14. The PEAgNPs significantly ($P<0.001$) boost up the content of antioxidant enzymes in DEN induced cancerous rats. Similar response like treated groups was seen in all antioxidant parameters after the introduction of standard drugs (silymarin) in DEN induced hepatic cancer groups animals and suggested about the scavenging of the production of free radicals.

4.7.8 Effect of PEAgNPs on the activities of membrane bound enzymes

Table 4.14 and figure 4.15 depicts the level of membrane-bound enzyme Na^+/K^+ATPase and Ca^{2+}ATPase in liver of all group rats. DEN group rats showed the reduced activity of membrane bound enzyme in term of Ca^{2+}ATPase and Na^+/K^+ATPase, which was significantly ($P<0.001$) increased by the both dose level treatment of PEAgNPs. Standard drug (silymarin) also significantly raised the level of membrane bound enzymes in DEN induced hepatocarcinogenesis rats.

4.7.9 Histopathological observation of liver in the control and experimental rats

The histopathological study of liver tissue was observed under a light microscope and images of liver section of control rat and experimental rats were depicted in figure 4.16. Normal control group show the well-defined architecture of liver cells without any pathological changes and having a small nuclei with granulated cytoplasm. DEN induced liver cancer group showed cell necrosis with mild dysplastic nuclei and high accumulation of fats leads to hepatocellular damage. Plant extract groups indicates loss of architecture with mild degenerative changes. Treatment with silver nanoparticles dose dependently showed a reduction of necrosis and decrease in hepatic cell damage with well-defined nuclei when compared with group I. Standard drug showed recovery of hepatic architecture with increased inter cellular space when compared to normal control group.

Table 4.13. Effect of PEAgNPs on enzymatic and non-enzymatic antioxidant profile in liver of control and DEN- induced HCC rats

Treatment	**Catalase**	**SOD**	**GPx**	**GSH**	**G6PD**	**Vitamin C**
Normal	90.11±2.88	8.14±0.46	10.72±0.14	45.55±2.93	8.36±0.08	3.68±0.10
DEN	47.45±3.17$^{###}$	2.00±0.58$^{###}$	3.69±0.19$^{###}$	18.81±2.89$^{###}$	2.35±0.16$^{###}$	1.47±0.15$^{###}$
DEN+PEE	62.63±2.60*	5.39±0.20***	6.55±0.18***	35.36±2.02**	4.57±0.14***	2.62±0.15***
DEN+ PEAgNPs 10	67.48±2.36**	5.48±0.15***	8.41±0.18***	34.34±2.33**	5.49±0.14***	2.71±0.08***
DEN+ PEAgNPs20	64.89±4.13**	6.01±0.11***	8.55±0.05***	36.52±2.02**	4.41±0.09***	2.94±0.02***
DEN+Sily	70.96±3.59***	6.49±0.15***	9.28±0.34***	40.96±2.50***	7.78±0.10***	3.34±0.22***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

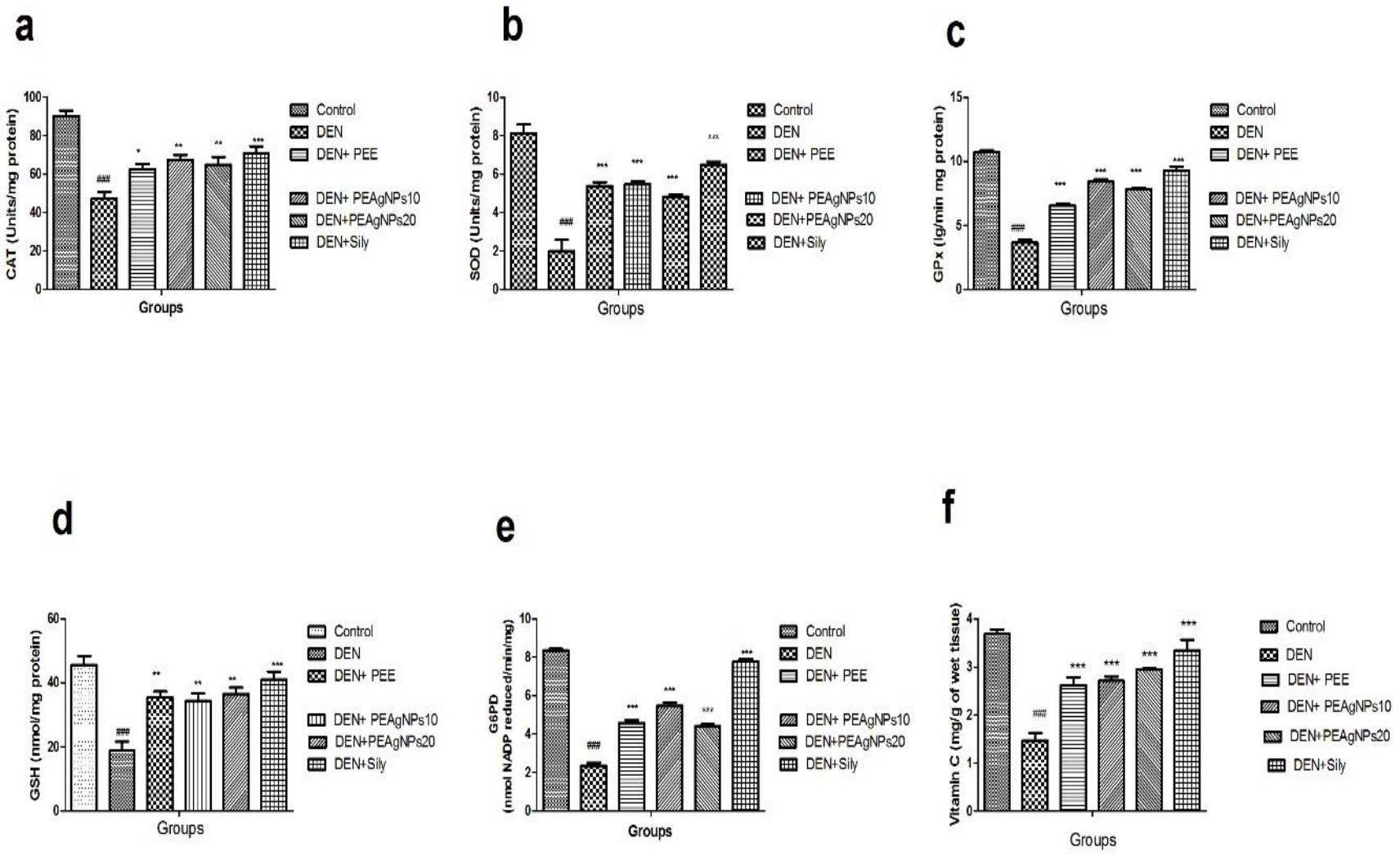


Figure 4.14 Effect of PEAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats

Table 4.14. Effect of PEAgNPs on Membrane bound activities in liver of control and DEN- induced HCC rats

Treatment	Na^+/K^+ATPase	Ca^{2+}ATPase
Normal Control	4.45±0.33	7.41±0.25
DEN	$2.14\pm0.32^{###}$	$3.23\pm0.21^{###}$
DEN+PEE	3.16 ± 0.35^{ns}	$4.96\pm0.49^{*}$
DEN+ PEAgNPs 10	$3.80\pm0.33^{**}$	$5.44\pm0.44^{**}$
DEN+ PEAgNPs20	$4.18\pm0.20^{**}$	$6.24\pm0.12^{***}$
DEN+Sily	$4.09\pm0.05^{**}$	$6.54\pm0.25^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

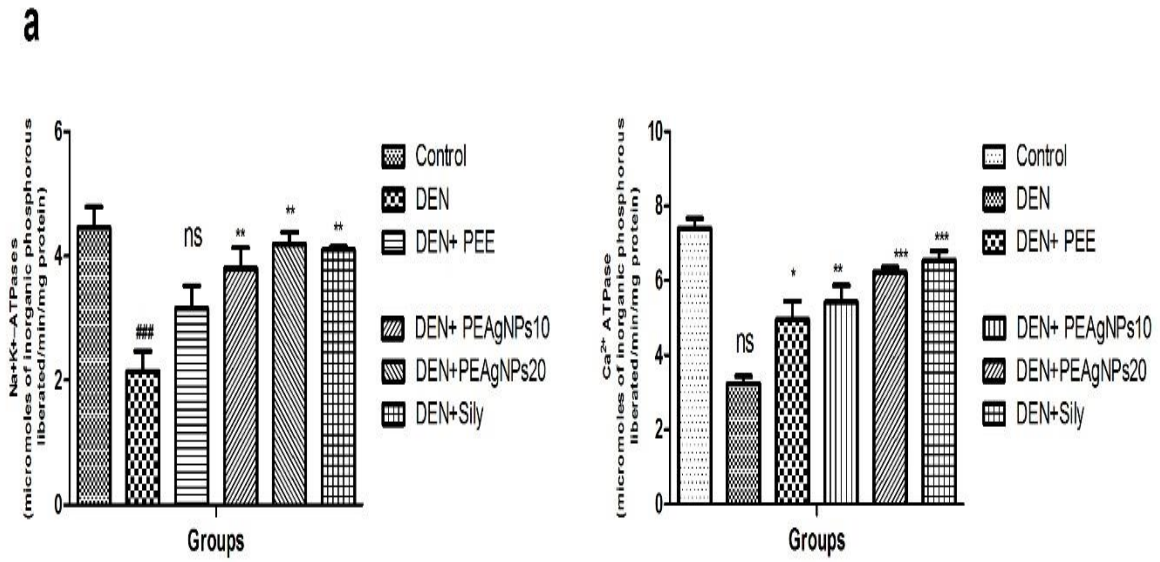


Figure 4.15 Effect of PEAgNPs on Membrane bound activities in liver of control and DEN-induced HCC rats

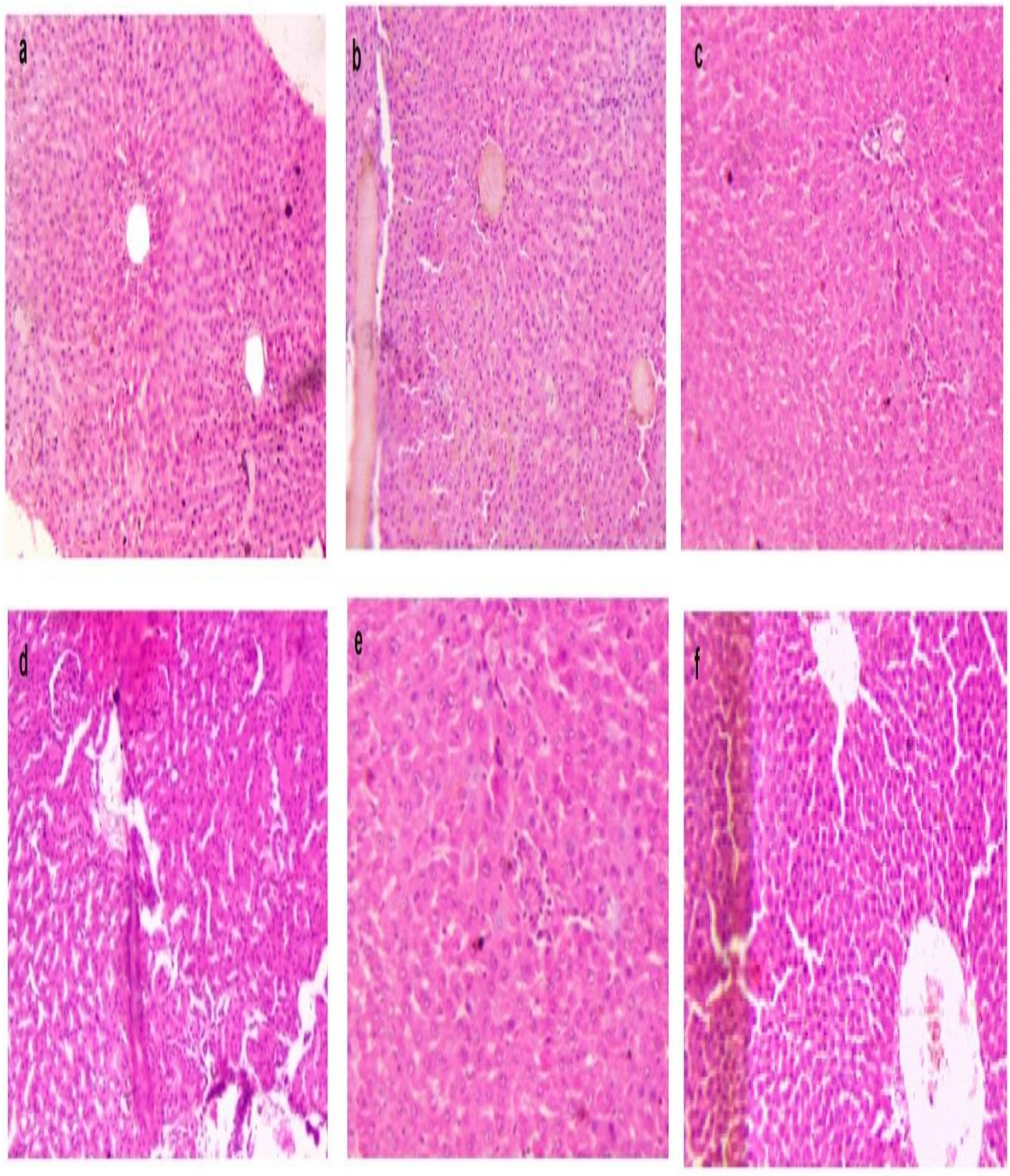


Figure 4.16 Photomicrograph of eosin-haematoxylin stained histological liver section area for (a) Normal control group, (b) DEN induced liver cancer group (c) DEN+ PEE (d) DEN +PEAgNPs (10mg/kg bw) (e) DEN +PEAgNPs (20mg/kg bw), and (f) DEN+ Silymarin

4.8 Renal cancer

4.8.1 Effect of PEAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats

Table 4.15 depict about the number of rats with RCC, total number of tumour, number of animals with bilateral and unilateral tumour, percentage incidence of tumour. Normal control group rat showed no detectable knob on the kidney structure till end of the study. Through visible observation, DEN induced group rats affirmed the arrangement of knob on kidney tissues. DEN induced group animals when treated with PEAgNPs, affirmed the chemo-defensive impact of PEAgNPs and established by means of decrease in tumour frequency in a dose dependent way.

Table 4.15 Effect of PEAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats

Groups	**No. of Rats with RCC**	**No. of rats with Unilateral tumors**	**No. of rats with bilateral tumors**	**Total no. of tumors**	**Incidence of tumors**
Normal	-	-	-	-	-
DEN	6	3	3	18	100
DEN+PEE	5	3	2	10	83.33
DEN+ PEAgNPs10	4	2	2	9	66.66
DEN+ PEAgNPs20	3	3	0	6	50

4.8.2 Effect of PEAgNPs on serum marker enzymes

Table 4.16 and figure 4.17 depicts the effect of serum marker enzyme viz. BUN, uric acid and creatinine. It was observed that the levels of all serum marker enzymes levels were elevated in DEN induced group. Prophylactic treatment with PEAgNPs demonstrated a marked reduction in the levels of BUN, creatinine and Uric acid in a dose dependent manner. No huge contrast were seen in control rat and PEAgNPs treated group.

Table 4.16 Effect of PEAgNPs on serum marker enzymes in rats

Treatment	Urea	Creatinine	Uric acid
Normal control	26.05±1.27	1.57±0.1	1.82±0.11
DEN	82.24±3.78$^{\#\#\#}$	4.56±0.29$^{\#\#\#}$	3.84±0.28$^{\#\#\#}$
DEN+ PEE	45.29±2.98***	2.65±0.09***	2.42±0.17***
DEN+PEAgNPs10	36.21±2.87***	2.39±0.14***	2.39±0.16***
DEN+ PEAgNPs20	30.1±2.45***	2.01±0.21***	2.01±0.09***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

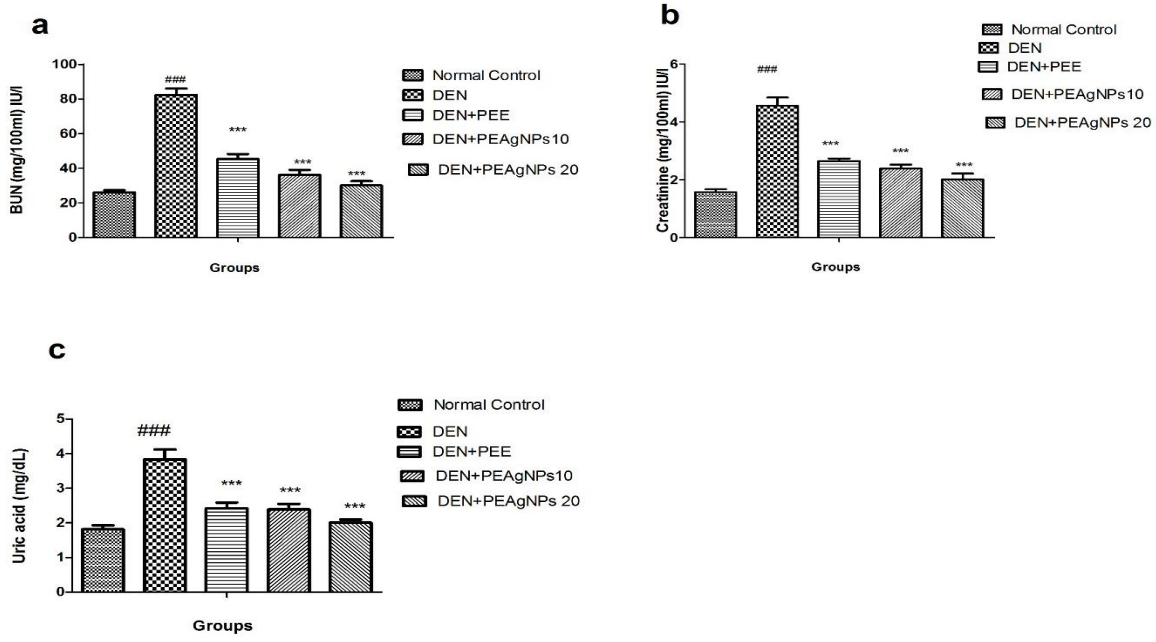


Figure 4.17 Effect of PEAgNPs on serum marker enzymes

4.8.3 Effect of PEAgNPs on tumour marker enzyme in experimental rats

The consequences of prophylactic treatment of rats with PEAgNPs against DEN induced group on tumour marker enzymes viz. XO, LDH, and GGT level were appeared in figure 4.17 and table.4.18. There was upregulation in the levels of XO, LDH and GGT in DEN induced group when contrasted with normal control. Prophylactic administration of PEAgNPs significantly ($p<0.001$) downregulated tumour marker enzyme level in all DEN induced renal cancer group.

Table 4.17 Effect of PEAgNPs on tumour marker enzyme in control and DEN induced RCC rats

Treatment	Xanthine oxidase	LDH	Gamma GTP
Normal control	0.42±0.03	218.21±6.29	350.42±6.28
DEN	$1.2\pm0.12^{###}$	$486.35\pm10.23^{###}$	$621.89\pm11.37^{###}$
DEN+ PEE	0.98 ± 0.01ns	$352.3\pm9.34^{***}$	$440.56\pm9.21^{***}$
DEN+PEAgNPs10	$0.82\pm0.06^{**}$	$290.6\pm7.56^{***}$	$425.91\pm8.9^{***}$
DEN+ PEAgNPs20	$0.78\pm0.09^{***}$	$260.09\pm7.23^{***}$	$405.9\pm9.01^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

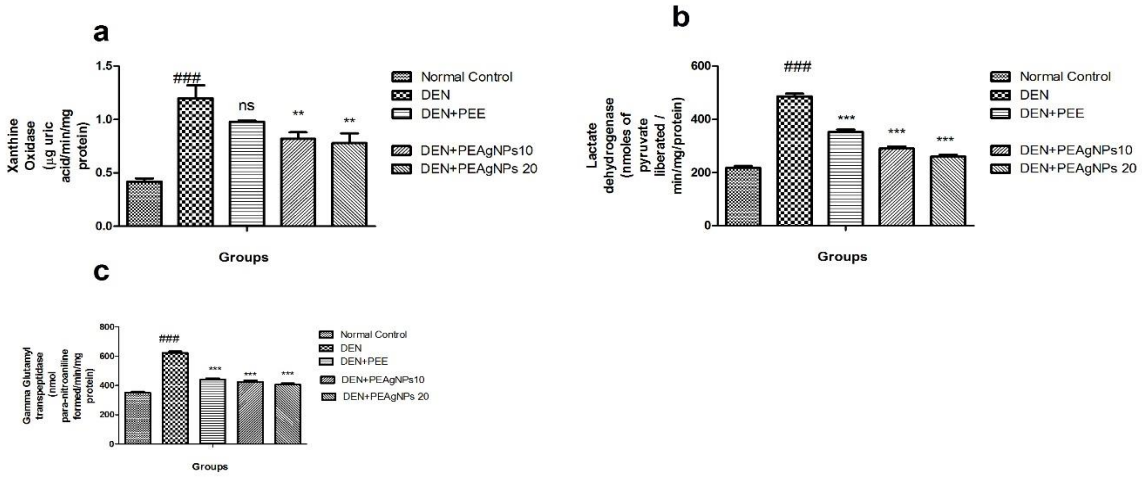


Figure 4.18 Effect of PEAgNPs on tumour marker enzyme

4.8.4 Effect of PEAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats

Malondialdehyde (MDA) levels in renal tissue, utilized as an indicator of lipid peroxidation. The level of MDA was significantly ($p<0.001$) enhanced (8.21± 0.10) in DEN induced groups as shown in table 4.18 and figure 4.19. In PE aqueous extract treated group, no huge change was seen. PEAgNPs 20 and 30mg/kg considerably ($p<0.001$) diminished the enhanced levels renal MDA content contrasted with DEN induced rats which serve as a negative control and significantly re-established the membrane integrity by lessening their levels in the renal tissue.

Table 4.18 Effect of PEAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats

Treatment	**MDA**
Normal control	2.53±0.02
DEN	$8.21\pm0.10^{\#\#\#}$
DEN+ PEE	$6.48\pm0.26^{***}$
DEN+PEAgNPs10	$4.92\pm0.27^{***}$
DEN+ PEAgNPs20	$3.86\pm0.17^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

4.8.5 Effect of PEAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

To explore antioxidant prospects of PEAgNPs and its effect on DEN induced renal tumour, enzymatic antioxidant parameter, for example, catalase, SOD, GSH, GPx, GST and GR were assessed and presented in table 4.19. There was alteration in the concentration of

antioxidant enzymes in DEN induced liver cancer group. Supplementation of PEAgNPs at both the dose levels and PEE significantly ($p<0.001$) ameliorated the content of all these parameters (figure 4.19). Similarly, significant reduction was observed in the content of endogenous antioxidant enzymes, in particular, reduced glutathione, glutathione S-transferase, Glutathione reductase and glutathione peroxidase were noted in the rats inebriated with DEN when contrasted with normal control rats. PEAgNPs restores all the level of endogenous antioxidant enzymes significantly ($p<0.001$) at both the dose level. DEN induced group show elevation in concentration of H_2O_2 as compared to normal control rat. Administration of PEAgNPs significantly ($p<0.001$) restore the level of H_2O_2 at both the doses level.

4.8.6 Effect of PEAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Proinflammatory cytokines and inflammatory mediator level were observed to be raised significantly ($p<0.001$) in DEN induced group and responsible for expansion of carcinogenesis (table 4.20 and figure 4.20). Treatment with PEAgNPs at both the levels significantly ($P < 0.001$) mitigated the level of inflammatory cytokine and mediators. It was apparent from the figure that higher dose of PEAgNPs was observed to be feasible against renal tumour.

4.8.7 Effect of PEAgNPs on ODC activity and thymidine incorporation in kidney of control and DEN- induced RCC in rats

DEN treatment show significant upregulation in ODC activity and ^3H thymidine incorporation activity in wistar rats as depicted in table 4.21 and figure 4.21. Treatment with biofabricated silver nanoparticles of *P.emblica* leaf aqueous extract caused mark ($p<0.001$) reduction in the raise level of ODC and ^3H thymidine incorporation activity till the end of experiment. No significant difference were observed in the in ODC activity and ^3H thymidine incorporation activity of DEN + PEAgNPs (20 mg/kg BW) and normal control.

Table 4.19 Effect of PEAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

Treatment	**SOD**	**GSH**	**Catalase**	**GPx**	**GST**	**GR**	**H_20_2**
Normal control	99.67±3.42	7.99±0.25	4.78±0.17	260.11±7.26	226.11±7.9	296.45±8.56	25.32±2.90
DEN	53.12±2.49$^{###}$	3.62±0.09$^{###}$	1.67±0.09$^{###}$	141.28±4.79$^{###}$	117.29±4.19$^{###}$	165.9±4.66$^{###}$	74.94±4.76$^{###}$
DEN+ PEE	63.86±2.78*	4.09±0.21^{ns}	2.09±0.02^{ns}	178.28±4.23***	156.72±5.02***	179.2±5.07^{ns}	61.62±4.12^{ns}
DEN+PEAgNPs10	75.96±2.76***	5.48±0.19***	3.18±0.2***	221.98±6.07***	178.36±6.56***	205.27±5.65***	48.39±3.56***
DEN+ PEAgNPs20	86.01±3.34***	6.41±0.23***	3.76±0.14***	243.86±6.29***	193.28±5.45***	238.31±6.34***	39.21±3.11***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

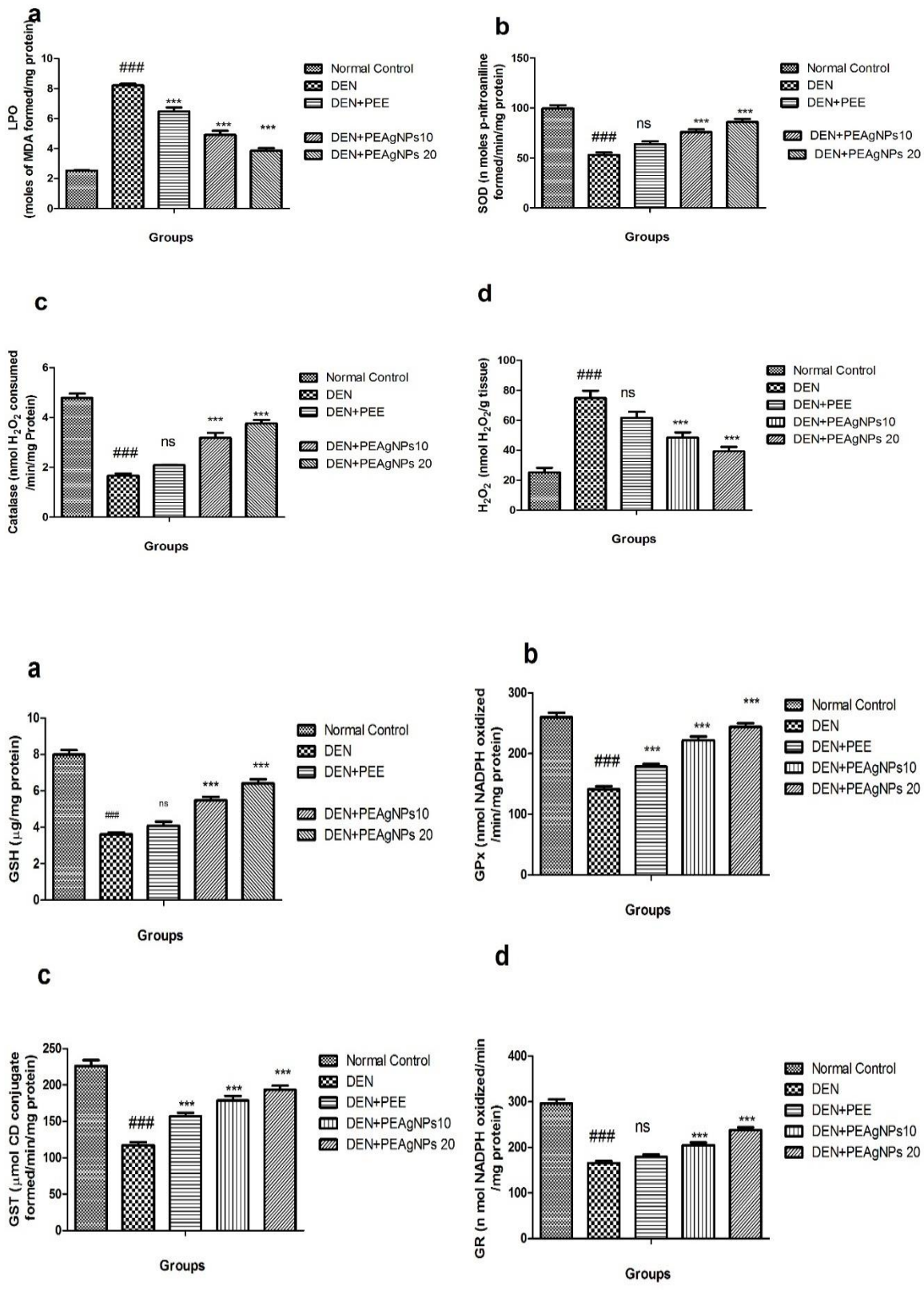


Figure 4.19 Effect of PEAgNPs on the levels of antioxidant enzymes

Table 4.20 Effect of PEAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Treatment	TNF-α	IL-6	IL-1β	NF-κB
Normal control	213.36±7.28	824.05±13.29	152.38±5.28	421.10±8.19
DEN	750.42±12.67$^{\#\#\#}$	1756.28±21.89$^{\#\#\#}$	790.62±9.39$^{\#\#\#}$	1262.31±20.56$^{\#\#\#}$
DEN+ PEE	426.3±8.49***	1290.92±18.34***	482.09±8.05***	856.11±15.48***
DEN+PEAgNPs10	336.68±9.40***	1050.42±16.43***	309.38±6.32***	555.46±11.87***
DEN+ PEAgNPs20	290.92±8.45***	926.32±16.79***	221.35±6.20***	502.62±9.67***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

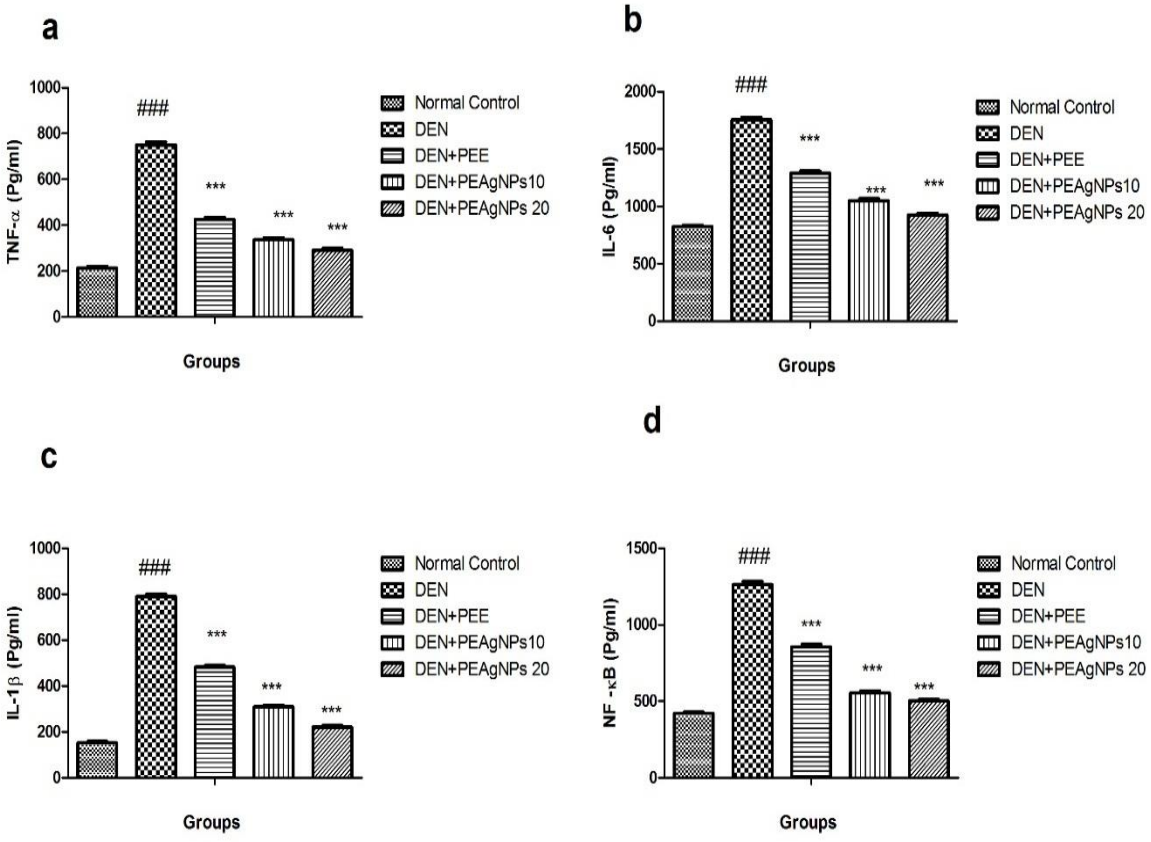


Figure 4.20 Effect of PEAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Table 4.21 Effect of PEAgNPs on ODC activity and thymidine incorporation in kidney of control and DEN- induced RCC in rats

Treatment	ODC activity	^{3}H thymidine incorporation
Normal control	723.15±18.47	1650.46±28.56
DEN	1876.90±29.56$^{\#\#\#}$	2790.70±37.69$^{\#\#\#}$
DEN+ PEE	1505.57±23.27***	2143.60±34.87***
DEN+PEAgNPs10	1112.47±22.34***	1932.47±30.40***
DEN+ PEAgNPs20	843.78±20.12***	1785.09±30.12***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

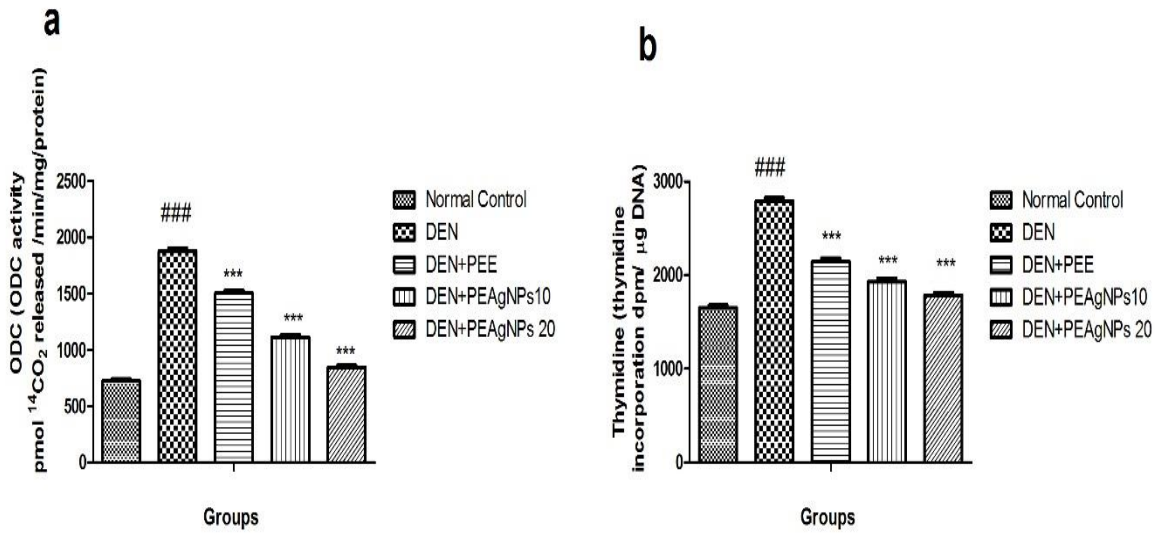


Figure 4.21 Effect of PEAgNPs on ODC activity and thymidine incorporation in kidney of control and DEN- induced RCC in rats

4.8.8 Histopathology observation of kidney of control and experimental rats

Figure 4.22 demonstrates the histopathological characteristics of renal tissue of normal control, DEN-induced, PEE and PEAgNPs treated group rats. Normal control group rat shows normal architecture of renal tissue such as urinary space, compact Bowman's capsules, distal

tubules, proximal tubules, medullary rays and collecting ducts and cubic epithelium. DEN induced rats revealed the autopsy of renal tissue, deformation of renal cortical portion, gapping in the space of Bowman's capsules, inflammatory blood cells, and tubule interstitial. Treatment with extract of Phyllanthus emblica to DEN induced rats showed improvement in inflammatory blood vessels, vacoulation of tubules, and gross proficiency of normal architecture by restoration of urinary space and Bowman's capsules. PEAgNPs treated rats revealed the tubules with normal structure and glomeruli. PEAgNPs (20mg/kg) treated animals showed reclamation of space of the Bowman capsules, fewer inflammatory blood vessels with normal tubules and collecting ducts with respect to DEN induced rats.

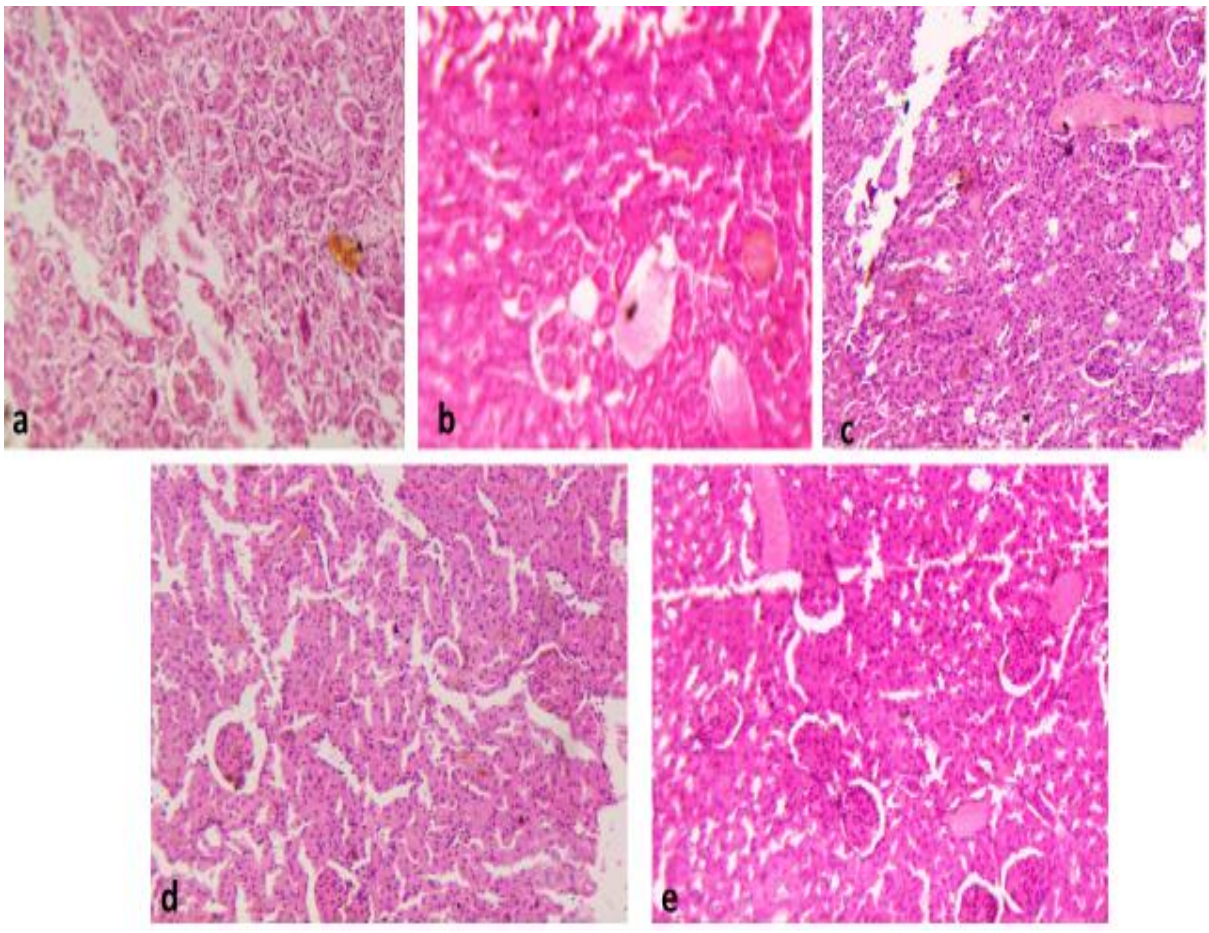


Figure 4.22 Photomicrograph of eosin-haematoxylin stained histological renal section area for (a) Normal Control group, (b) DEN induced liver cancer group (c) DEN+ PEE (d) DEN +PEAgNPs (10mg/kg bw) (e) DEN +PEAgNPs (20mg/kg bw)

Results of *Madhuca Longifolia*

4.9 Pharmacognostical character of leaf of *Madhuca Longifolia*

4.9.1 Macroscopical characters of leaf of *Madhuca Longifolia*

The colour of the leaf is green, characteristic odour and bitter taste. They are clustered at the end of the branches. The size of leaf is 11-15cm long and 6-8 cm wide. The shape of leaves is lanceolate to ovate, petiole of leaves is short and easily fracture. The apex of leaves is acute, base is cuneate and arrangement is opposite (figure 1.10).

4.9.2 Microscopic characters of leaf of *Madhuca Longifolia*

The T.S. showed the existence of dorsiventral cellular arrangement between the lamina and prominent midrib. The upper layer of epidermis is made up of compactly arrange thin cell walled parenchyma cells. Mesophyll is divided into palisade and spongy parenchymatous cells. Palisade cells are single layered, compactly packed, elongated and are not continuous over the vascular bundles of midrib. Underlying the epidermis, polygonal parenchymatous cells is present. Vascular bundle is fibrous in appearance, encircled by many layers of cortex and distinct sclerenchymatous cells of midrib and lamina portion. Xylem was present in the middle of the leaf and surrounded by the phloem. Calcium oxalate crystals, starch grains present in the region of cortex. The trichome is unicellular and stomata is anomocytic type (Figure 4.23).

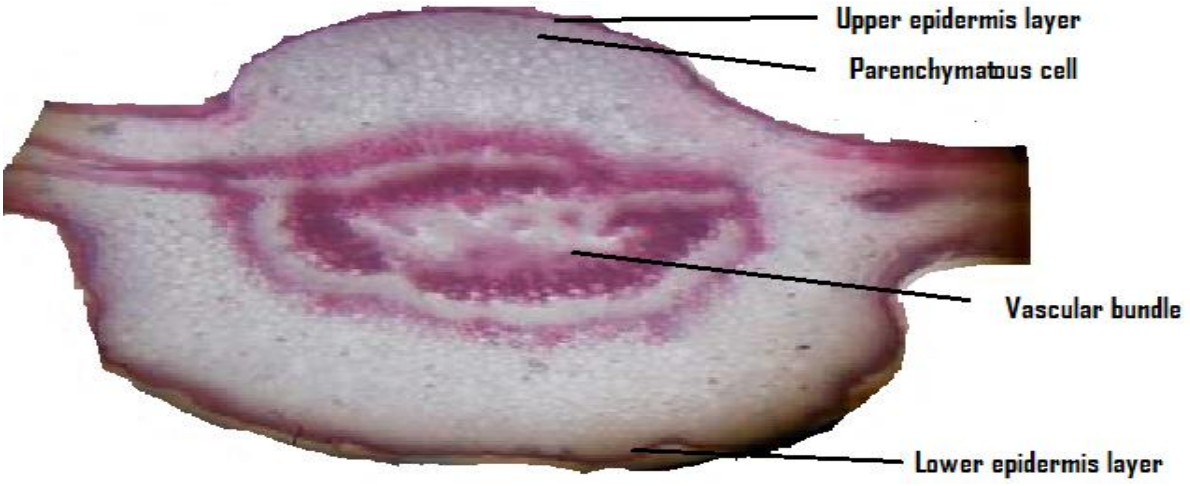


Figure 4.23 Transverse section of leaf of *Madhuca Longifolia*

The results of leaf constant and physicochemical parameter is displayed in table no 4.22-4.23.

Table 4.22 Determination of leaf constant of *Madhuca longifolia*

Leaf Constant	**Range**	**Average**
Stomatal Number		
Upper surface	0	
Lower surface	10-16	13
Stomatal Index		
Upper Surface	0	
Lower Surface	17.4-19.2	18.3
Vein islet number	4-6	5
Vein termination number	16.5-18.2	17.35

Table 4.23 Physico-chemical parameters of leaves of *Madhuca Longifolia*

S. No.	**Parameter**	**Observation (% w/w)**
1	Ash value	
a	Total ash	5.2 ±0.31
b	Acid soluble ash	1.2
c	Acid insoluble ash	0.7
2.	Moisture content	8.09
3	Foreign matter	0.7

4.10 Extraction of aqueous extract of *Madhuca longifolia*

The leaves of *Madhuca longifolia* were subjected to double distilled water and the percentage yield was obtained as 10.05 % (w/w) which is depicted in table 4.24.

Table 4.24 Percentage yield of extract of leaves of *Madhuca Longifolia*

Botanical name	**family**	**Part used**	**Solvent used for extraction**	**% yield (Extract)**
Madhuca Longifolia	Sapotaceae	leaves	water	10.05

4.11 Preliminary phytochemical studies

Phytochemical studies were performed to determine the presence of various chemical compounds present in the plant extract. The different chemical tests were done on the aqueous extract of *Madhuca longifolia*. *Madhuca longifolia* revealed positive test for various chemicals constituents such as and presented in table 4.25

Table 4.25 Qualitative chemical test on the aqueous extract of *Madhuca longifolia*

S. No.	Chemical Test	Aqueous extract
1.	**Alkaloids** Mayers Reagent Dragendroff Reagent Hagner reagent Wagner reagent	+ + + +
2.	**Glycosides** Legal test Keller Killani Test Borntragers Test	+ - -
3.	**Flavonoids** Ammonia Test Shinoda/ Paw test	+ +
4.	**Sterols** Liebermann-Buchard test Salwoski Test	- -

5.	**Phenolic compounds and tannins** Ferric chlorides test Lead acetate test	 + +
6.	**Proteins** Millions test Biuret test	+ +
7.	**Coumarins**	-

4.12 Synthesis of Silver nanoparticles of *Madhuca Longifolia*

Briefly, 10 ml of aqueous plant extract (*Madhuca Longifolia*) was mixed with 90 ml of $AgNO_3$ solution under vigorous stirring in Erlenmeyer flask and incubated overnight at RT for the reduction of silver ions. The solution turns from yellow to black colour which is represented in Figure 4.24.

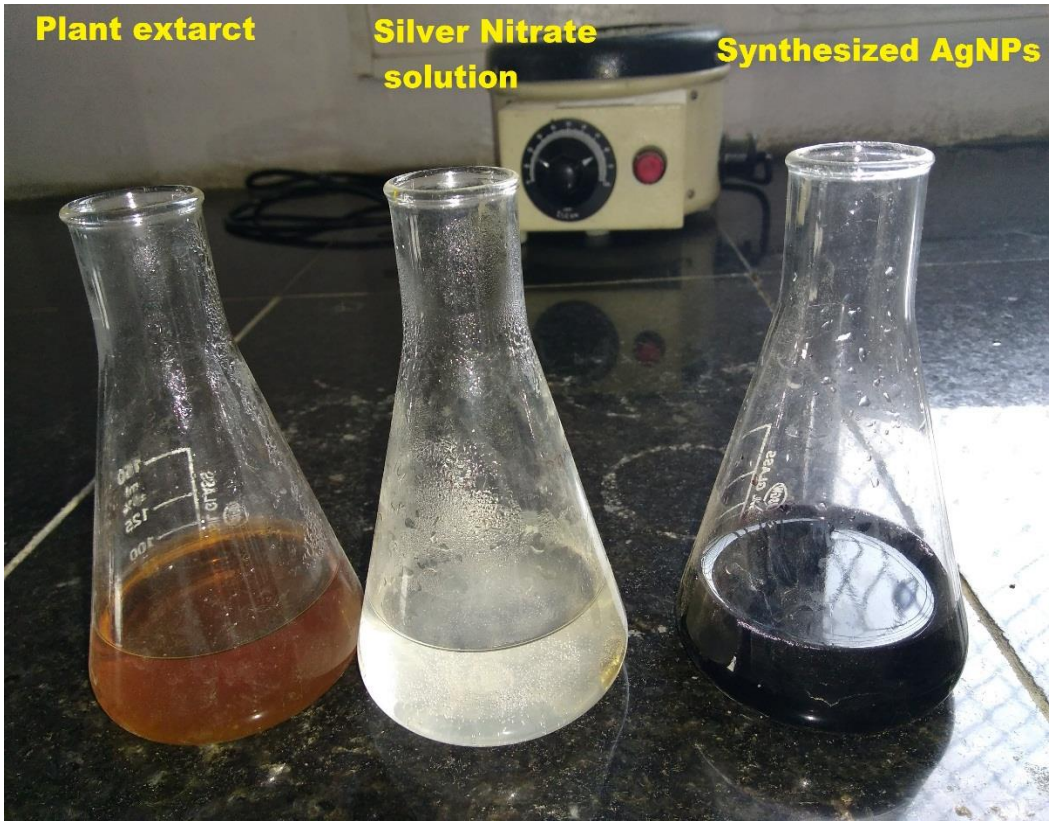


Figure 4.24 Biosynthesis of Silver nanoparticles of *Madhuca Longifolia* aqueous extract

4.13 Characterization of biosynthesized AgNPs of *Madhuca Longifolia extract*

4.13.1 UV spectral analysis of MLAgNPs

The formation of AgNPs using aqueous extract of *Madhuca longifolia* was confirmed by the visual appearance of black colour in the suspension as shown in figure 4.24. After 1 hour of colour change, a single intense peak was obtained at 484 nm in the UV spectrophotometer due to excitation of the surface plasmon resonance (SPR) and spectrum is represented in fig.4.25 Visual colour change was obtained after the addition of plant aqueous extract to silver nitrate solution and the phytoconstituents present in aqueous extract reduce the silver ion in AgNPs during synthesis. Higher the concentration of plant extract, more reductive process occur.

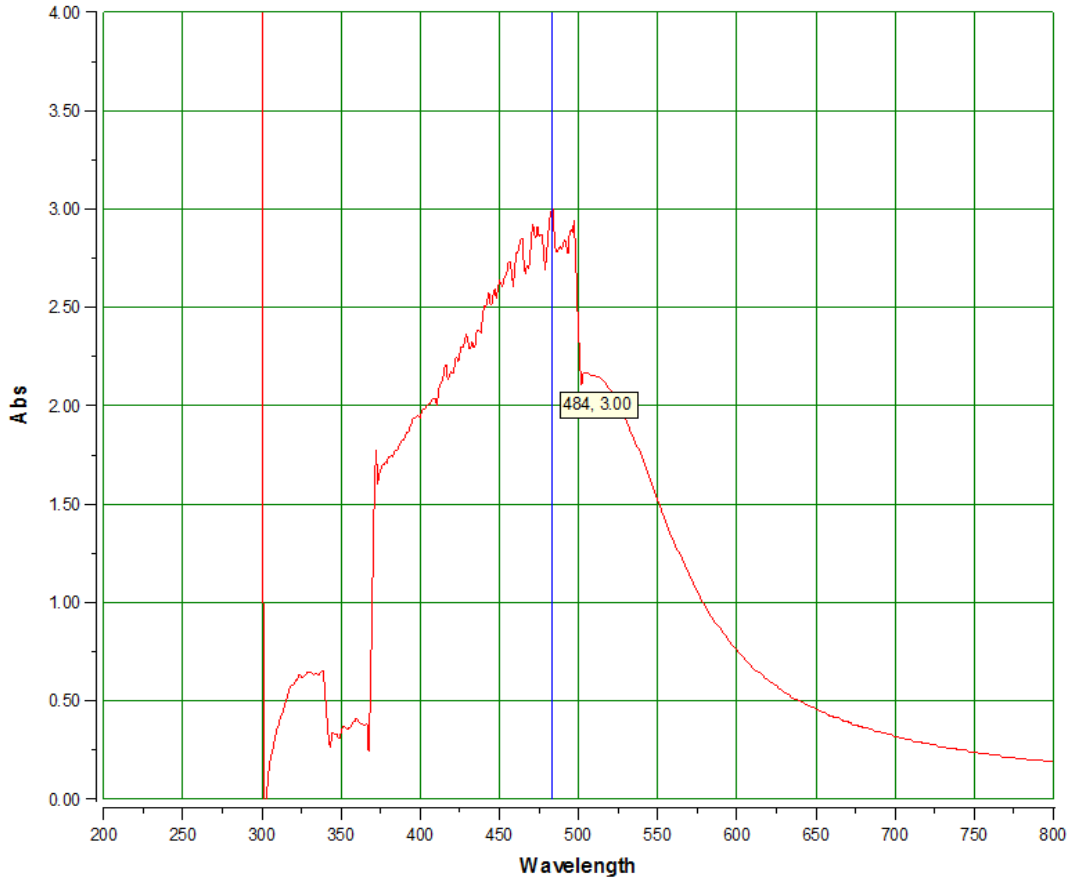


Figure 4.25 UV-Vis spectrum of MLAgNPs

4.13.2 FTIR spectra analysis of MLAgNPs

Through phytochemical screening, it was observed that aqueous leaf extract of *Madhuca longifolia* contains a carbohydrate, glycosides and flavonoid. These capping and reducing agent play a major role in the reduction of silver ion during reaction. Presence of different functional groups and biomolecule was revealed through FTIR spectroscopy. Chemical composition of silver nanoparticles surface was also identified FTIR analysis. In silver nanoparticles, intense peaks was observed at 3180 cm^{-1} assigned to Phenolic O-H stretch (broad, s), 2919 cm^{-1} assigned to C-H (s) stretch, 1605cm^{-1} assigned to C=C aromatic stretch, 1441cm^{-1} assigned to aromatic C=C bending, 1112cm^{-1} assigned to C=O stretch (s), 824cm^{-1} assigned to C-H bending (Figure 4.26).

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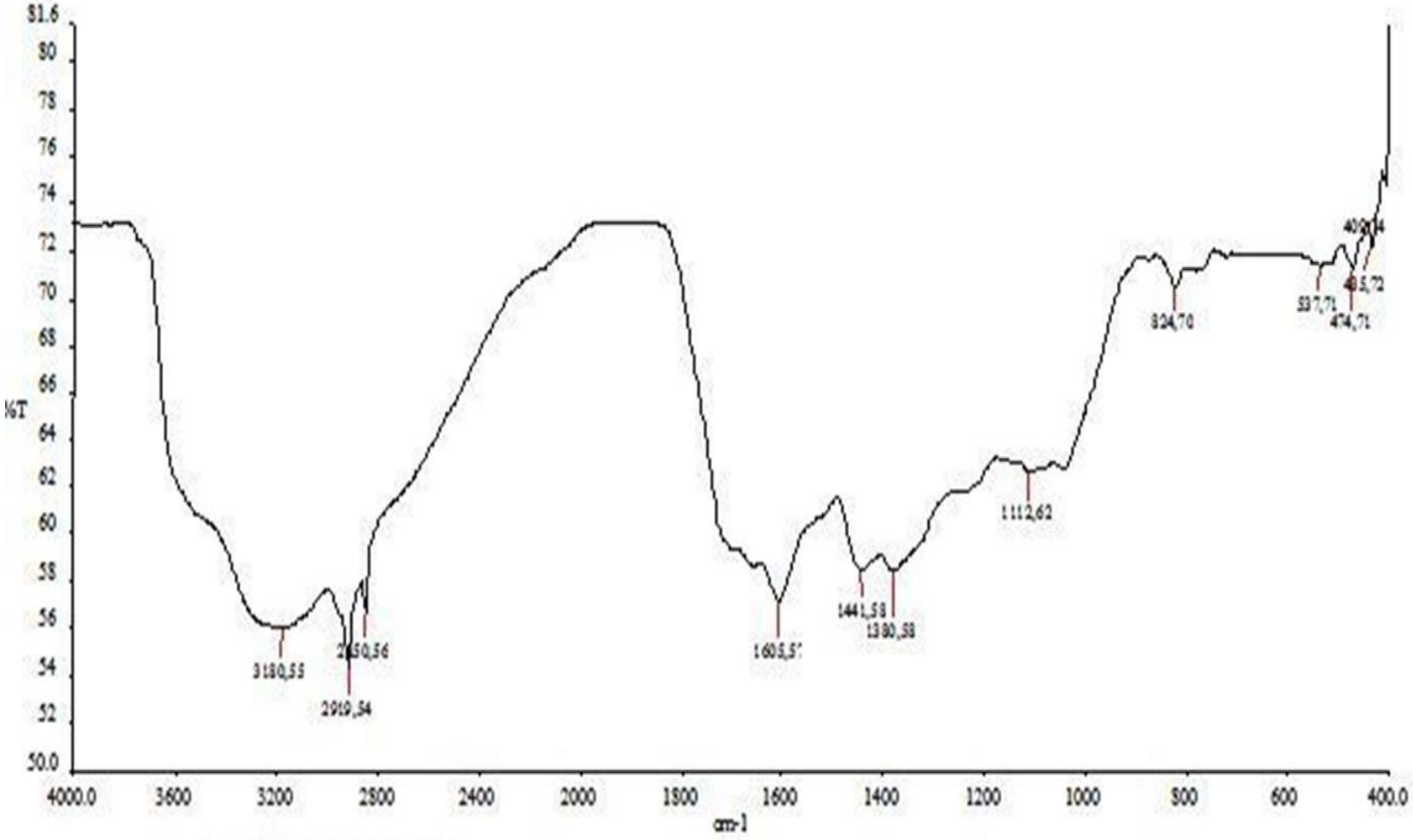


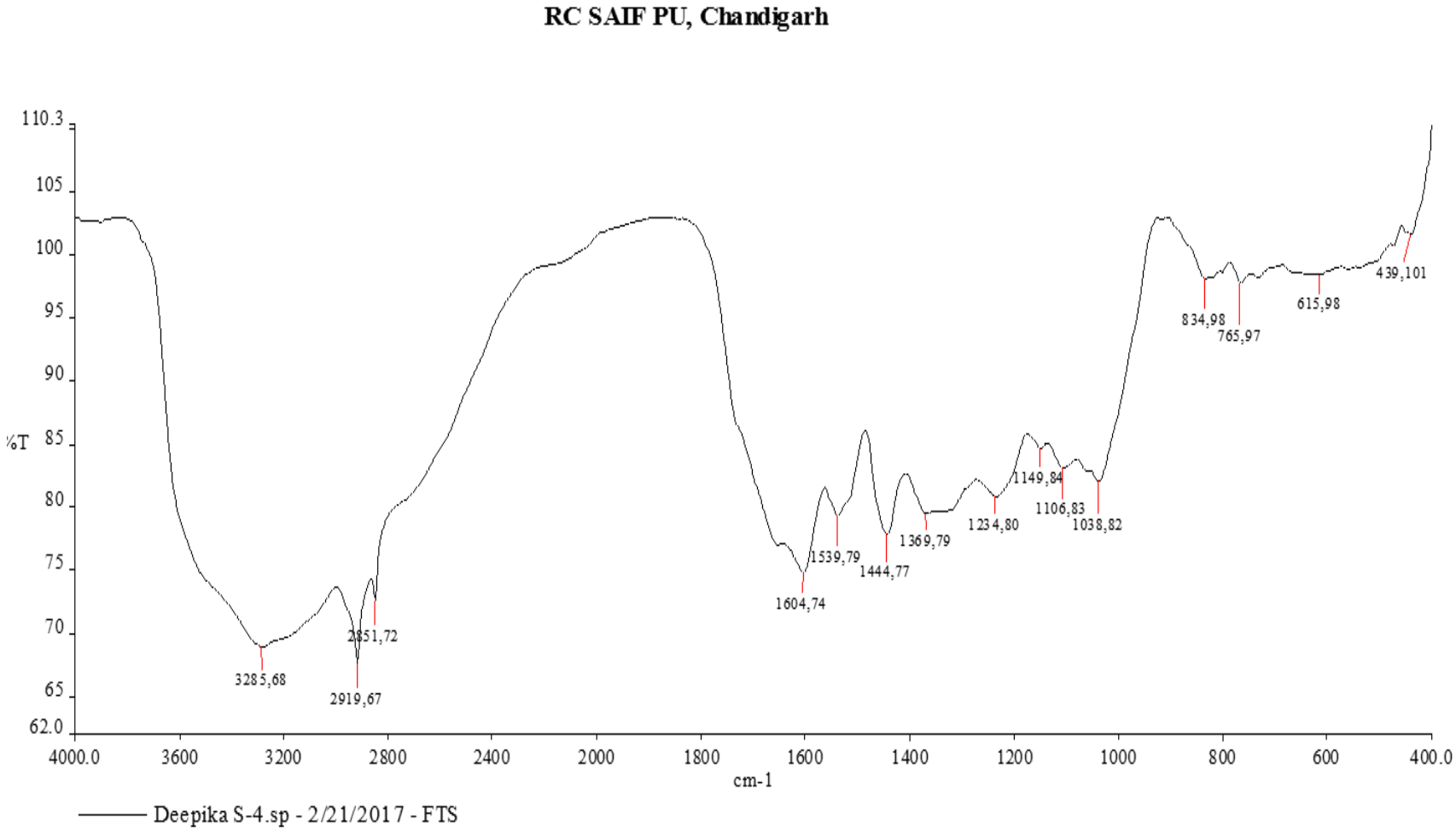


Figure 4.26 FTIR spectra a) MLE b) MLAgNPs

4.13.3 XRD pattern of MLAgNPs

The XRD pattern of MLAgNPs was depicted in table 4.26 and figure 4.27. The XRD diffraction peaks at 2θ degree of 38.03, 44.03, 64.49 and 77.53 corresponded to the (111), (200), (220), and (311) planes as mentioned in standard data (JCPDS file No.01-071-4613). All the peaks were present in the figure shows the crystalline structure of silver and crystalline planes of the face centered cubic.

Table 4.26 XRD pattern of MLAgNPs

Pos. [°2θ]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2θ]
32.1686	0.1673	2.78265	39.03	33.53
38.0339	0.3346	2.36594	100.00	171.79
44.0351	0.9368	2.05643	16.75	80.58
46.2214	0.2676	1.96413	17.43	23.96
54.7712	0.4015	1.67603	4.98	10.27
57.3343	0.8029	1.60705	3.03	12.49
64.4973	0.4684	1.44480	23.31	56.06
77.5311	0.2676	1.23126	23.28	32.00
81.6643	0.5353	1.17908	5.71	15.69

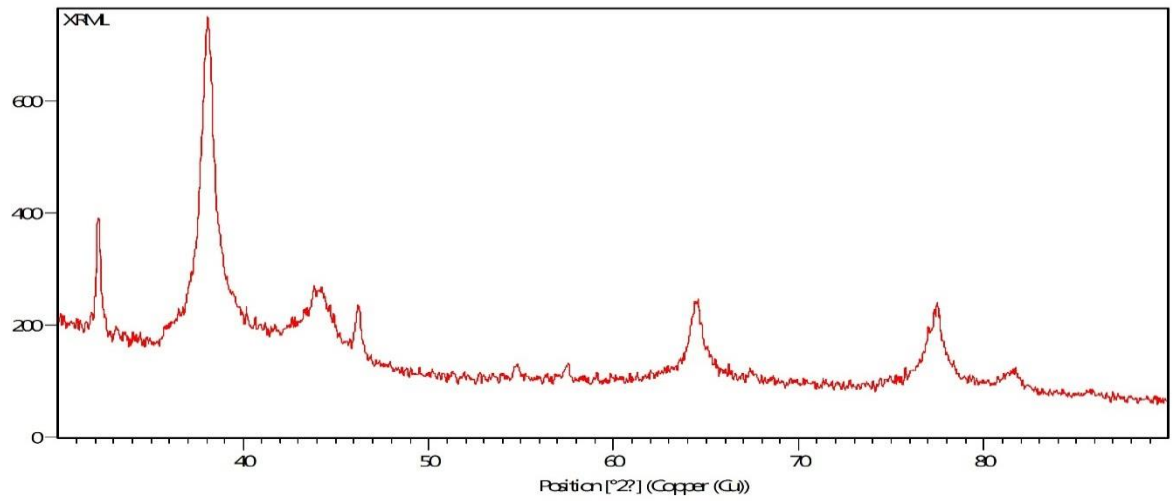


Figure 4.27 XRD spectrum of synthesized MLAgNPs

4.13.4 Field Emission Scanning Electron Microscope (FESEM) of MLAgNPs

The surface morphology of AgNPs was carried out by using FE-SEM instrument (SUPRA-55, CARL ZEISS, GERMANY) and images are depicted in figure 4.28. The suspension of AgNPs was placed in clean glass plate, and water was evaporated to characterize the surface morphology, size and size distribution. The images showed spherical shape of silver nanoparticles in which particles was enclosed by the different bicompound and particle size ranges between 20 to 65 nm. The results confirmed the production of silver nanoparticles in which leaf aqueous extract act as capping and reducing agent due to presence of biomolecule.

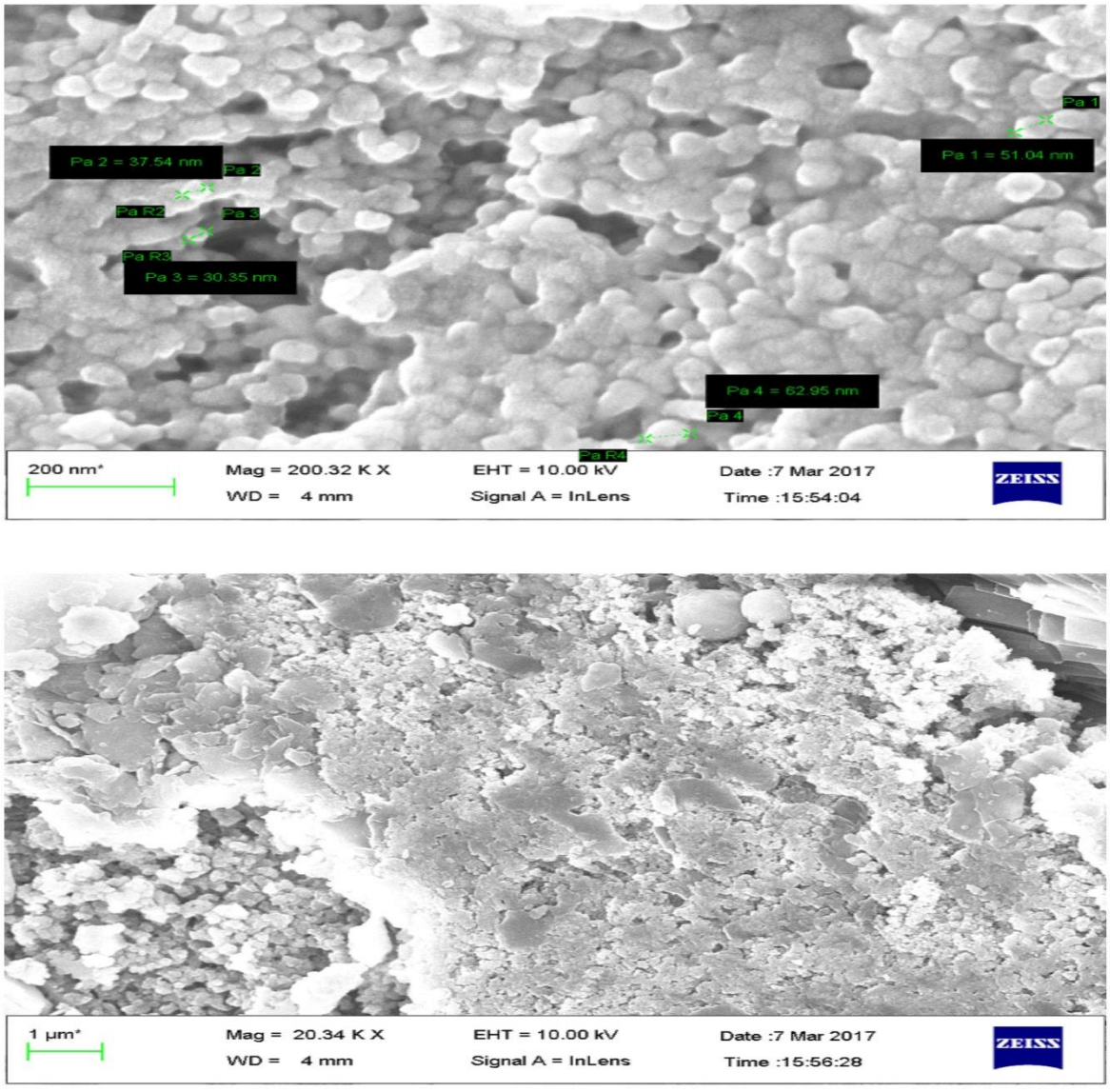


Figure 4.28 FESEM images of Synthesized MLAgNPs

4.13.5 **Energy dispersive X-ray (EDX) analysis of MLAgNPs**

The EDX confirms the presence of stoichiometry, purity and elemental composition profile in functionalized silver nanoparticles (Figure 4.29). It revealed the strong signal of silver in the range of 2.8–3.4 keV due to excitation of surface plasmon resonance (SPR). The other elemental such as Cl, Oxygen, Nitrogen etc. were also detected and might be due to presence of bioactive molecule present in leaf extract of *M.Longifolia*. The elemental mapping was also done by EDX to confirm the presence of silver in silver nanoparticle. The distribution of silver on the surface of the nanoparticles confirmed the formation of AgNPs and represented by green dots.

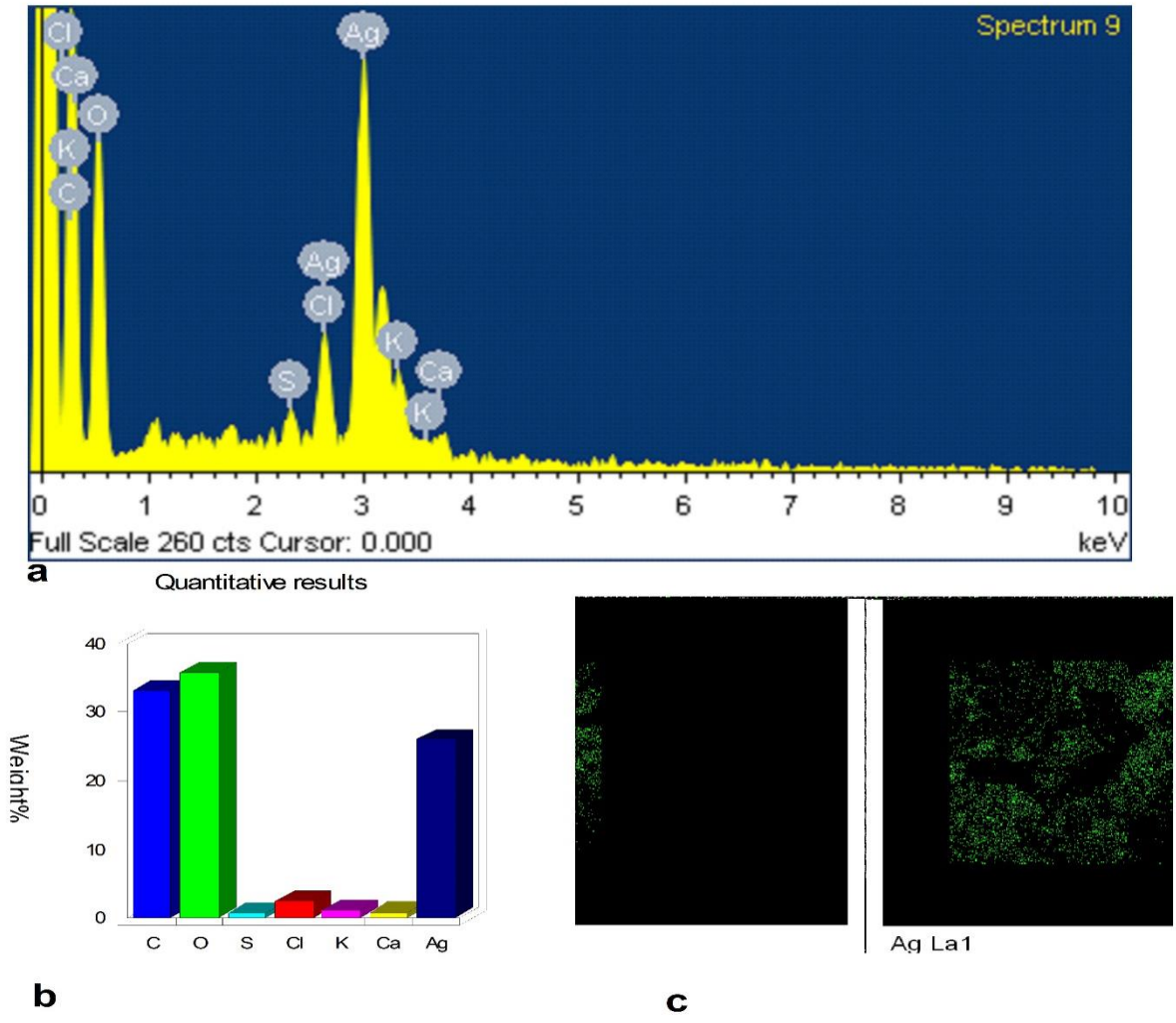


Figure 4.29 EDX spectrum of synthesized MLAgNPs

4.13.6 TEM study of MLAgNPs

Surface morphology and size of MLAgNPs was determined by TEM (Figure 4.30). The TEM images represent the spherical shape of silver nanoparticle and in the size range of 5 to 20 nm, confirm the existence of silver nanoparticles. Surface of nanoparticles covered with a dark shades represent secondary products and corresponds to biomolecule present in plant leaf extract. These biomolecule responsible for the reduction of silver salts to silver nanoparticles, obstruct the formation of cluster and initiate nucleation.

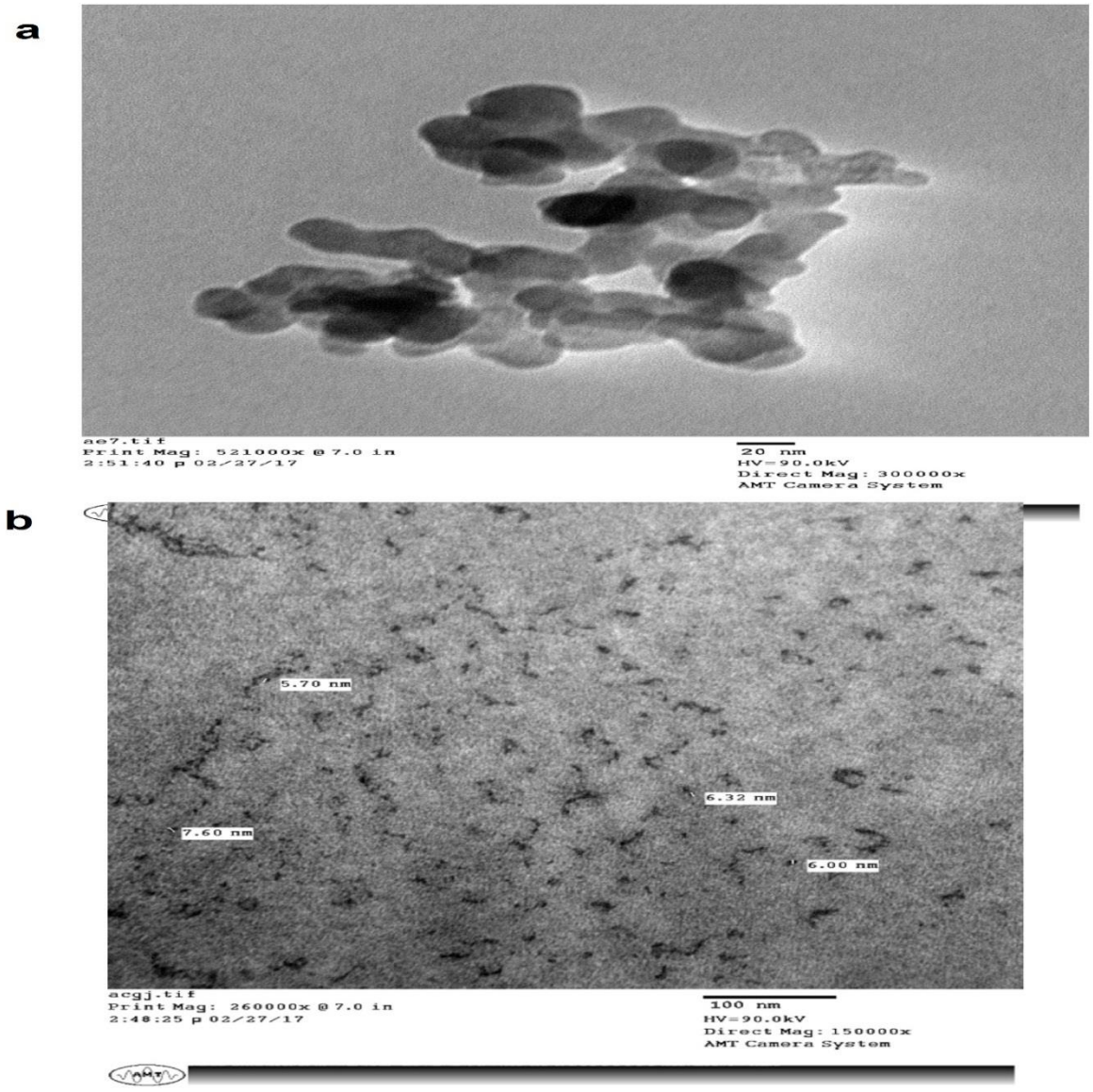


Figure 4.30 TEM images of MLAgNPs

4.14 *In vitro* cytotoxic activity of MLAgNPs against HuH-7 Cell line

In vitro cytotoxic activity of prepared silver nanoparticles embedded *Madhuca Longifolia* extract at different concentration against hepatic cancer cell lines (HUH-7) was tested by using by MTT assay. On increasing concentration of MLAgNPs, the cell proliferation activity decreases. The IC_{50} cell inhibition value for MLAgNPs was obtained at 41.01 µg/ml. Figure 4.31 and table 4.27 represent the percentage growth inhibition of HUH-7 cell line at different dilutions of silver nanoparticles of *Madhuca longifolia*.

Table 4.27 *in vitro* cytotoxic activity of MLAgNPs on hepatic cancer (HuH-7 cell line)

Log concentration	% Growth inhibition
1.000	0.000
1.30103	38.000
1.39794	64.000
1.477121	95.000
1.69897	128.000

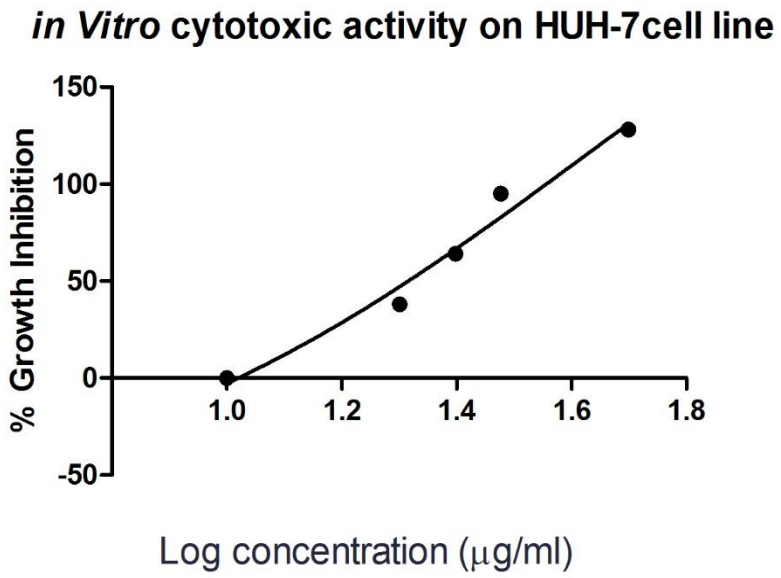


Figure 4.31 *in vitro* cytotoxic activity of MLAgNPs on hepatic cancer

4.15 *In vivo* hepatic cancer studies of MLAgNPs on rats

4.15.1 Effect of green synthesized MLAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats

A significant enhancement was observed in body weight (320.62±1.34) of normal control whereas reduction (270.21±0.34) was found in DEN induced group and depicted in table 4.28. DEN induced group when treated with MLAgNPs at a dose of 20 and 30 mg/kg b.w. show up regulation ($p < 0.001$) in the weight of the body. Similar result was also assessed in liver weight and mean liver weight of the DEN induced group. Treated group with different doses of MLAgNPs reduces the mean liver weight in a dose dependent manner. DEN induced group when treated with a silymarin improved body weight and relative liver weight when contrast to positive control group.

4.15.2 Effect of MLAgNPs on the development of liver nodules in control and DEN-induced HCC rats

Table.4.29 depicts the total numbers of hepatic knob, average number of nodules on hepatic and percentage of tumour incidence. Expansion of knobs on hepatic was found in DEN induced groups as compared to MLAgNPs which lowers the knob present on liver in a dose dependent manner.

Table 4.28: Impact of MLAgNPs on the Body weight and relative liver weight in control and DEN-induced HCC rats

Treatment	**Initial body weight**	**Final Body weight (g)**	**Liver weight (g)**	**Relative liver weight**
Control	172.54±0.10	320.62±1.34	8.26± 0.28	2.57 ± 0.28
DEN	176.62±0.29	270.21±0.34$^{###}$	19.32±0.45	7.15±1.78$^{###}$
DEN+ Sily	165.83±0.08	301.46±1.50***	9.98±0.07	3.31 ±0.61^{ns}
DEN+MLE	162.35±0.59	294.21±1.32***	17.36±0.78	5.90 ±1.34 ^{ns}
DEN+ MLAgNPs20	167.90±0.01	301.86±0.72***	14.05±0.13	4.65 ±0.48 ^{ns}
DEN+ MLAgNPs30	163.45±0.78	306.56±1.89***	11.09±0.01	3.61 ±0.67 ^{ns}

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

Table 4.29 Effect of MLAgNPs on the development of macroscopic hepatic nodules in different groups of rats.

Treatment	Total no. of nodules	Tumour incidence (%)	Average number of nodules
Control	0	0	0
Toxic	107	100	$54.86 \pm 3.01^{###}$
DEN+PEE	83	75.32	$39.18 \pm 2.10^{***}$
DEN+ MLAgNPs20	32	34.03	$9.65 \pm 1.54^{***}$
DEN+ MLAgNPs30	29	31.97	$8.05 \pm 1.26^{***}$
DEN+ Sily	14	21.38	$4.16 \pm 1.56^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

4.15.3 Effect of MLAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

Table 4.30 illustrates impacts of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and alpha feto protein in the serum of control and experimental group of animals. Wistar rats when induced with DEN showed upregulation in all parameters with respect to control group. MLAgNPs at different doses levels significantly ($p < 0.001$) downregulated the effects of all serum marker enzyme when contrast to toxic control group. Significance enhancement was observed in the activity of serum AFP in DEN induced group with respect to normal group. Different doses strength of MLAgNPs show deduction in the levels of serum AFP when treated in a dose dependent manner. Significant reduction ($p < 0.001$) was found in DEN + Silymarin treated group when compared with DEN induced group.

Table 4.30. Effect of MLAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

Treatment	ALT	AST	ALP	AFP
Control	45.85±2.41	57.23±3.38	43.24±2.38	132.96±3.24
DEN	239.52±5.17$^{###}$	224.73±6.89$^{###}$	217.4±7.73$^{###}$	207.04±5.59$^{###}$
DEN+ Sily	38.93±2.23***	69.33±3.62***	61.26±3.34***	146.99±5.1***
DEN+MLE	160.23±4.78***	178.89±4.37***	156.07±4.97***	187.54±4.09**
DEN+ MLAgNPs20	98.23±3.78***	97.77±3.45***	89.78±2.28***	154.38±3.23***
DEN+ MLAgNPs30	68.23±2.9***	89.56±0.78***	76.34±3.9***	149.89±1.02***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

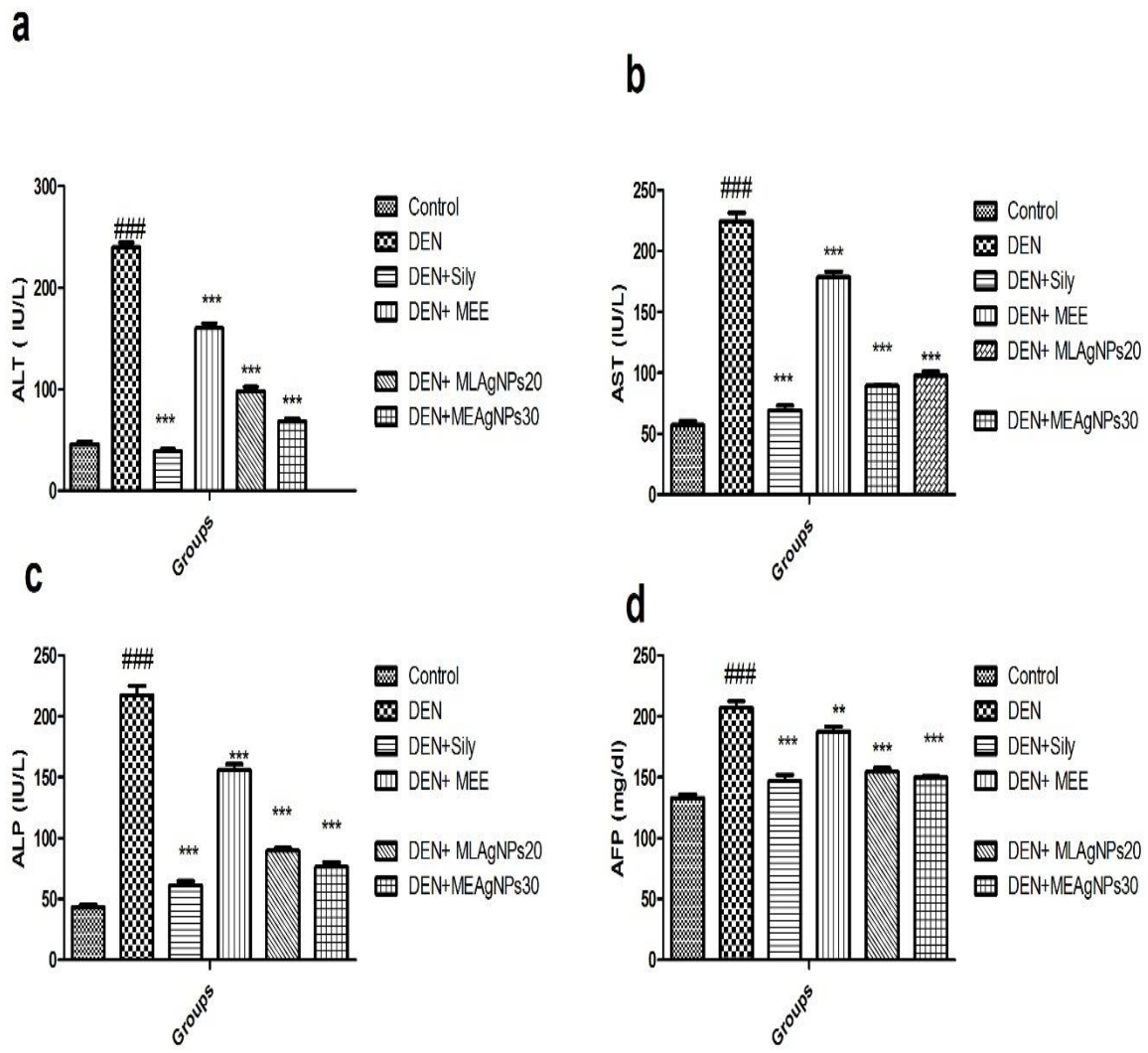


Figure 4.32 Effect of MLAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

4.15.4 Effect of MLAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

The parameter of all non-hepatic enzymes of control and experimental groups of rats is shown in table 4.31 and figure 4.33. There was down-regulation in the activity of all these parameters of DEN induced group whereas enhancement and enzymes restoration was done by MLAgNPs at both the dose levels ($p < 0.001$).

Table 4.31 Effect of MLAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

Treatment	TB	TP	Albumins	Globulins	A/G ratio
Control	0.72±0.09	9.78±0.56	6.67±0.29	3.11±0.01	2.14±0.03
DEN	4.02±0.12$^{###}$	5.36±0.18$^{###}$	2.78±0.29$^{###}$	2.58±0.11$^{##}$	1.08±0.02$^{###}$
DEN+ Sily	0.87±0.11***	8.95±0.34***	5.78±0.67***	3.17±0.21**	1.83±0.07***
DEN+MLE	1.89±0.13***	6.29±0.09^{ns}	4.22±0.26*	2.04±0.05*	2.03±0.09***
DEN+ MLAgNPs20	1.21±0.02***	7.63±0.5**	4.85±0.12***	2.78±0.04^{ns}	1.87±0.05***
DEN+ MLAgNPs30	0.98±0.06***	8.19±0.54***	5.1±0.12***	3.09±0.16*	1.65±0.01***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

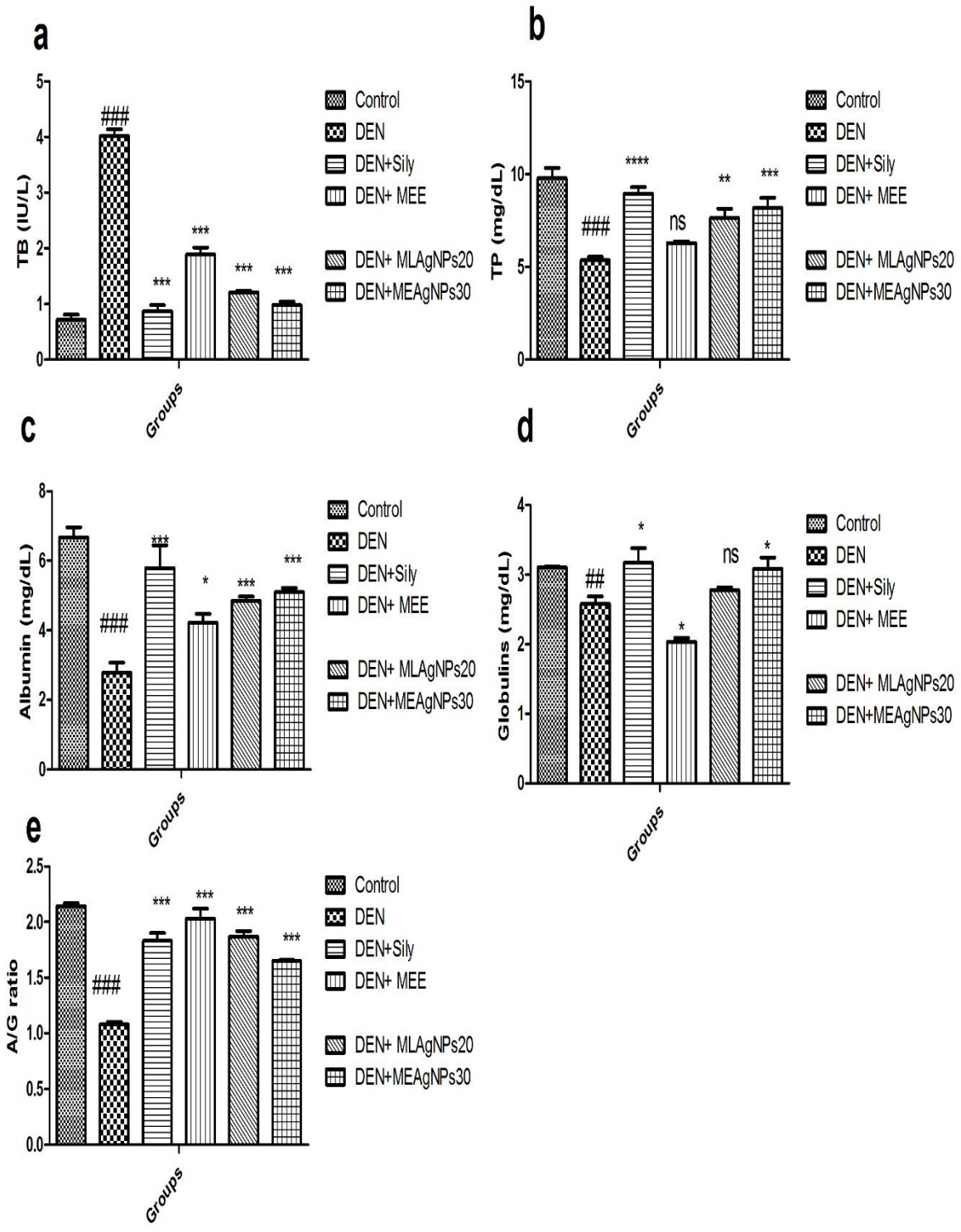


Figure 4.33 Effect of MLAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

4.15.5 Effect of MLAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

Table 4.32 and fi demonstrated the impact of the proinflammatory cytokines on the normal control group and DEN induced liver cancer group. DEN induced toxic group rats explained expanded content of proinflammatory cytokines, which was lessened by the MLAgNPs treatment at both the dose level which was close to the normal control rats.

Table 4.32 Effect of MLAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

Treatment	**TNF-α**	**NF-κB**	**IL-6**	**IL-1β**
Control	50.21±2.28	159.2±4.09	103.2±4.29	23.26±1.35
DEN	169.09±4.45$^{\#\#\#}$	283.12±8.98$^{\#\#\#}$	305.19±8.09$^{\#\#\#}$	86.99±3.98$^{\#\#\#}$
DEN+ Sily	60.28±2.29***	158.78±3.56***	106.2±4.4***	40.02±2.28***
DEN+MLE	100.23±3.05***	203.89±4.34***	200.29±7.9***	70.12±3.08***
DEN+ MLAgNPs20	73.95±2.97***	176.76±4.78***	128.11±3.9***	48.2±1.55***
DEN+ MLAgNPs30	69.29±0.36***	170.56±3.5***	115±3.36***	45.29±1.3***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

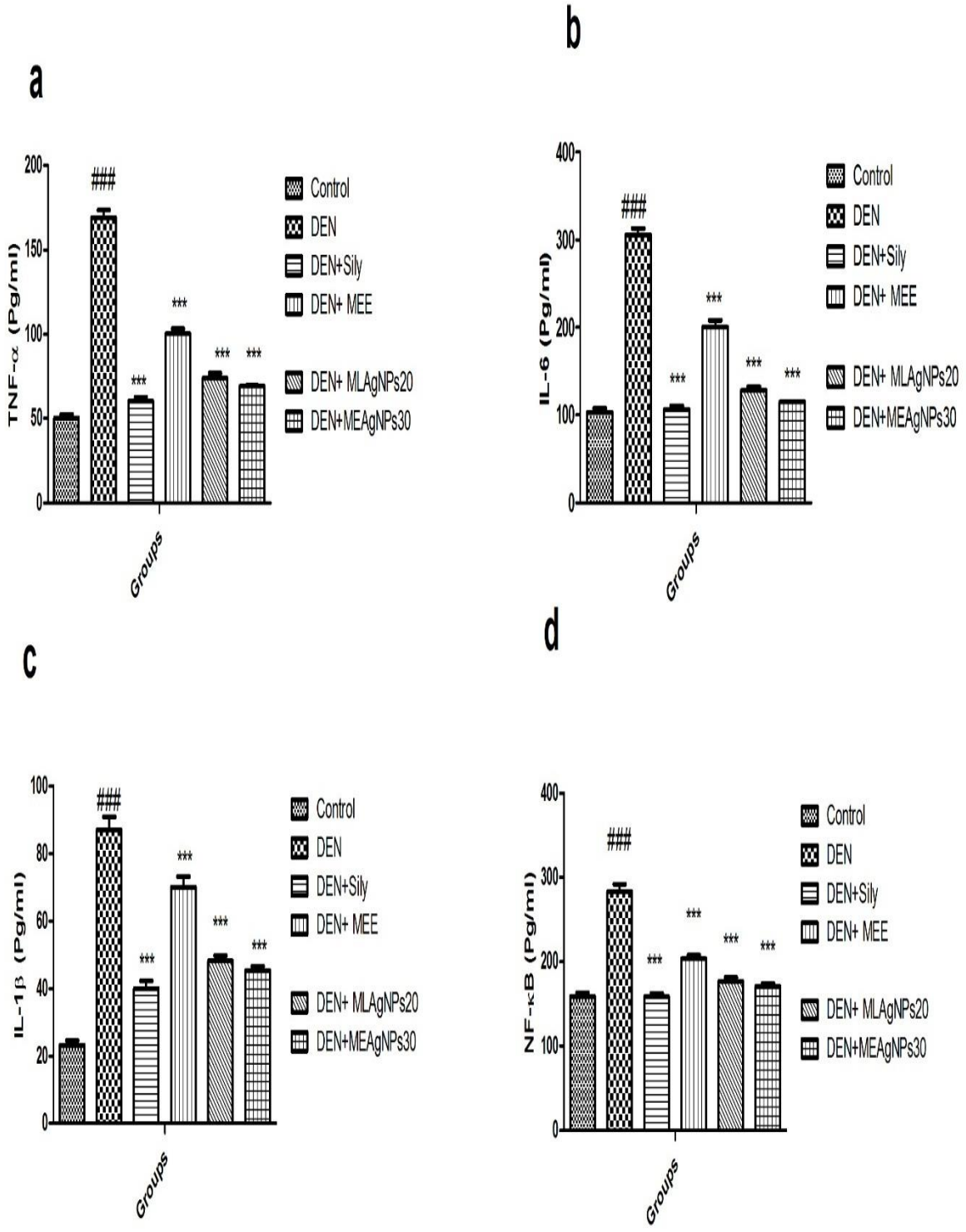


Figure 4.34 Effect of MLAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

4.15.6 Effect of MLAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats

A significant enhancement was observed in MDA content (a biomarker of LPO), during DEN induced hepatic cancer in wistar rats. Administration of MLAgNPs at different doses significantly ($p < 0.001$) down regulates the LPO level in DEN induced hepatic cancer wistar rats (Table 4.33 and figure 4.35). When LPO level was determined, DEN + Sily demonstrates the down direction of MDA level with respect to normal group.

Table 4.33 Effect of MLAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats

Treatment	**MDA**
Control	22.01±1.02
DEN	$42.18\pm0.15^{\#\#\#}$
DEN+ Sily	$20.11\pm0.07^{***}$
DEN+MLE	$31.78\pm1.08^{***}$
DEN+ MLAgNPs20	$22.89\pm1.02^{***}$
DEN+ MLAgNPs30	$20.22\pm1.67^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

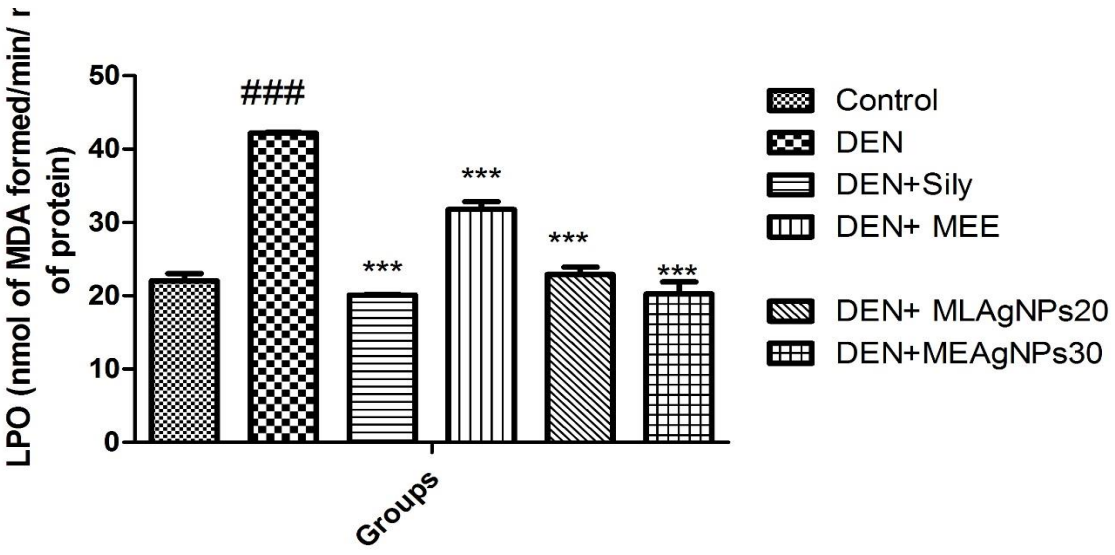


Figure 4.35 Effect of MLAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats

4.15.7 Effect of MLAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats

The levels of SOD, Catalase, GPx, GSH, G6PD and vitamin C were significantly decreased ($P < 0.001$) in the DEN induced toxic group with respect to control group rats (Table 4.34 and figure 4.36). After administration of MLAgNPs in a dose graded response, significantly ($p < 0.001$) increment in the levels of all the enzymatic and non-enzymatic antioxidants were noted. A better response was also seen in the enzymes after the administration of standard drug in DEN induced group.

4.15.8 Effect of MLAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats

Significant reduction were seen in the level of Na^+/K^+ ATPase and Ca^{2+}ATPase of the DEN induced liver cancer group rats when contrasted with normal control wistar rats. Administration of MLAgNPs in a dose graded response shows enhancement in Ca^{2+}ATPase levels. A practically comparative result of lessening was observed for the Na^+/K^+ ATPase

activity in the liver tissue of DEN induced group rats as shown in Table 4.35 and figure 4.37. Silymarin (standard drug) when introduced to DEN induced group showed significantly raised level of both ATPase and restored the content of membrane bound enzymes in rats.

4.15.9 Histology of liver tissue

The liver section of rat was stained with eosin and haematoxylin illustrated in figure 4.38. The liver of normal control rat showed well defined structure of liver in which mono-nucleated cells which have regular border and in contact of its surrounding cells. DEN induced liver cancer group revealed irregular cellular structure with divided nuclei which lost their spherical shapes, focal necrosis, hepatocellular adenoma and hyperplastic knob was present on it. When this group exposed or treated with silymarin it made a huge changes in the section of liver and only show dilation of blood sinusoids. DEN induced rat when treated with different doses level of MLAgNPs seemed to invert the auxiliary changes observed in liver, neoplastically transformed cells and showed certain level of improvement when compared to DEN induced rats group

Table 4.34. Effect of MLAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats

Treatment	Catalase	SOD	GPx	GSH	G6PD	Vitamin C
Control	93.99±3.36	7.38±0.18	11.56±0.56	50.29±0.37	9.89±0.22	4.28±0.06
DEN	50.28±2.36$^{###}$	2.63±0.09$^{###}$	4.9±0.28$^{###}$	15.38±0.37$^{###}$	2.56±0.14$^{###}$	40.67±0.75$^{###}$
DEN+ Sily	74.28±3.78***	7.56±0.15***	10.06±0.09***	45.38±0.57***	8.06±0.1***	22.11±0.11***
DEN+MLE	60.11±2.9^{ns}	4.78±0.12***	7.04±0.65***	31.66±0.78***	5.37±0.34***	28.2±1.28***
DEN+ MLAgNPs20	67.12±3.09**	6.03±0.27***	8.56±0.23***	37.2±0.79***	6.69±0.07***	21.81±1.03***
DEN+ MLAgNPs30	69.36±1***	6.24±0.15***	9.05±0.58***	40.12±0.02***	7.13±0.67***	20.98±0.36***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

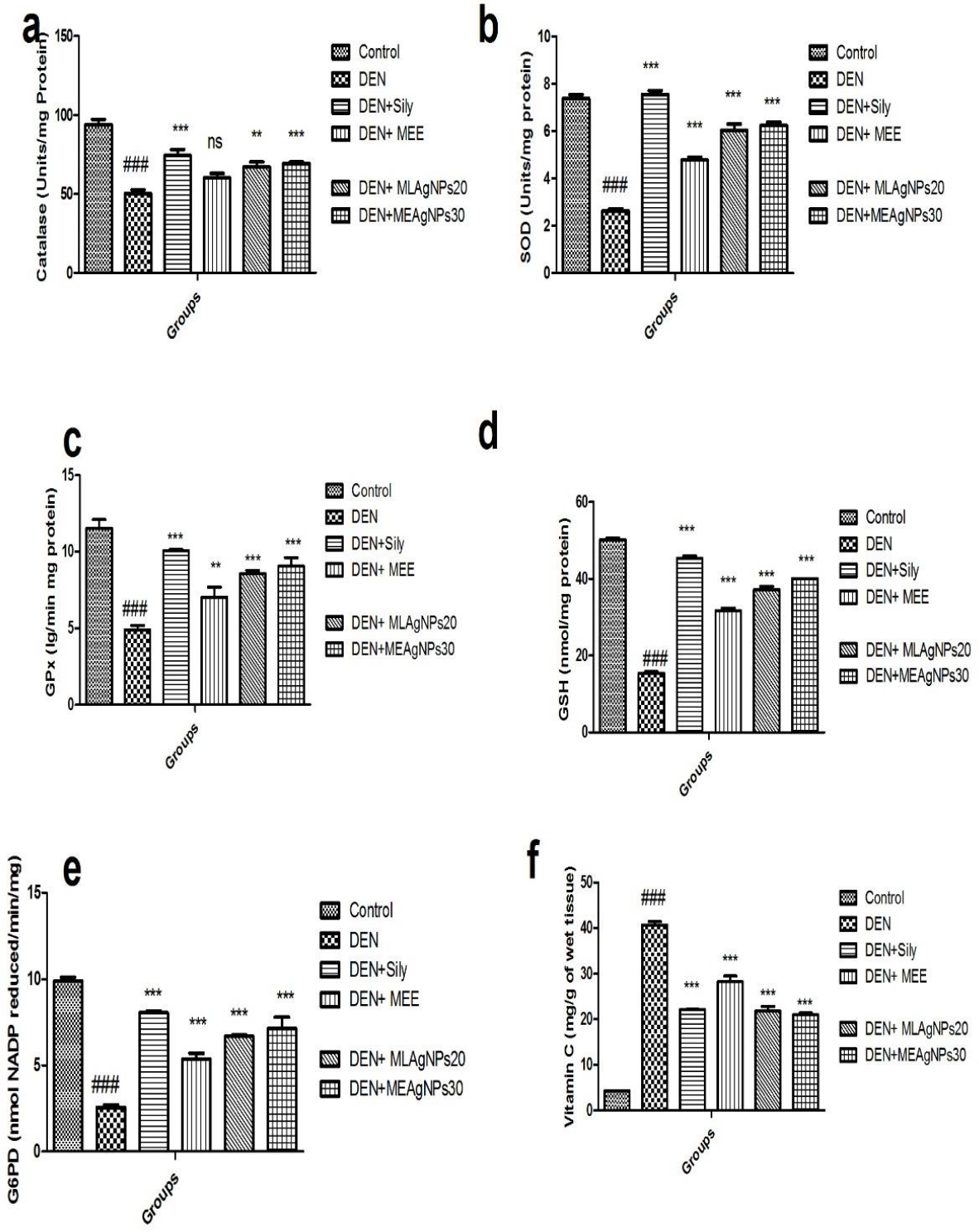


Figure 4.36 Effect of MLAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats

Table 4.35 Effect of MLAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats

Treatment	Na^+/K^+ ATPase	Ca^{2+}ATPase
Control	4.42±0.12	9.93±0.28
DEN	$2.57±0.18^{\#\#\#}$	$4.29±1.02^{\#\#\#}$
DEN+ Sily	$4.17±0.05^{***}$	$8.01±0.09^{***}$
DEN+MLE	$3.45±0.23^{*}$	$5.66±0.21^{ns}$
DEN+ MLAgNPs20	$3.97±0.03^{***}$	$7.23±0.59^{**}$
DEN+ MLAgNPs30	$4.02±0.2^{***}$	$7.76±0.28^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

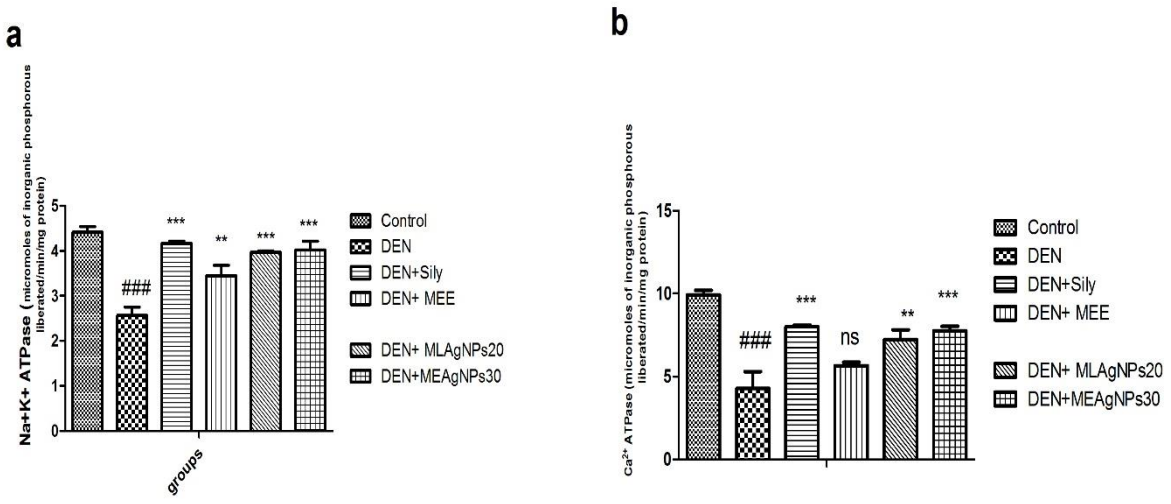


Figure 4.37 Effect of MLAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats

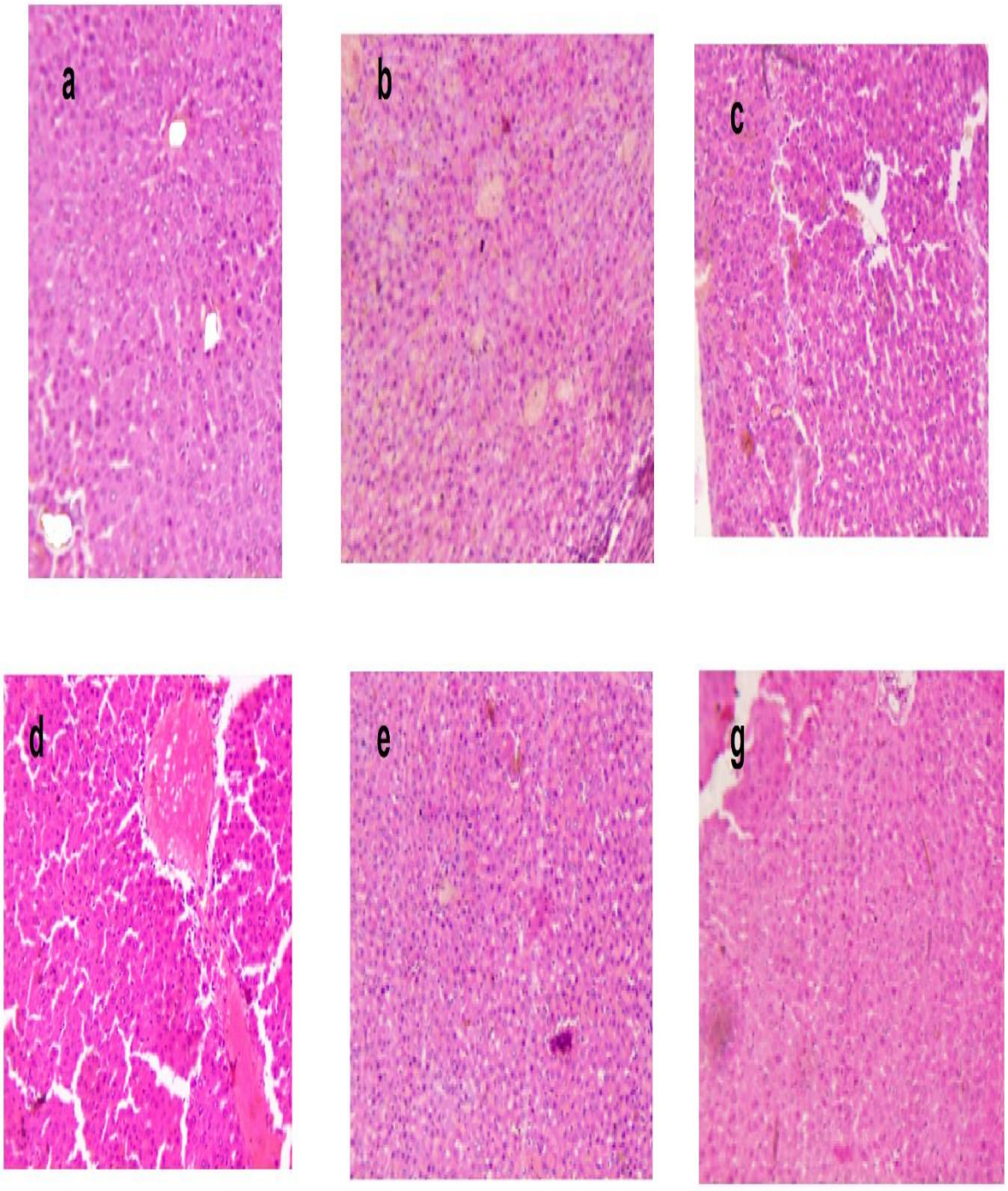


Figure 4.38 Photomicrograph of eosin-haematoxylin stained histological liver section area of (a) Normal control group, (b) DEN induced liver cancer group (c) DEN+ Silymarin (d) DEN+ MLE (e) DEN +MLAgNPs (20mg/kg bw) , and (f) DEN +MLAgNPs (30mg/kg bw)

4.16 Renal cancer

4.16.1 Effect of MLAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats

Table 4.36 depict about the number of rats with RCC, total number of tumour, number of animals with bilateral and unilateral tumour, percentage incidence of tumour. Normal control group rat shows w no detectable knob on the kidney structure till end of the study. Through visible observation, DEN induced group rats affirmed the arrangement of knob on kidney tissues. DEN induced group animals when treated with MLAgNPs, affirms the chemo-defensive impact of MLAgNPs and established by means of decrease in tumour frequency in a dose dependent way.

Table 4.36 Effect of MLAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats

Groups	No. of Rats with RCC	No. of rats with Unilateral tumors	No. of rats with bilateral tumors	Total no. of tumors	Incidence of tumors
Normal control	-	-	-	-	-
DEN	6	3	3	18	100
DEN+ MLE	5	3	2	10	83.33
DEN+ MLAgNPs20	3	2	1	9	50
DEN+ MLAgNPs30	2	1	1	6	33.33

4.16.2 Effect of MLAgNPs on serum marker enzymes of control and DEN induced RCC rats

Table 4.37 and figure 4.39 delineates the effect of MLAgNPs on BUN, Creatinine and uric acid on DEN induced group and treated groups. There was significant increase was

observed in all serum enzymes of DEN induced renal cancer when compared to normal control group. Pre-treatment with MLAgNPs significantly down regulated the effect of all these serum specific markers in at both the dose level.

Table 4.37 Effect of MLAgNPs on serum marker enzymes of control and DEN induced RCC rats

Treatment	Urea	Creatinine	Uric acid
Normal control	27.09±1.57	1.26±0.06	1.67±0.21
DEN	83.29±2.76$^{###}$	4.02±0.25$^{###}$	4.01±0.34$^{###}$
DEN+ MLE	52.89±1.67***	2.98±0.15**	2.67±0.17***
DEN+ MLAgNPs20	37.21±1.78***	2.23±0.19***	2.21±0.06***
DEN+ MLAgNPs30	34.23±1.56***	1.99±0.21***	1.78±0.08***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

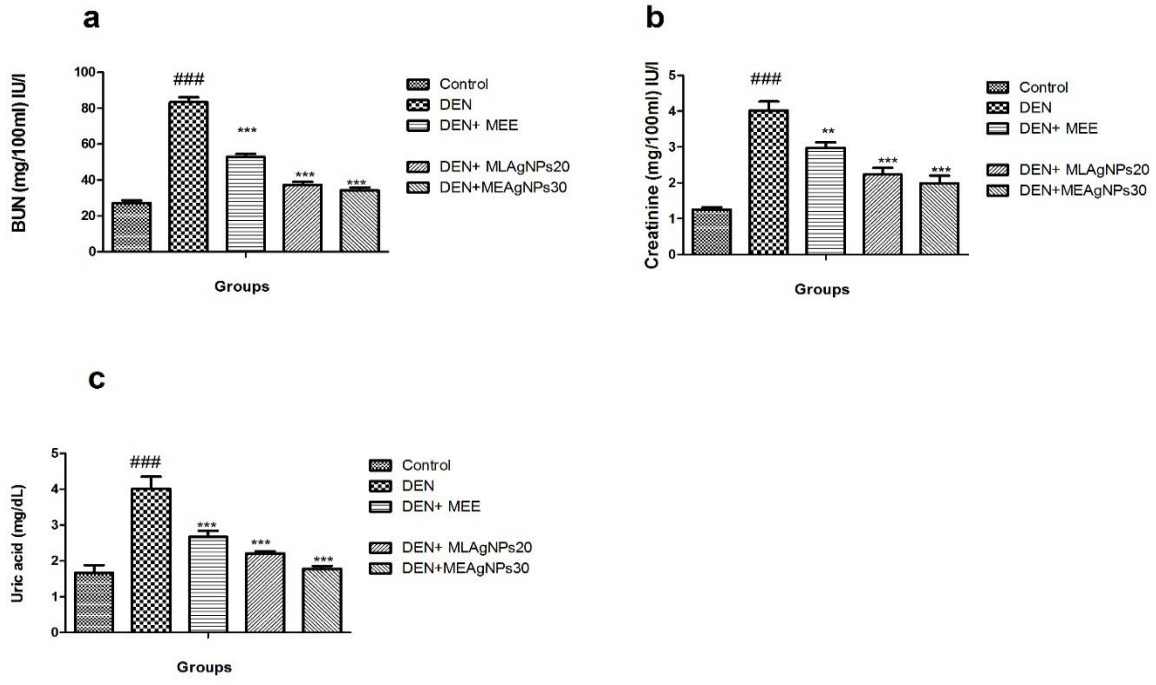


Figure 4.39 Effect of MLAgNPs on serum marker enzymes of control and DEN induced RCC rats

4.16.3 Effect of MLAgNPs on tumour marker enzyme in rats

Table 4.38 and figure 4.40 show the level of xanthine oxidase, γ-glutamyl transpeptidase and Lactate dehydrogenase were significantly restored by MLAgNPs. DEN induced groups rats, showed significant raised level of Xanthine oxidase, c-glutamyl transpeptidase and Lactate dehydrogenase. Marked reduction was recorded in all the level of enzymes after the treatment with MLAgNPs at the both drug doses. Not much significant changes were observed in DEN + MLE group.

Table 4.38 Effect of MLAgNPs on tumour marker enzyme

Treatment	Xanthine oxidase	LDH	γ-GTP
Normal control	0.68±0.06	220.21±5.67	372.42±7.24
DEN + Fe-NTA	2.01±0.21$^{###}$	450.28±9.78$^{###}$	624.89±11.21$^{###}$
DEN+ MLE	1.01±0.09***	354.6±8.06***	480.36±9.56***
DEN+ MLAgNPs20	0.8±0.05***	321.57±6.49***	405.23±9.77***
DEN+ MLAgNPs30	0.76±0.09***	299.97±7.86***	396.9±8.01***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

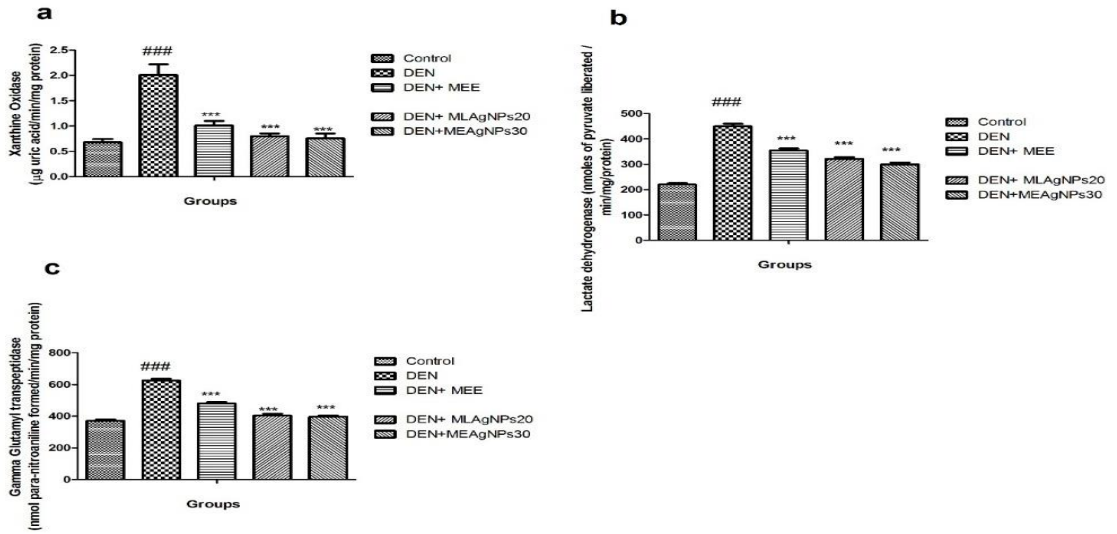


Figure 4.40 Effect of MLAgNPs on tumour marker enzyme

4.16.4 Effect of MLAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats

In DEN induced group, lipid peroxidation was estimated by the formation of MDA which harm the oxidative parameter. A notable increment was found in the level of oxidative parameter of DEN induced group as compared to normal control. It was observed that treatment with MLAgNPs at the dose levels of (20mg/kg and 30mg/kg BW) showed reclamation of membrane integrity when contrast with toxicant group (table 4.39 and figure 4.41).

Table 4.39 Effect of MLAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats

Treatment	MDA
Normal control	2.67±0.08
DEN + Fe-NTA	$9.63\pm0.56^{###}$
DEN+ MLE	$7.05\pm0.49^{***}$
DEN+ MLAgNPs20	$6.07\pm0.39^{***}$
DEN+ MLAgNPs30	$5.29\pm0.09^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

4.16.5 Effect of MLAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

Wistar rat when treated with DEN, it downregulated the activity of kidney tissue antioxidant parameter and endogenous antioxidant enzymes when contrast to normal control wistar rat. This exhaustion of renal enzymatic antioxidants enzymes prompts the interruption of other oxidative pathway enzymes. Treatment with MLAgNPs at both the dose levels re-established the antioxidant profile. Minor significance changes were found in the kidney tissue antioxidant parameters of DEN + MLAgNPs (30 mg/kg BW) and normal control.

There was significant elevation in the concentration of H_2O_2 was found in the DEN induced group of wistar rat. At all doses level of MLAgNPs, it diminished the concentration of H_2O_2 when compared to normal control rats (table 4.40 and figure 4.41).

4.16.6 Effect of MLAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

There was significant enhancement of proinflammatory cytokine and inflammatory mediators in DEN induced rat when compare with the normal control (table 4.41 and figure 4.42). MLAgNPs treatment at a higher dose was found to be effective and restored inflammatory cytokines and mediator levels to normal.

4.16.7 Effect of MLAgNPs on tumour promotion markers in kidney of control and DEN-induced RCC in rats

Wistar rats when subjected to DEN treatment show significant enhancement in thymidine activity as depicted in table 4.42 and figure 4.43. Treatment with biofabricated silver nanoparticles of *M.longifolia* leaf aqueous extract caused mark reduction in the raise level of ODC and thymidine in a dose dependent manner till the end of experiment. No significant changes were observed in the he was found in ODC and thymidine of DEN + MLAgNPs (30 mg/kg BW) and normal control.

Table 4.40 Effect of MLAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

Treatment	**SOD**	**GSH**	**Catalase**	**GPx**	**GST**	**GR**	**H_2O_2**
Normal control	100.02±3.67	7.96±0.57	4.62±0.22	255.25±7.23	221.1±8.01	301.37±9.02	32.12±3.01
DEN + Fe-NTA	52.46±1.1$^{###}$	3.45±0.29$^{###}$	1.54±0.04$^{###}$	132.12±4.12$^{###}$	119.37±3.78$^{###}$	175.38±5.9$^{###}$	96.23±5.95$^{###}$
DEN+ MLE	64.29±3.09*	4.09±0.78^{ns}	2.71±0.02***	176.98±3.29***	156.2±4.67***	197.86±4.03^{ns}	74.32±5.89*
DEN+ MLAgNPs20	85.59±3.2***	5.48±0.32^{ns}	3.48±0.20***	210.38±4.2***	187.2±4.57***	239.09±5.28***	58.66±4.37***
DEN+ MLAgNPs30	89.87±2.45***	6.41±0.67***	4.09±0.11***	226.9±5.67***	195.9±5.98***	267.86±6.03***	47.12±4.23***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

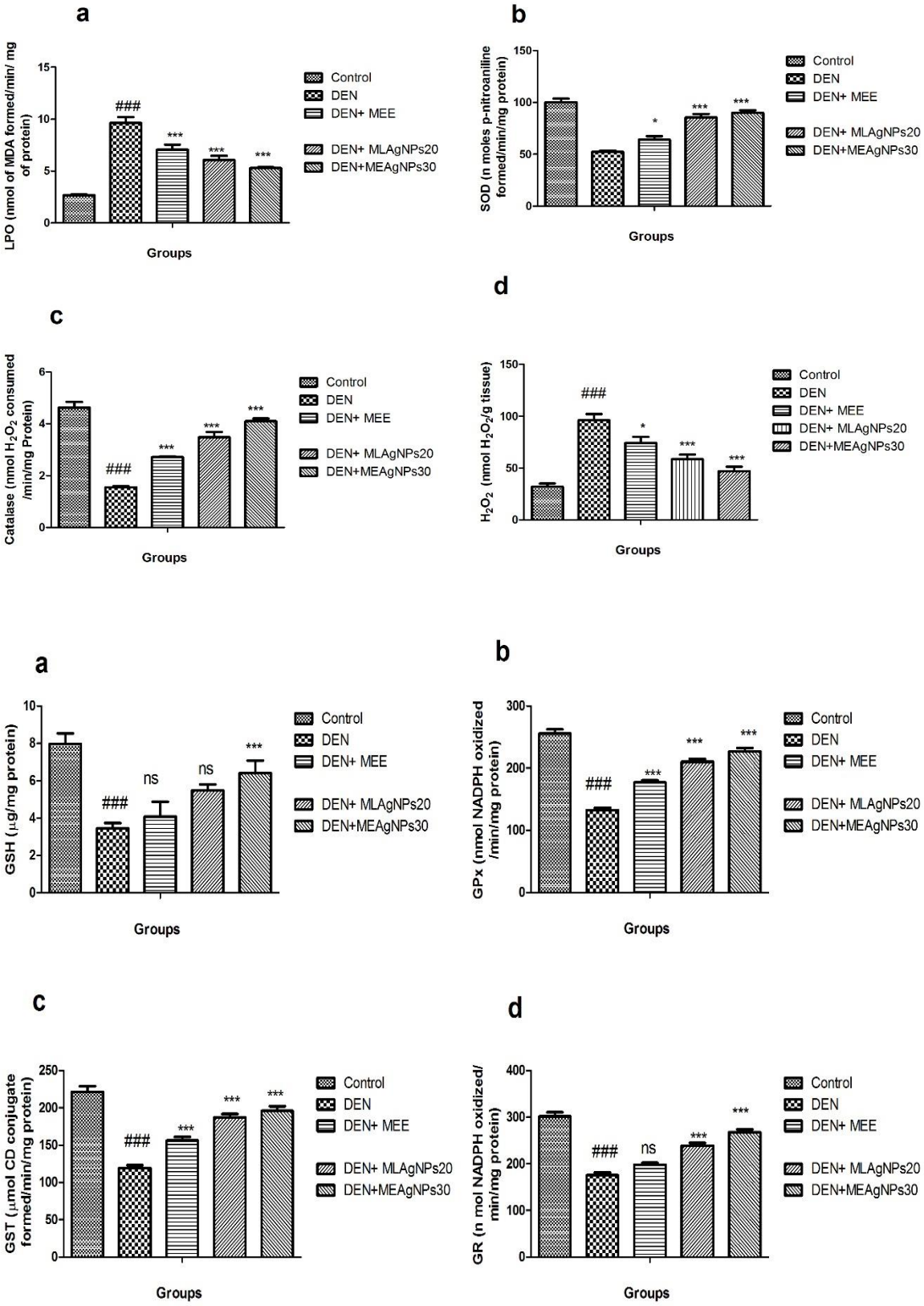


Figure 4.41 Effect of MLAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

Table 4.41 Effect of MLAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Treatment	TNF-α	IL-6	IL-1β	NF-κB
Normal control	257.78±7.98	813.9±012.78	167.32±5.09	500.23±8.39
DEN + Fe-NTA	837.98±13.07$^{###}$	2021.34±22.34$^{###}$	801.35±9.23$^{###}$	1500.20±27.39$^{###}$
DEN+ MLE	503.89±9.21***	1489.30±21.89***	480.78±7.34***	1208.37±21.65***
DEN+ MLAgNPs20	368.34±9.68***	1289.22±17.34***	339.20±7.01***	876.99±16.78***
DEN+ MLAgNPs30	285.78±8.20***	1003.45±16.21***	234.70±5.98***	732.20±13.22***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

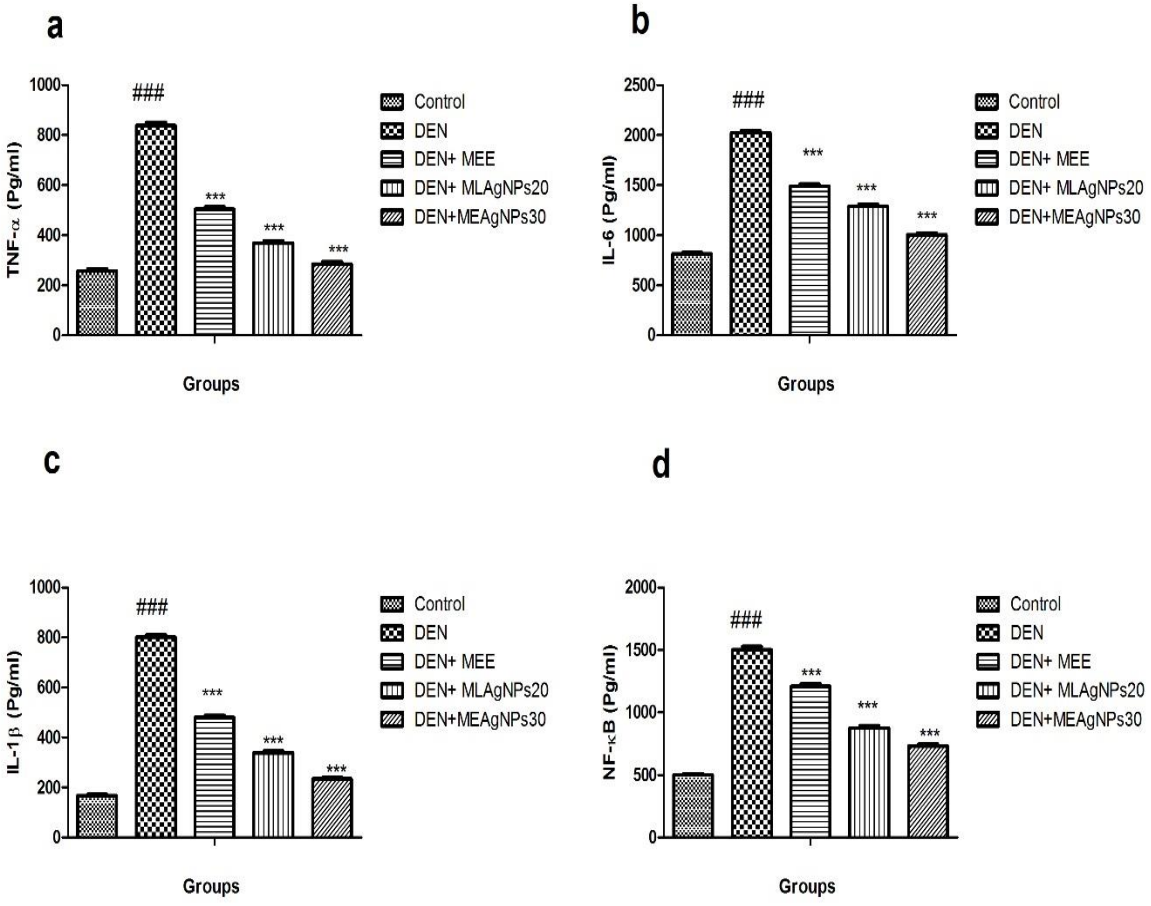


Figure 4.42 Effect of MLAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Table 4.42 Effect of MLAgNPs on tumour promotion markers in kidney of control and DEN-induced RCC in rats

Treatment	ODC	Thymidine incorporation
Normal control	856.20±23.12	1839.12±33.29
DEN	2509.56±32.24$^{###}$	3106.29±41.04$^{###}$
DEN+ MLE	1703.00±26.20***	2523.56±36.29***
DEN+ MLAgNPs20	1398.39±22.34***	2109.56±30.12***
DEN+ MLAgNPs30	1109.07±21.78***	2007.65±29.34***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

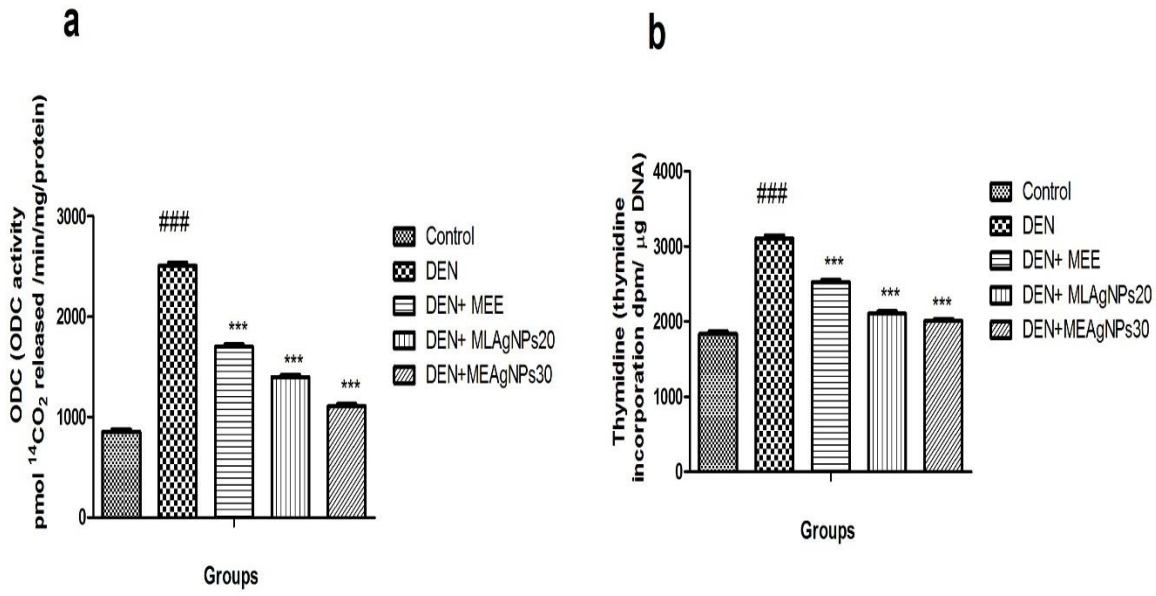


Figure 4.43 Effect of MLAgNPs on tumour promotion markers in kidney of control and DEN-induced RCC in rats

4.16.8 Histopathological studies of renal tissue

Figure 4.44 displays the normal structural design of renal tissue of normal control group rats such as Usual glomerulus which have cluster of blood capillaries), urinary space,

complete bowman's capsules, squamous epithelium, medullary rays, distal tubules, proximal tubules and collecting ducts in normal control group rats. DEN induced tumorigenesis rat shows hydropic weakening, distortion of cytoarchitecture, proliferation in space of Bowman's capsules, less corpuscles and hyperplastic glomeruli. DEN induced animals treated with MLAgNPs (20mg/kg) showed the normal tubular structure with proper glomeruli. MLAgNPs (30mg/kg) treated rats showed the regaining of space of the Bowman capsules, fewer inflammatory blood vessels, normal glomerular structure, normal structure of proximal convoluted tubules and distal convoluted tubule.

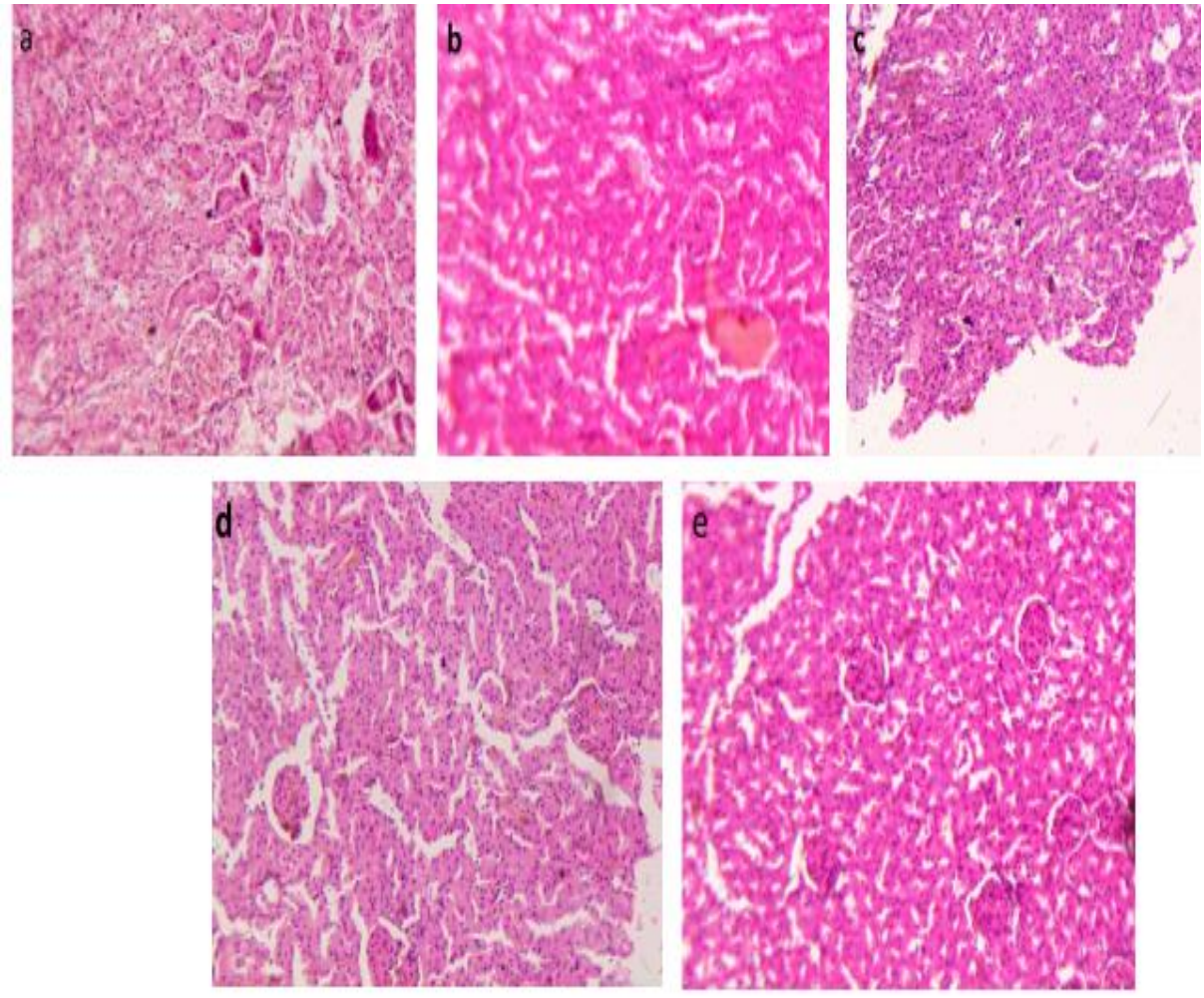


Figure 4.44 Photomicrograph of eosin-haematoxylin stained histological liver section area for (a) Normal Control group, (b) DEN induced liver cancer group (c) DEN+ MEE (d) DEN +MLAgNPs (20mg/kg bw) (e) DEN +MLAgNPs (30mg/kg bw)

Results of *Carissa carandas*

4.17 Pharmacognostical character of leaf of *Carissa carandas*

4.17.1 Macroscopical characters of leaf of *Carissa carandas*

The colour of the upper surface of leaves are dark green and lower surface is light green in colour. It has characteristics odour with 5.1-6.5 cm long and 1.9-3 cm wide. It has oval shaped with reticulate venation. The leaves are simple, opposite, short petiole, obtuse apex and rounded base. The base is asymmetric with leathery glabrous texture and prominent veins (Figure 1.12).

4.17.2 Microscopic characters of leaf of *Carissa carandas*

The lamina of epidermis is covered by the thin layer of cuticle as shown in figure 4.45. Below the upper epidermis, a single layer of parenchymatous hypodermis is present in continuation with bi-layer radially palisade parenchyma. Upper epidermis followed by single layer of parenchymatous hypodermis in mid rib and below the hypodermis collenchyma is present. In the ground tissue, chlorenchymatous cells are present under the collenchyma. Circular cavities are present and distributed in the ground tissue which specify fragmented parenchymatous cells. Stele comprises of bi-collateral vascular bundles enclosed by calcium oxalate cluster sheath. Glandular trichomes are less in number.

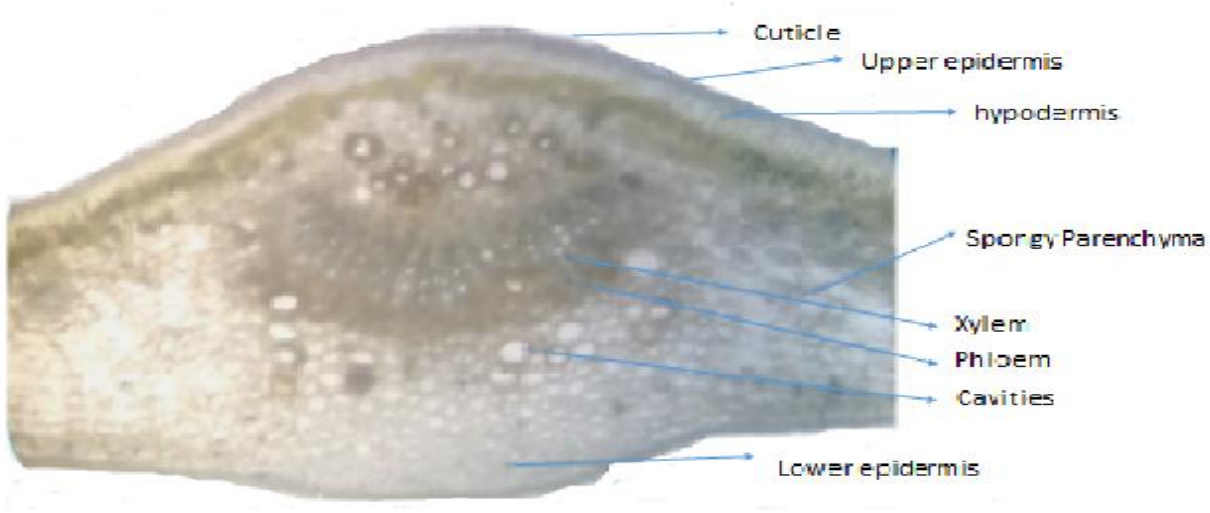


Figure 4.45 Transverse section of leaf of *Carissa carandas*

The results of leaf constant and physicochemical parameter is displayed in table no 4.43 and 4.44.

Table 4.43 Determination of leaf constant of *Carissa carandas*

Leaf Constant	**Range**	**Average**
Stomatal Number		
Upper surface	0	0
Lower surface	15-17	16
Stomatal Index		
Upper Surface	0	0
Lower Surface	12-15	13.5
Vein islet number	17-22	19.5
Vein termination number	45-50	47.5

Table 4.44 Physico-chemical parameters of leaves of *Carissa carandas*

S. No.	**Parameter**	**Observation (% w/w)**
1	Ash value	
A	Total ash	8.4
B	Acid soluble ash	
c	Acid insoluble ash	3.5
2.	Moisture content	9.89
3	Foreign matter	0.9

4.18 Extraction of Aqueous extract of *Carissa carandas*

The leaves of P.emblica were subjected to double distilled water and the percentage yield was obtained as 9.01% (w/w) which is depicted in table 4.45

Table 4.45 Percentage yield of extract of leaves of Carissa *carandas*

Botanical name	**family**	**Part used**	**Solvent used for extraction**	**% yield (Extract)**
Carissa *carandas*		leaves	water	9.03

4.19 Preliminary phytochemical studies

Phytochemical studies were performed to determine the presence of various chemical compounds present in the plant extract. The different chemical tests were done on the aqueous extract of selected medicinal plants. *Carissa carandas* revealed positive test for various chemicals constituents such as and presented in table 4.46

Table 4.46 Qualitative chemical test on the aqueous extract of *Carissa carandas*

S. No.	Chemical Test	Aqueous extract
1.	**Alkaloids**	
	Mayers Reagent	+
	Dragendroff Reagent	-
	Hagner reagent	+
	Wagner reagent	-
2.	**Glycosides**	
	Legal test	+
	Keller Killani Test	-
	Borntragers Test	+
3.	**Flavonoids**	
	Ammonia Test	+
	Shinoda/ Paw test	+
4.	**Sterols**	
	Liebermann-Buchard test	-
	Salwoski Test	-

5.	**Phenolic compounds and tannins** Ferric chlorides test Lead acetate test	 + +
6.	**Proteins** Millions test Biuret test	 - -
7.	**Coumarins**	-

4.20 Synthesis of Silver nanoparticles of Carissa *carandas*

Briefly, 10 ml of plant extract (Carissa *carandas*) was mixed with 90 ml of $AgNO_3$ solution under vigorous stirring in Erlenmeyer flask and incubated overnight at RT for the reduction of silver ions. The solution turns from brown to dark-brown colour which is represented in figure 4.46. The solution was centrifuged at 8000 rpm for 15 min to remove the unwanted organic matter, washed twice with deionized water and dried in an oven.

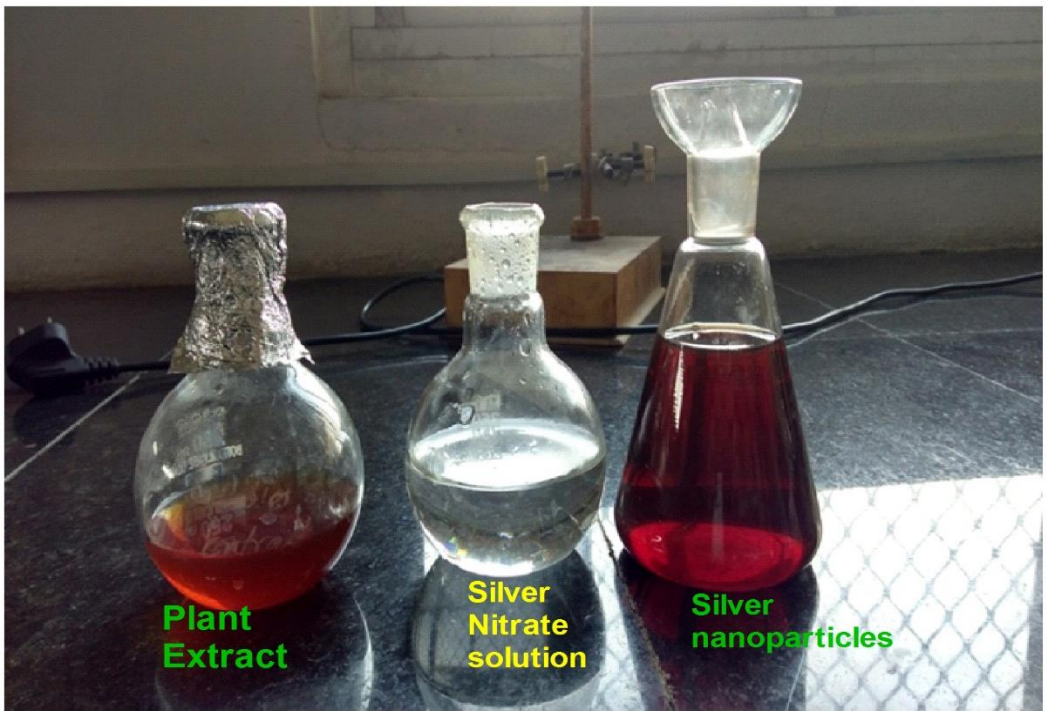


Figure 4.46 Biosynthesis of Silver nanoparticles of Carissa *carandas* aqueous extract

4.21 Characterization of silver nano-drugs of Carissa *carandas*

4.21.1 Structural characterization of CCAgNPs by Absorbance spectroscopy

Absorbance spectroscopy is a straightforward, yet powerful method to determine the production of AgNPs. The UV-visible spectrum of the synthesized AgNPs was delineated in figure 4.47. The absorption spectrum depicts the importance of silver nitrate and the chemical constituents found in the leaves *C. carandas* responsible for the development of silver nanoparticles. After the addition of silver nitrate, continuous stirring and upon heating, the colourless solution was changed to brown color, flagging the arrangement of AgNPs. The silver nanoparticle shows the absorption spectra at 420 nm.

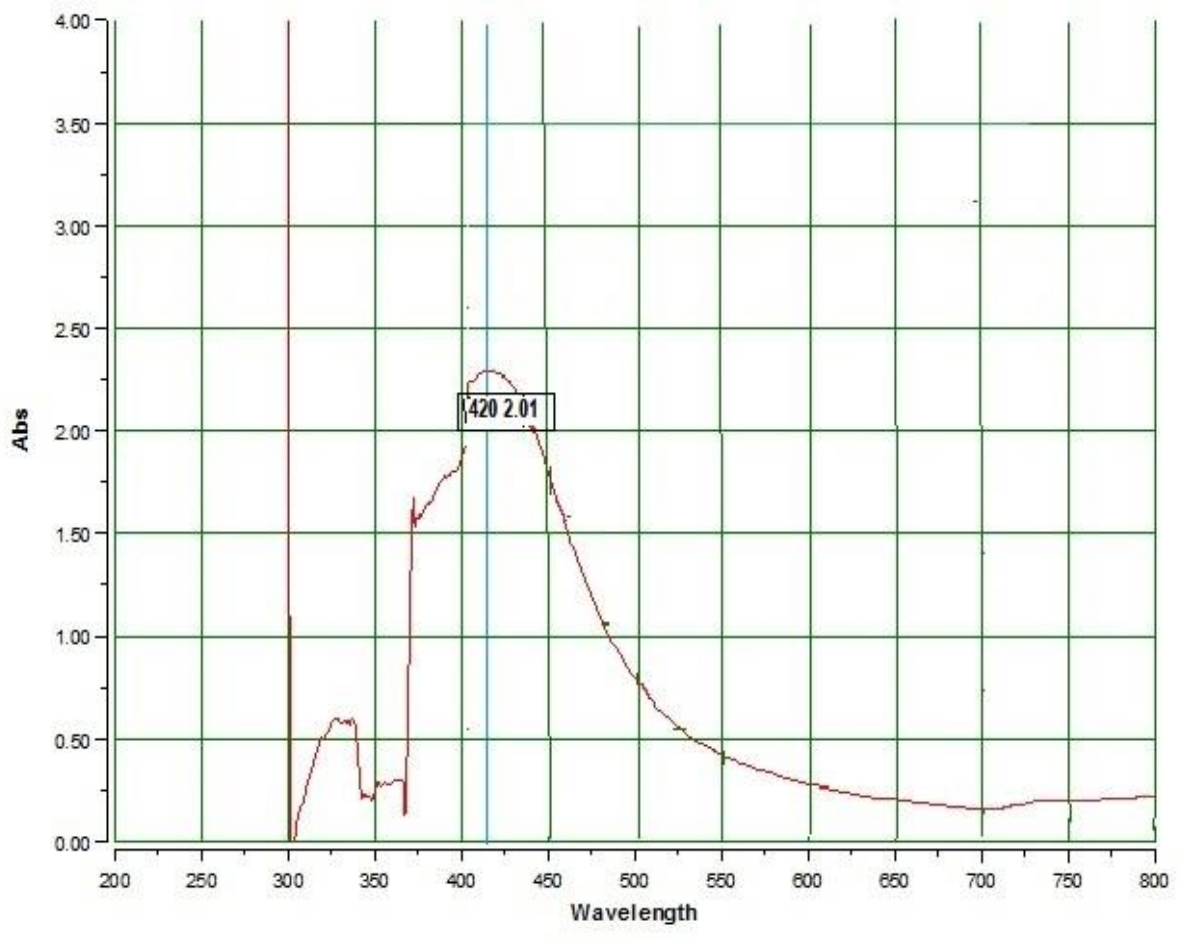
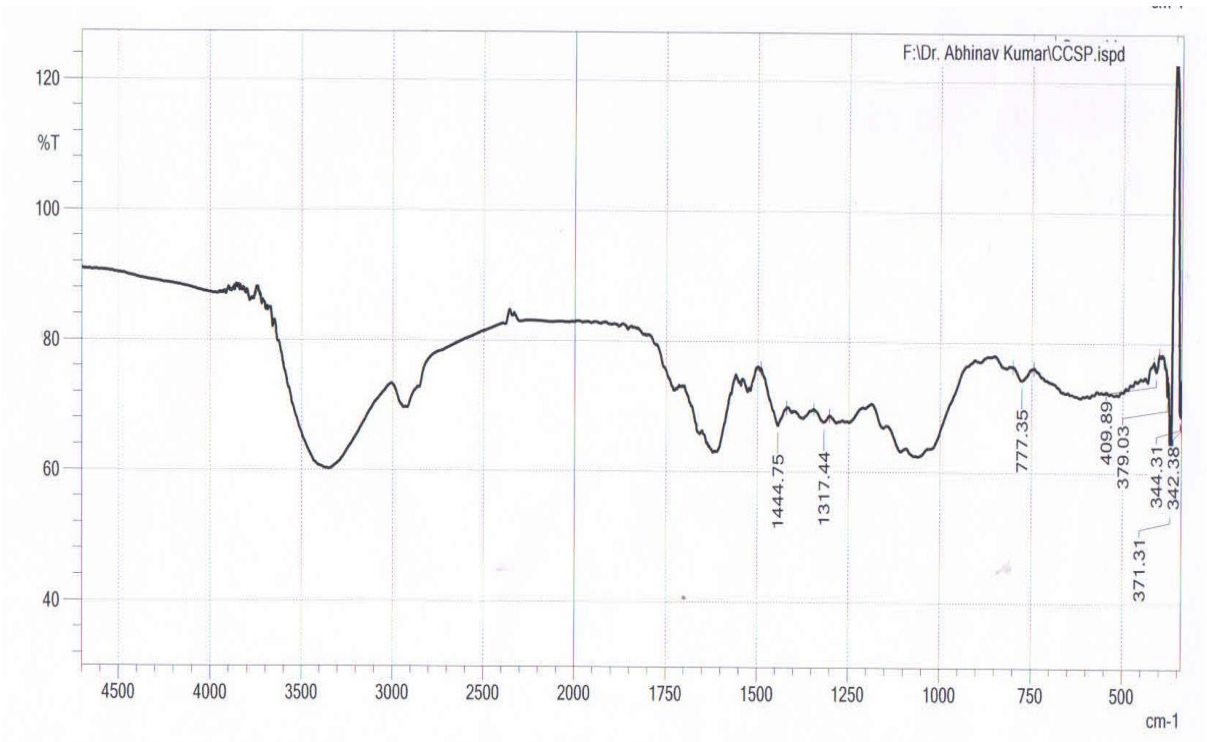


Figure 4.47 UV-Vis spectrum of CCAgNPs

4.21.2 FTIR spectroscopy of CCAgNPs

FTIR analysis of CCAgNPs were done to detect the possible association between silver and the bioactive moieties involved in the formation and the stability of silver nanoparticles. The spectra reveals the intense peak at 3426, 2905, 1610, 1444, 1381, 776, 343cm^{-1} in silver nanoparticles and 3370, 2960, 1620, 1444, 1317, 777, 409, 342 cm^{-1} in plant extract. Figure 4.48 shows the FTIR spectrum of CCE and CCAgNPs. The vibrations band found at 3426 cm^{-1} in CC-AgNPs spectra assigned the OH stretching of the phenolic group. In same case, the vibration bands at 2905 cm^{-1} assigned to the stretching vibration of aliphatic C-H group, 1610 cm^{-1} attributed to the bending vibrations modes of aromatic ring C=C, 1444 cm^{-1} due to aliphatic C-H groups, 1381 cm^{-1} due to aromatic stretching of C N (The peptide bonds of proteins), 776 cm^{-1} denoted to bending of the aromatic carbon-hydrogen bond respectively, observed in the past reports.



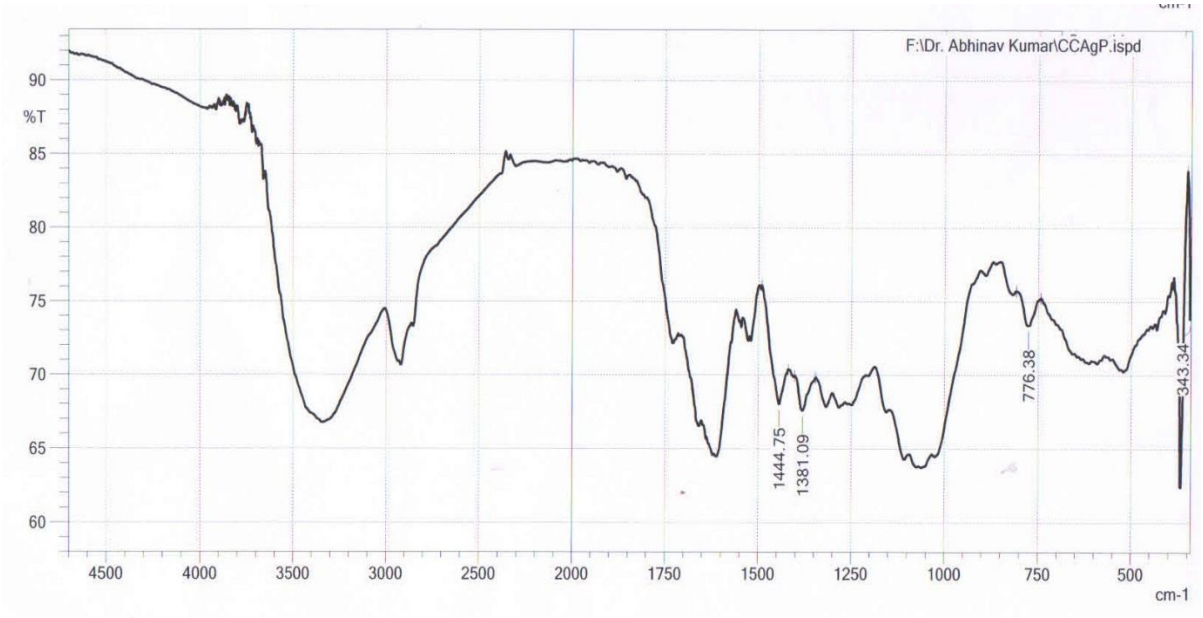


Figure 4.48 FTIR spectra a) CCE b) CCAgNPs

4.21.3 XRD analysis of CCAgNPs

XRD analysis was performed to determine the geometric structure and gapping between atoms of AgNPs. Figure 4.49 delineates the XRD pattern of the incorporated AgNPs by the leaves of CC. Results suggest the crystalline planes that indicate peaks of silver nanoparticles. It shows main characteristic peaks for silver which is found at 38.12°, 44.18°, 64.53°, 77.48°, 81.07°.and might be corresponding with (111), (200), (220) and (311) alluded from the JCPDS card no. 040783.

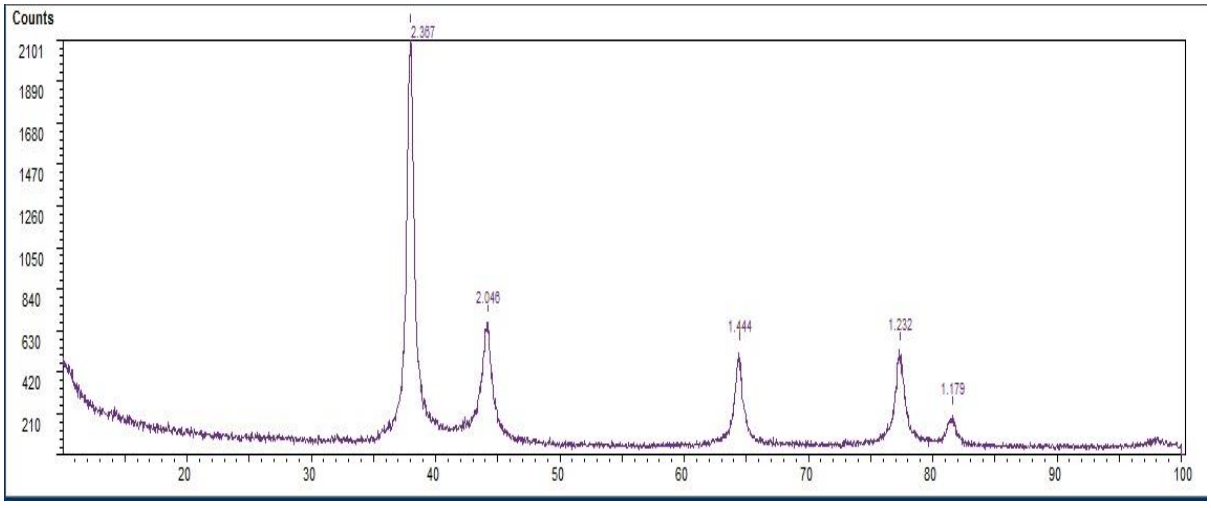


Figure 4.49 XRD analysis of CCAgNPs

Table 4.47 XRD analysis of CCAgNPs

Pos. [°2θ]	d-spacing [Å]	Area [cts*°2θ]
28.879	3.0892	74.0
37.876	2.3734	194.0
41.324	2.1830	134.0
48.809	1.8844	59.0
57.722	1.5958	46.0
64.366	1.4462	518.0
77.568	1.2297	420.0
81.353	1.1818	163.00
86.819	1.1209	47.0

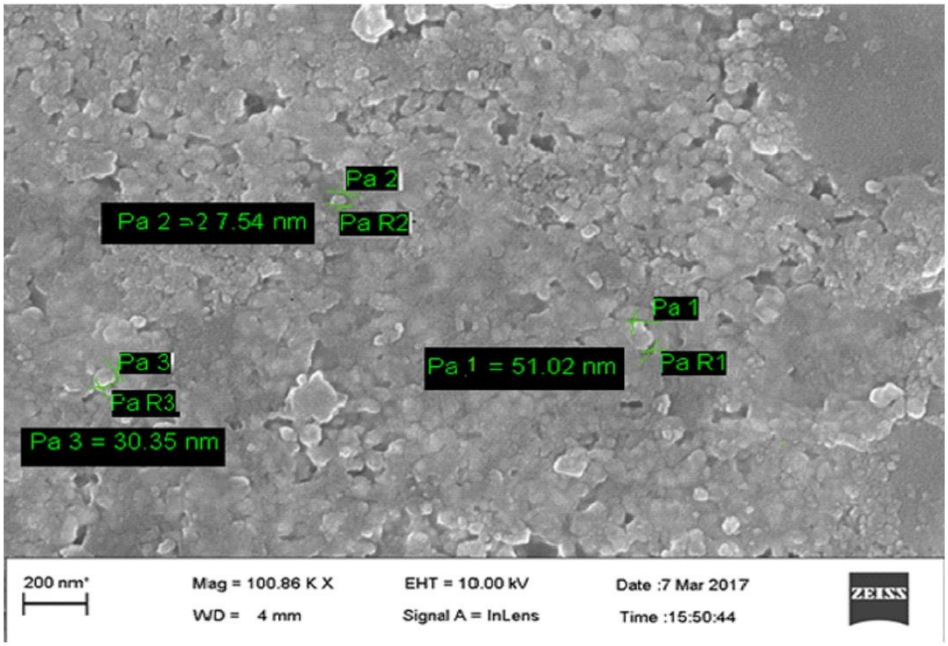
4.21.4 FESEM of CCAgNPs

FESEM provide images of samples on the submicron level and provide higher resolution pictures when compared to TEM. It reveals that observed images were distinct and spherical in shape. The size of silver nanoparticle ranges from 28-60 nm (Figure 4.50).

4.21.5 EDX analysis of CCAgNPs

The qualitative elemental detection of silver nanoparticles was confirmed by using EDX which was coupled with FESEM. It envisaged higher amount of silver content at 3 keV which confirms the synthesis of silver nanoparticles (Figure 4.50). Perception of a few peaks in the region of the silver shows various valence conditions of Ag in the silver nanoparticles. Outstandingly, peak represent to other elements are normally distinguished in the specimens gathered on a carbon-coated copper grid or might be the presence of bioactive in the leaves extract which was responsible for the formation of AGNPs.

a



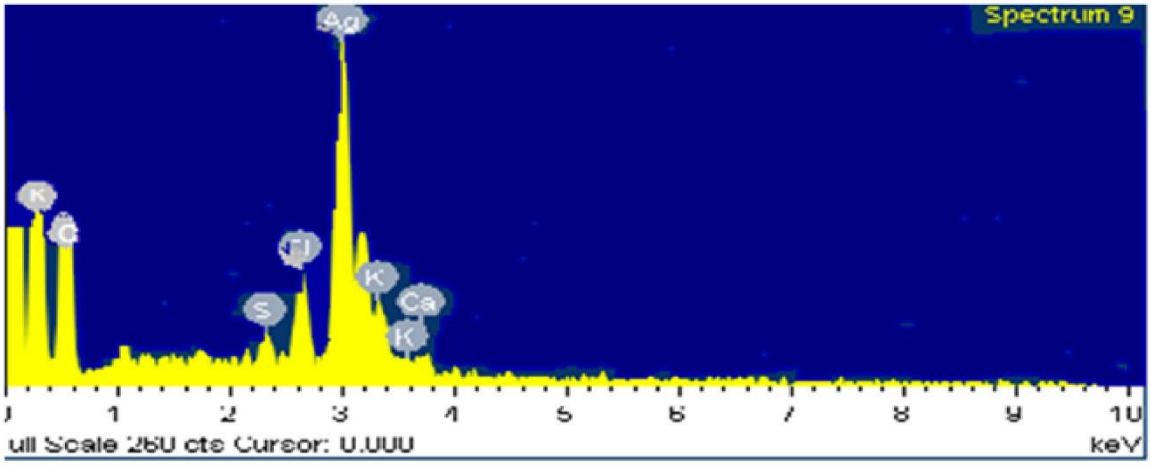


Figure 4.50 FESEM with EDX analysis of CCAgNPs

4.21.6 TEM analysis of CCAgNPs

TEM examination provides data about the size and development of silver nanoparticles. This technique is the initial step for deciding the morphology and exact size of synthesized silver nanoparticles. The TEM picture of silver nanoparticle demonstrated that morphology of synthesizing silver nanoparticles was dominatingly spherical in shape and furthermore

presence of phytocompounds which acts as a capping agent on the surface of the silver nanoparticles which adds to its higher stability [43]. The TEM pictures of the AgNPs appear in (Figure 4.51) where all the NPs were observed to be a circular shape.

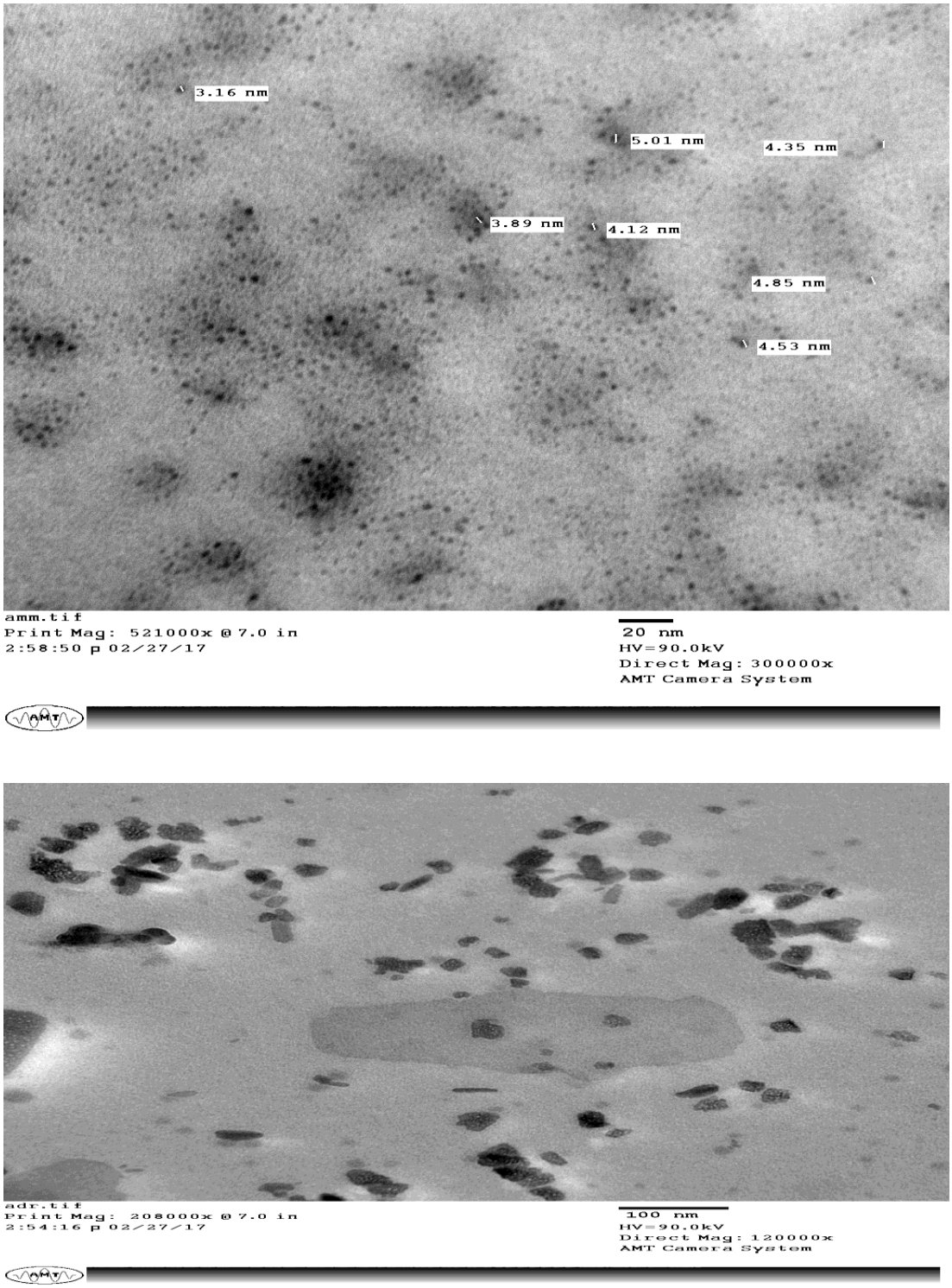


Figure 4.51 TEM analysis of CCAgNPs

4.22 *In vitro* cytotoxic activity of CCAgNPs against HuH-7 Cell line

The anticancer impact of silver nanoparticles was studied on human cell lines, HUH-7 by utilizing MTT test and reactions are exhibited as the percent cell inhibition after treating with different dilution of CCAgNPs from 10-50 µg/ml for 24 hours. It is obvious from figure 4.52 and table 4.48 that elevated levels of % cell inhibition of HUH-7 cell lines were seen, on increasing the concentration of formulation. The IC_{50} cell inhibition of CCAgNPs against HuH-7 cell lines was found at 43.87µg/ml. The debilitated survival cell lines demonstrate a potential cytotoxic impact of CCAgNPs.

Table 4.48

Log concentration	% Growth inhibition
1.000	12.37
1.30103	34.93
1.39794	67.29
1.477121	76.47
1.69897	82.34

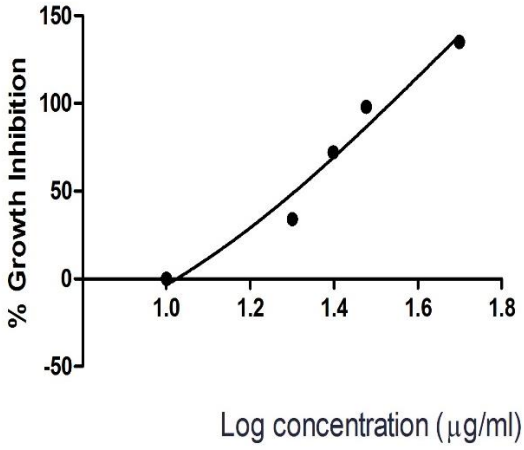


Figure 4.52 *In vitro* cytotoxic activity of CCAgNPs against HuH-7 Cell line

4.23 *In vivo* hepatic cancer Studies of CCAgNPs on rats

4.23.1 Effect of green Synthesized CCAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats

The body weight of DEN group rats was significantly (P>0.001) decreased when compared to normal control rats as depicted in figure 4.49. When silver nanoparticles were orally administered to DEN groups rats, the body weight was significantly enhanced (P>0.001) at both the dose levels. It was seen that, the liver weight of DEN induced hepatocarcinogenesis animals was significantly higher as compared to normal groups and silver nanoparticles treated groups.

4.23.2 Effect of CCAgNPs on the development of liver nodules in control and DEN-induced HCC rats

The total number of rats, liver knobs, knob incidences, and an average number of knobs/knob bearing liver was exemplified in table 4.50. DEN control rats showed the presence of hepatic knobs, while there were no noticeable knobs observed in the normal control group rats. DEN rats when treated with CCAgNPs declined the hepatic knobs when contrasted with DEN induced groups

4.23.3 Effect of CCAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

It was evident from the figure 4.53 and table 4.51 that DEN control group showed elevated level of all hepatic parameters AST (201.36±3.85), ALP (203.58±2.07), ALT (221.67±3.67), and AFP (245.20±2.09) with respect to normal control group. DEN induced hepatic cancer group when treated with CCAgNPs significantly declined the level of all hepatic serum marker enzymes in a dosage dependent way**.**

Table 4.49: Effect of green Synthesized CCAgNPs on body weight, liver weight and relative liver weight in control and DEN-induced HCC rats

Treatment	**Initial body weight**	**Final Body weight (g)**	**Liver weight (g)**	**Relative liver weight**
Control	168.28±0.10	301.54±2.64	10.06± 0.12	3.33±0.32
DEN	182.35±0.87	298.29±2.12	22.73±0.78	7.62±0.21
DEN+ Sily	172.78±0.85	327.65±1.90***	12.65±0.56	3.86±0.11
DEN+CCE	165.30±0.76	293.23±2.05***	20.08±0.89	6.84±0.07
DEN+ CCAgNPs20	162.45±0.65	310.44±1.82***	18.35±0.94	5.91±0.23
DEN+ CCAgNPs30	169.96±0.39	299.67±2.75***	16.24±0.35	5.42±0.37

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

Table 4.50 effect of CCAgNPs on the development of macroscopic hepatic nodules in different groups of rats.

Treatment	Total no. of nodules	Tumour incidence (%)	Average number of nodules
Control	0	0	0
DEN	110	100	58.78 ± 2.90
DEN+ Sily	17	25.38	6.28 ± 1.28
DEN+CCE	86	79.98	42.57 ± 2.68
DEN+ CCAgNPs20	37	39.43	11.23 ± 0.97
DEN+ CCAgNPs30	25	34.67	$9.98 \pm 1.16^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

Table 4.51. Effect of CCAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

Treatment	ALT	AST	ALP	AFP
Control	54.37±3.28	61.06±2.45	50.21±2.89	142.65±2.68
DEN	221.67±3.67$^{###}$	201.36±3.85$^{###}$	203.58±2.07$^{###}$	245.20±2.09$^{###}$
DEN+Sily	41.52±3.67***	86.90±4.62***	63.78±1.87***	169.89±4.37***
DEN+CCE	86.28±3.09***	87.90±3.07***	136.60±1.09***	201.38±3.07***
DEN + CCAgNPs20	132.56±2.67***	138.78±3.90***	86.00±1.57***	175.45±2.28***
DEN + CCAgNPs30	102.23±1.89***	98.08±2.67***	74.56±3.99***	173.78±3.98***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

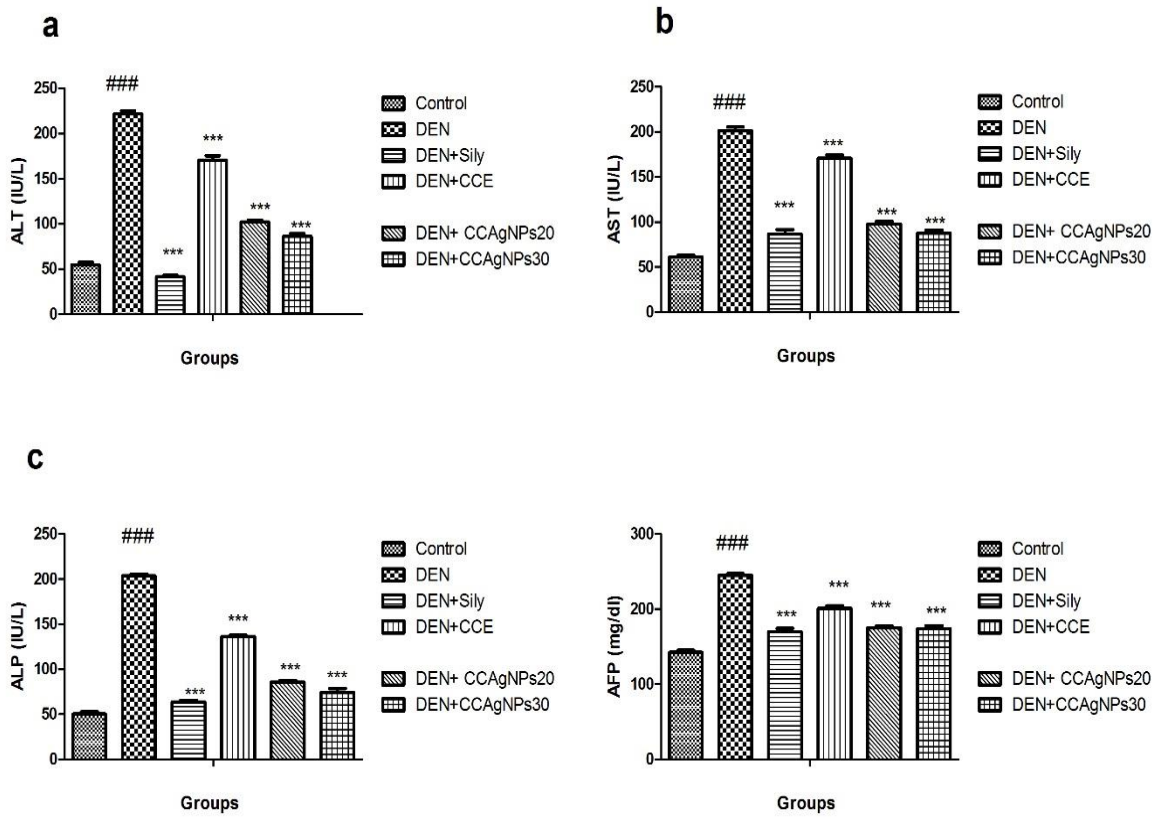


Figure 4.53 Effect of CCAgNPs on serum marker enzyme of hepatic parameter in control and DEN- induced HCC rats

4.23.4 Effect of CCAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

Table 4.52 and figure 4.54 portrayed decreased levels of total protein, albumin, total bilirubin and A/G ratio in DEN control rats, along with remarked level of albumin and total protein which suggested the hypo function of liver and inability to battle diseases. When DEN induced liver cancer group rats received a different dose level of CCAgNPs significantly raised the level of total protein, albumin, and total bilirubin. The increased levels of non-hepatic enzymes show the restoration of liver function in hepatoma bearing animals.

Table 4.52 Effect of CCAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

Treatment	TB	TP	Albumins	Globulins	A/G ratio
Control	0.53±0.11	8.9±0.62	5.4±0.31	3.5±0.21	1.54±0.09
DEN	4.21±0.32$^{\#\#\#}$	5.2±0.15$^{\#\#\#}$	2.68±0.26$^{\#\#\#}$	2.52±0.23$^{\#}$	1.06±0.12$^{\#}$
DEN+Sily	0.94±0.25***	8.29±0.34*	5.24±0.31***	3.05±0.2***	1.71±0.17***
DEN+CCE	1.67±0.02***	6.11±1.09ns	4.03±0.49*	2.08±0.17ns	1.93±0.06***
DEN + CCAgNPs20	1.06±0.21***	7.05±1.09ns	4.9±0.02***	2.15±0.12ns	2.28±0.14***
DEN + CCAgNPs30	0.98±0.36***	7.88±0.29***	5.16±0.38***	2.72±0.31ns	1.89±0.18***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

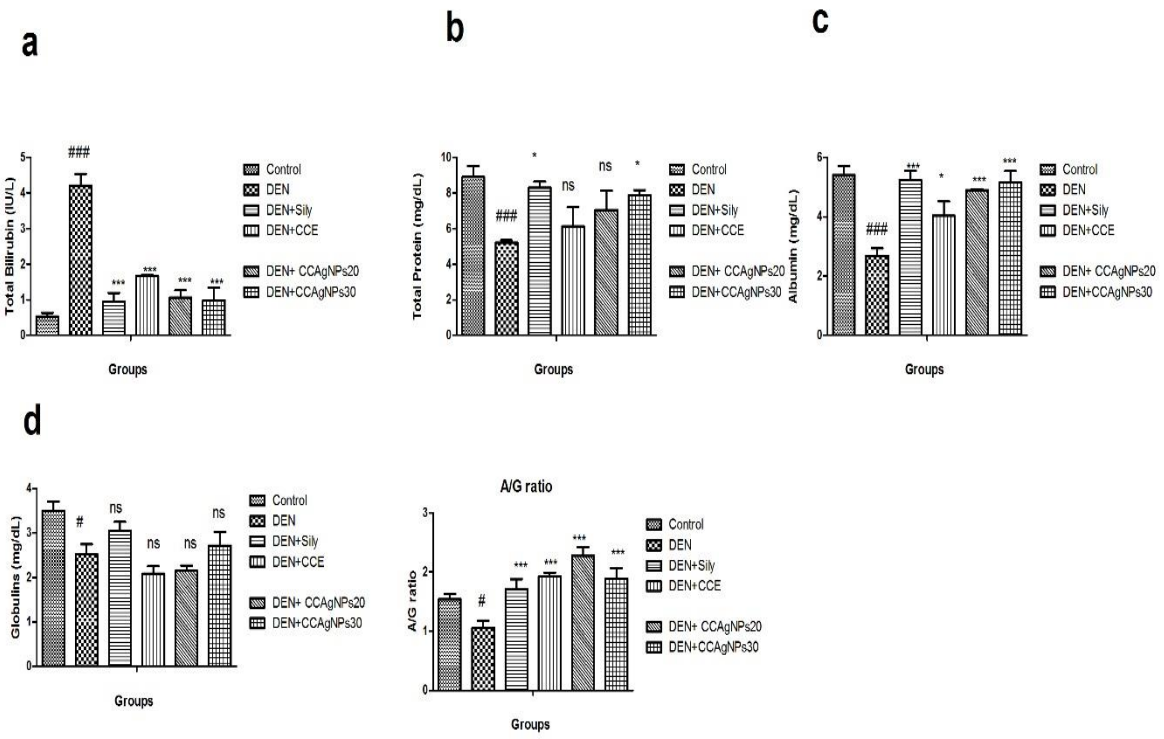


Figure 4.54 Effect of CCAgNPs on serum marker enzyme of non-hepatic parameter in control and DEN- induced HCC in rats

4.23.5 Effect of CCAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

Table 4.53 and Figure 4.55 speaks about the proinflammatory cytokines, for example, TNF-α, IL-6, and IL-1β. The raised level of TNF-α in DEN Induced rats was significantly (P>0.001) decreased by CCAgNPs at both a dose levels toward the finish of the experiment. Proinflammatory cytokines, in other word, IL-6, showed a same result as that of TNF-α. DEN induced hepatocarcinogenesis demonstrated the elevated level of IL-6 with respect to normal control rats. Silymarin treated groups confirmed the significant (P>0.001) reduction in IL-6 level. DEN induced liver cancer group demonstrated the enhanced level of IL-1β when contrasted with normal control, which was significantly (P>0.001) re-established by CCAgNPs and standard drug Silymarin.

Table 4.53 Effect of CCAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

Treatment	TNF-α	NF-κB	IL-6	IL-1β
Control	48.98±2.34	154.76±3.02	97.05±5.38	23.26±1.35
DEN	167.95±4.62$^{\#\#\#}$	278.12±3.05$^{\#\#\#}$	298.29±3.47$^{\#\#\#}$	86.99±1.98$^{\#\#\#}$
DEN+Sily	57.52±3.61***	162.99±3.28***	108.5±5.40***	35.75±1.70***
DEN+CCE	84.2±3.07***	198.2±4.25***	197.9±4.67***	61.78±2.97***
DEN + CCAgNPs20	70.23±2.56***	173.47±3.9***	124.67±3.2***	43.29±3.55***
DEN + CCAgNPs30	67.83±2.23***	168.2±0.38***	117.19±3.67***	40.19±3.56***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

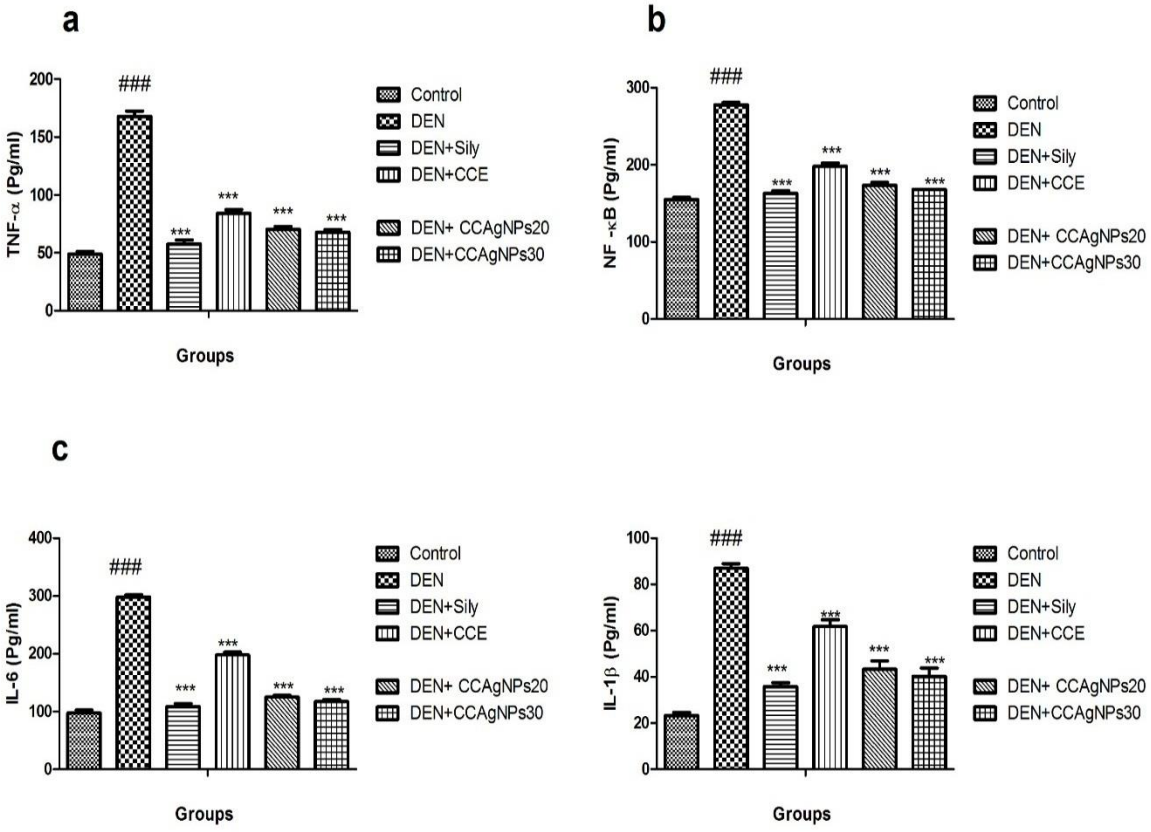


Figure 4.55 Effect of CCAgNPs on proinflammatory cytokines and inflammatory mediators in control and DEN- induced HCC in rats

4.23.6 Effect of CCAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats

DEN induced rats affirmed the enhanced level of LPO, decided in term of malonaldehyde (MDA) when contrasted with normal control rats. CCAgNPs treatment revealed the dose-dependent lessening in LPO, which confirms the antioxidant property of drug. Silymarin group rats managed the power of prevention against lipid peroxides which is caused by DEN in liver tissue (table 4.54 and figure 4.56).

Table 4.54 Effect of CCAgNPs on lipid peroxidation activity

Treatment	MDA
Control	17.68±2.26
DEN	$39.47\pm3.29^{###}$
DEN+Sily	$19.51\pm1.15^{***}$
DEN+CCE	$27.9\pm1.67^{**}$
DEN + CCAgNPs20	$21.38\pm1.45^{***}$
DEN + CCAgNPs30	$20.12\pm1.09^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

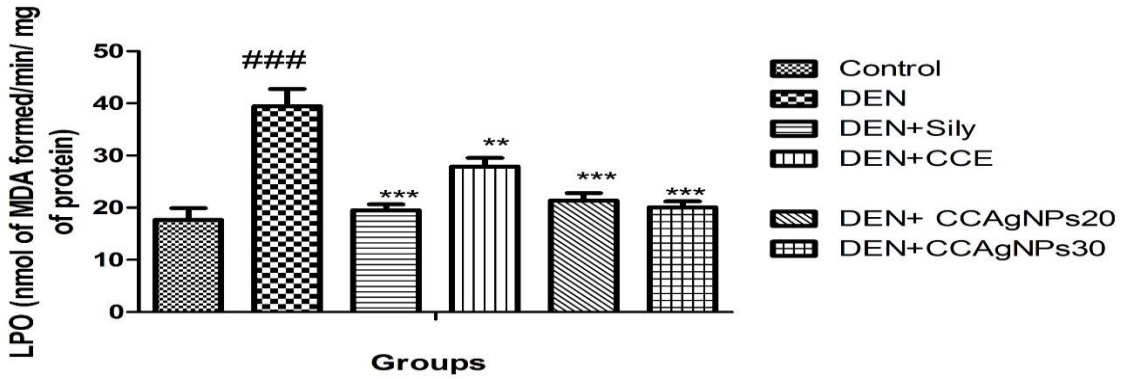


Figure 4.56 Effect of CCAgNPs on lipid peroxidation in liver of control and DEN- induced HCC rats

4.23.7 Effect of CCAgNPs on the levels of antioxidant enzymes in liver of rats

It was confirmed from the table 4.55 and figure 4.57 that DEN group animals demonstrated the significant (P>0.001) diminished content of antioxidant parameters, for example, SOD, catalase, GPx, GSH, G6PD and vitamin C, which were significantly (P>0.001) ameliorated by CCAgNPs at both the dose level. The standard drug treatment affirmed the noticeable modification in the substance of all these enzymes. The outcomes suggested that the CCAgNPs significantly (P>0.001) regulated the DEN-induced hepatocarcinogenesis in antioxidant parameters.

4.23.8 Effect of CCAgNPs on the activities of membrane bound enzymes in rats

DEN induced group revealed the downregulation of Na+/K+ ATPase and Ca^{2+}ATPase as compare to normal control wistar rats. Introduction of CCAgNPs boosted the levels of membrane bound enzymes in a dose dependent manner. A similar trend of enhancement was observed in DEN+ Silymarin treated group (table 4.56 and figure 4.58).

4.23.9 Histopathology observation of hepatic tissue

Normal structure with well-defined architecture contains a small uniform nuclei scattered in cytoplasm and uniform polyhedral-shape hepatocytes was observed in liver tissue of normal control rats. DEN induced cancer rats showed cell necrosis, cytoplasm forms an irregular shape, multiplication was found in the portal area of hepatic stellate cells and confirmed the presence of focal proliferation. DEN+CCE groups showed improvement in histological features of hepatic tissue with improving cytoplasm and altered hepatocytes. When liver induced rats treated with CCAgNPs affirmed the improvement by lessening inflammatory cells, regularize the cytoplasm shape, reduced binucleated cells and restored the architecture of hepatic tissue with altered hepatocytes in a dose dependent manner. Silymarin treated animals exhibited less number of micro droplets and necrosis cell, hepatocellular architecture seems superior when compared to DEN induced groups (Figure 4.59).

Table 4.55 Effect of CCAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats

Treatment	Catalase	SOD	GPx	GSH	G6PD	Vitamin C
Control	90.55±3.25	7.38±0.18	10.72±1.13	46.15±2.54	8.36±0.57	3.68±0.1
DEN	48.04±2.4$^{###}$	2.63±0.09$^{###}$	3.68±1.19$^{##}$	18.59±1.23$^{###}$	2.35±0.16$^{###}$	39.47±0.29$^{###}$
DEN+Sily	71.3±3.83***	6.49±0.15***	9.28±1.34*	41.77±2.68***	7.78±0.78***	19.51±0.15***
DEN+CCE	59.78±2.78*	4.9±0.78*	6.89±1.37^{ns}	29.58±2.99**	4.78±0.67^{ns}	32.56±1.67***
DEN + CCAgNPs20	63.89±2.35**	6.11±0.95***	8.27±1.57^{ns}	36.28±1.06***	6.8±0.87***	26.28±1.9***
DEN + CCAgNPs30	66.9±1.00***	6.38±0.65***	8.89±1.09*	38.9±1.28***	6.99±0.59***	23.19±0.08***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

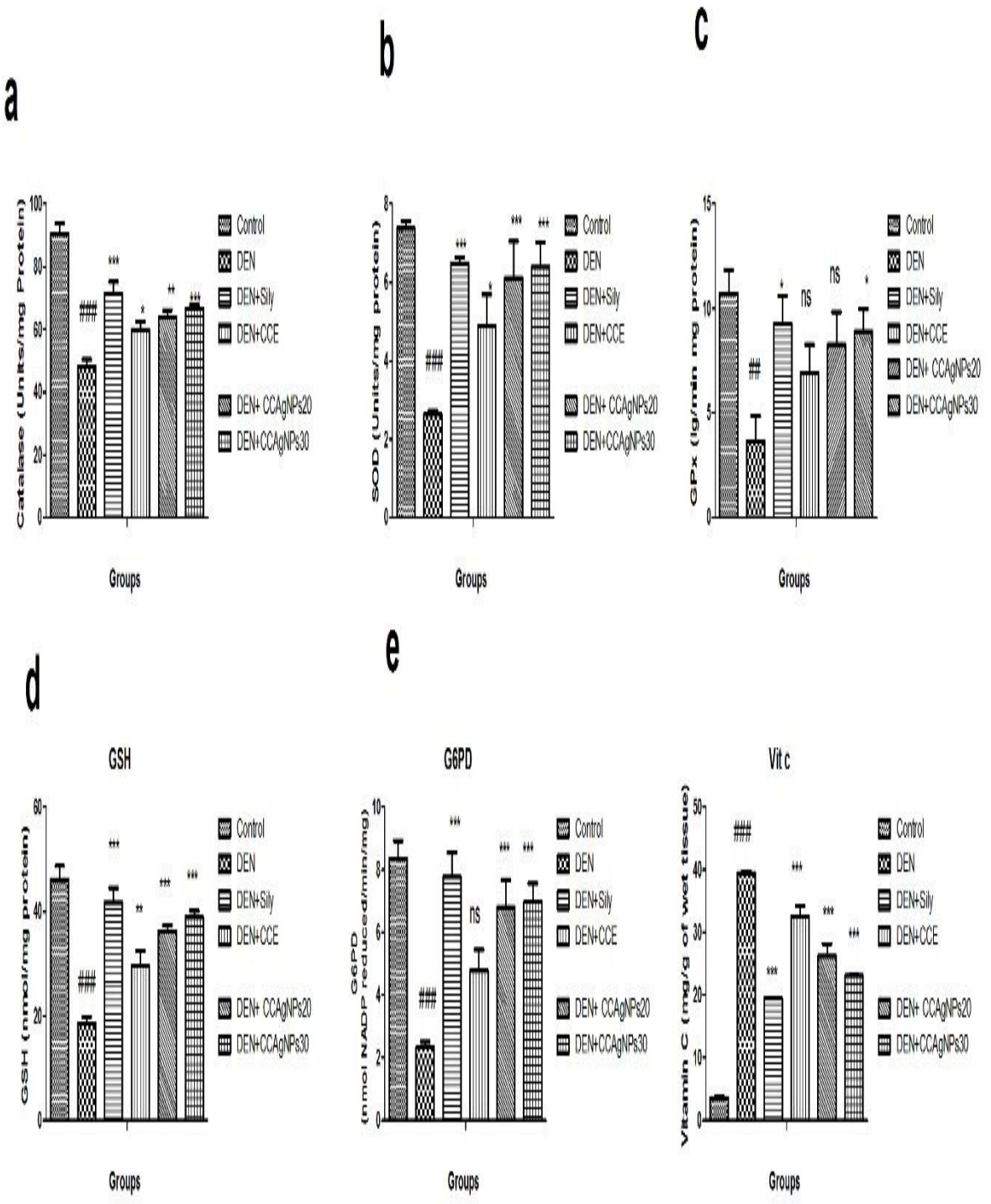


Figure 4.57 Effect of CCAgNPs on the levels of antioxidant enzymes in liver of control and DEN- induced HCC in rats

Table 4.56 Effect of CCAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats

Treatment	Na+K+–ATPases	Ca2+ ATPase
Control	4.37±0.05	8.56±0.09
DEN	2.33±0.08$^{\#\#\#}$	3.67±0.11$^{\#\#\#}$
DEN+Sily	4.09±0.07***	7.43±0.24***
DEN+CCE	3.18±0.13***	4.85±0.15**
DEN + CCAgNPs20	3.91±0.12***	6.57±0.27***
DEN + CCAgNPs30	3.99±0.09***	7.25±0.12***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

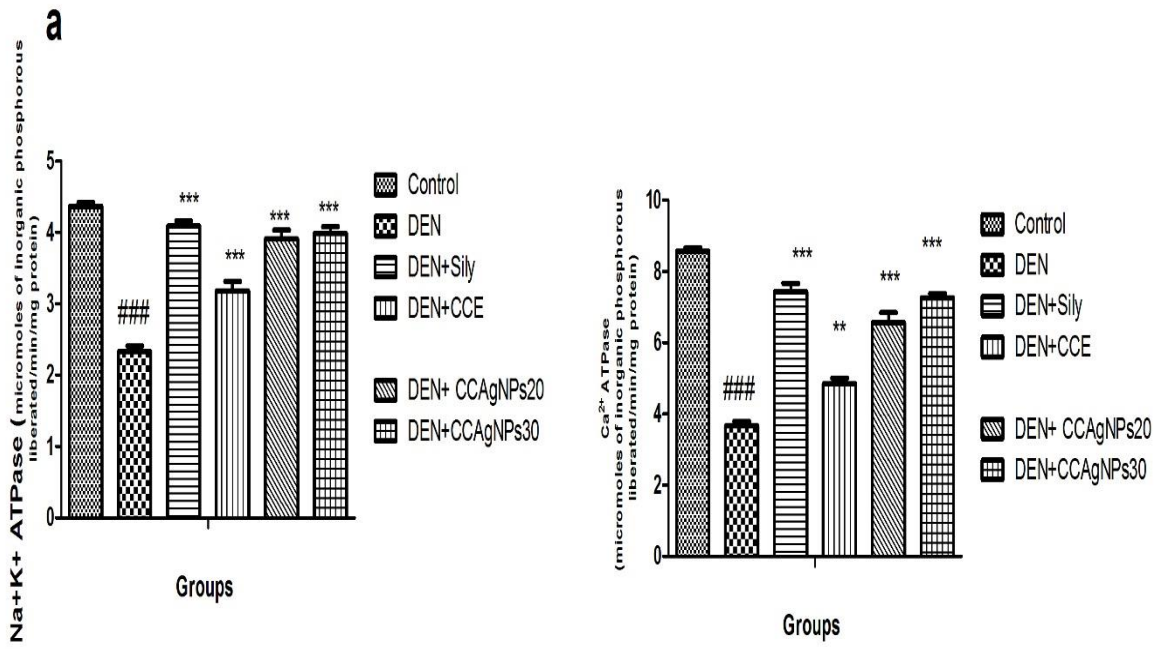


Figure 4.58 Effect of CCAgNPs on the activities of membrane bound enzymes in liver of control and DEN- induced HCC in rats

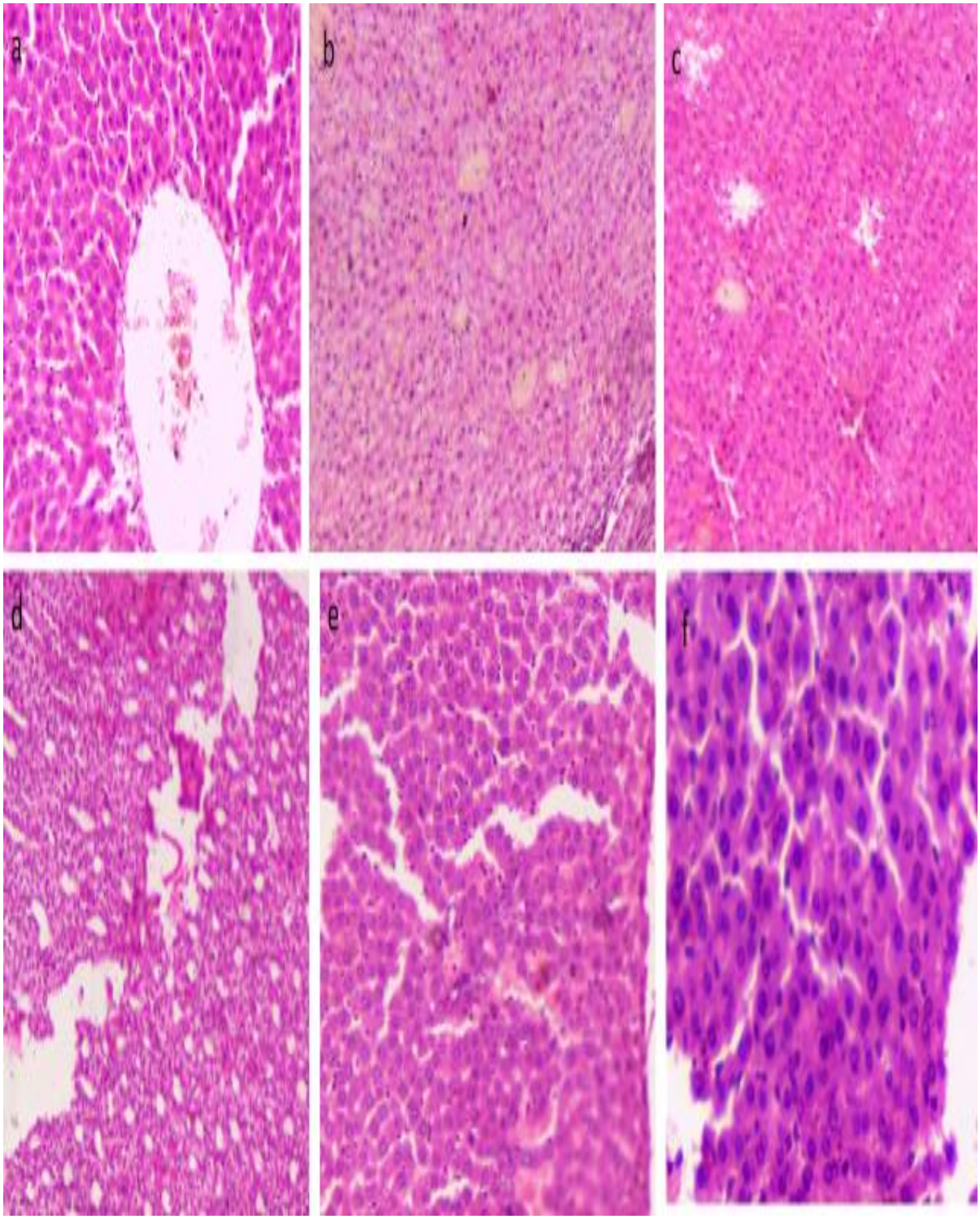


Figure 4.59 Photomicrograph of eosin-haematoxylin stained histological liver section area for (a) Normal control group, (b) DEN induced liver cancer group (c) DEN+ Silymarin (d) DEN+ CCE (e) DEN +CCAgNPs (20mg/kg bw) , and (f) DEN +CCAgNPs (30mg/kg bw)

4.24 Renal cancer

4.24.1 Effect of CCAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats

Table 4.58 depict about the number of rats with RCC, total number of tumour, number of animals with bilateral and unilateral tumour, percentage incidence of tumour. Normal control group rat showed no detectable knob on the kidney structure till end of the study. Through visible observation, DEN induced group rats affirmed the arrangement of knob on kidney tissues. DEN induced group animals when treated with CCAgNPs, affirmed the chemo-defensive impact of CCAgNPs and established by means of decrease in tumour frequency in a dose dependent way.

Table 4.57 Effect of CCAgNPs on macroscopic evaluation in kidney of control and DEN induced RCC (renal cancer carcinoma) rats

Groups	**No. of Rats with RCC**	**No. of rats with Unilateral tumors**	**No. of rats with bilateral tumors**	**Total no. of tumors**	**Incidence of tumors**
Control	-	-	-	-	-
DEN	6	3	3	18	100
DEN+CCE	6	3	3	10	100
DEN + CCAgNPs20	4	3	1	9	66.66
DEN + CCAgNPs30	4	2	2	6	66.66

4.24.2 Effect of CCAgNPs on serum marker enzymes of control and DEN induced RCC rats

The result of anticancer treatment with CCAgNPs on DEN induced renal cancer in the level of urea, creatinine, and BUN is shown in Table 4.59 and figure 4.60. Treatment with DEN produced an increment in the levels of renal markers such as urea, creatinine, and BUN when related to that of the control group. On treatment with CCAgNPs at both doses, a prominent reclamation of the all serum marker enzyme levels was remarked as compared with the DEN treated group.

4.24.3 Effect of CCAgNPs on tumour marker enzyme in control and DEN induced RCC rats

It is observe from table 4.60 and figure 4.61 that the level of gamma glutamyl transpeptidase, xanthine oxidase, and lactate dehydrogenase were significantly upgraded in DEN induced group II animals when compared to the control rats. CCAgNPs showed a noticeable down regulation in the levels of all enzymes in a dose dependent manner.

Table 4.58 Effect of CCAgNPs on serum marker enzymes of control and DEN induced RCC rats

Treatment	BUN	Creatinine	Uric acid
Control	30.05±1.27	1.26±0.10	1.45±0.06
DEN	73.24±3.78$^{\#\#\#}$	4.02±0.36$^{\#\#\#}$	3.29±0.28$^{\#\#\#}$
DEN+CCE	45.29±1.98***	2.65±0.25**	2.42±0.23*
DEN + CCAgNPs20	39.21±1.87***	2.39±0.32***	2.39±0.13**
DEN + CCAgNPs30	36.10±0.45***	2.01±0.21***	2.01±0.18***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

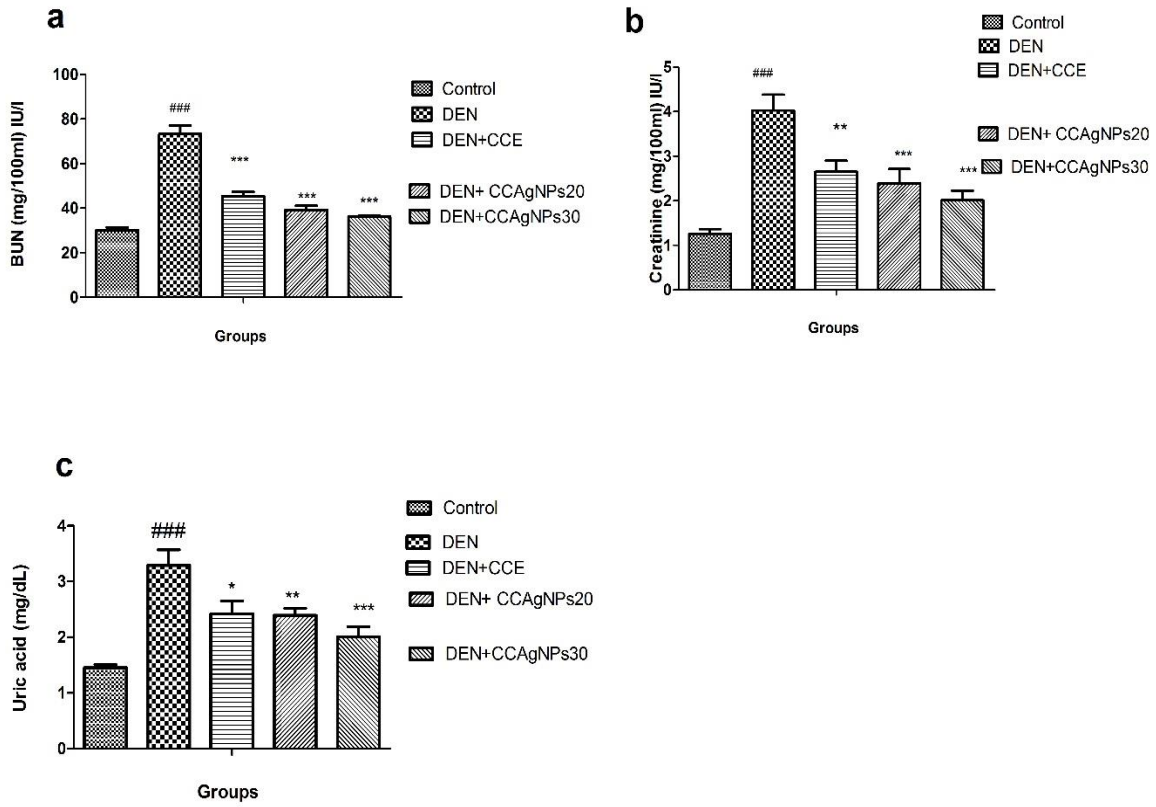


Figure 4.60 Effect of CCAgNPs on serum marker enzymes of control and DEN induced RCC rat

Table 4.59 Effect of CCAgNPs on tumour marker enzyme in control and DEN induced RCC rats

Treatment	**Xanthine oxidase**	**LDH**	**γ-GGT**
Control	0.52±0.03	218.40±6.03	356.42±6.36
DEN	1.24±0.09$^{\#\#\#}$	489.67±9.78$^{\#\#\#}$	624.89±12.09$^{\#\#\#}$
DEN+CCE	0.98±0.04*	398.78±8.34***	440.56±9.21***
DEN + CCAgNPs20	0.82±0.06***	294.60±7.56***	425.91±8.90***
DEN + CCAgNPs30	0.78±0.05**	275.09±6.23***	405.901±.01***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

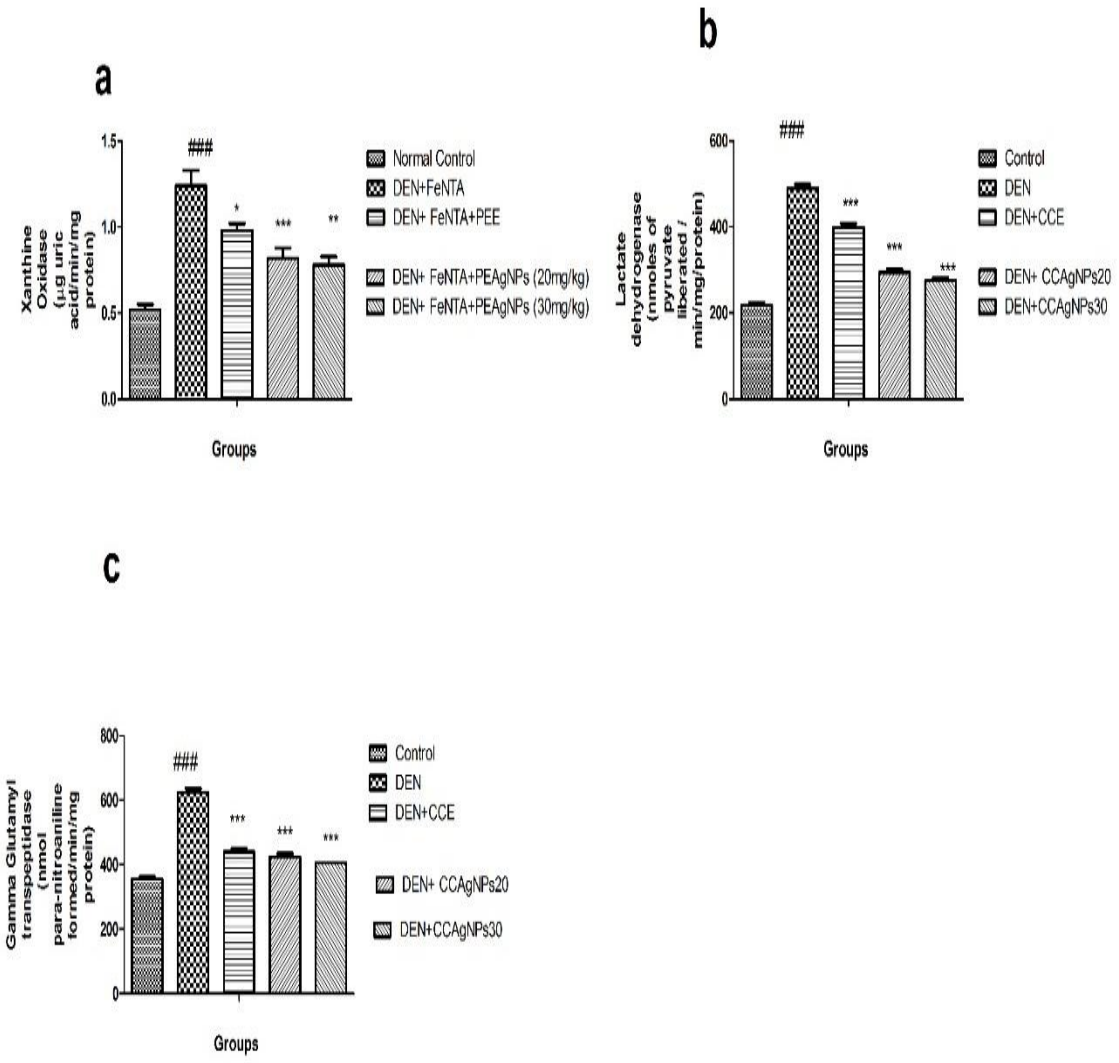


Figure 4.61 Effect of CCAgNPs on tumour marker enzyme in control and DEN induced RCC rats

4.24.4 Effect of CCAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats

It is declared from table 4.61 and figure 4.62 that the level of lipid peroxidation was raised significantly (P>0.001) in DEN induced group II animals with respect to the control (group I) animals. Introduction of the CCAgNPs significantly ((P>0.001)) declined the lipid peroxidation index. DEN induced group when treated with the plant no significant modification was seen and the values of silver nanoparticles are also similar to that of control.

Table 4.60 Effect of CCAgNPs on lipid peroxidation in kidney of control and DEN- induced RCC rats

Treatment	LPO
Control	2.24±0.02
DEN	$8.61\pm0.10^{###}$
DEN+CCE	$6.48\pm0.25^{***}$
DEN + CCAgNPs20	$4.92\pm0.54^{***}$
DEN + CCAgNPs30	$3.86\pm0.38^{***}$

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

4.24.5 Effect of CCAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

There was significant reduction was found in the activities of antioxidant parameter namely, superoxide dismutase, catalase, glutathione peroxidase, and glutathione S transferase, and endogenous antioxidant enzymes was recorded in rats intoxicated with DEN when compared to control as shown in table. However rats treated with CCAgNPs with the dose levels of 20mg and 30 mg /kg bw, the ranges of all these enzymes were significantly upregulated when contrast with normal control (table 4.62 and figure 4.62).

Table 4.61 Effect of CCAgNPs on the levels of antioxidant enzymes in kidney of control and DEN- induced RCC in rats

Treatment	SOD	GSH	catalase	GPX	GST	GR	H_20_2
Control	96.92±3.67	7.83±0.25	3.84±0.09	248.28 ±7.16	205.28 ±7.76	295.43±6.90	21.89±2.45
DEN	54.46±2.10$^{\#\#\#}$	3.09±0.21$^{\#\#\#}$	1.63 ±0.05$^{\#\#\#}$	129.79 ±5.80$^{\#\#\#}$	100.28 ±3.11$^{\#\#\#}$	182.78±4.86$^{\#\#\#}$	68.20±4.27$^{\#\#\#}$
DEN+CCE	62.86±1.98^{ns}	4.09±0.56^{ns}	2.69 ±0.05***	157.57 ±3.68**	114.41±3.61^{ns}	205.67±5.67*	54.65±4.01*
DEN + CCAgNPs20	82.96±2.76***	5.48±0.89*	3.09± 0.04***	195.36 ±3.14***	156.15 ±3.73***	256.12±4.53***	38.34±3.76***
DEN + CCAgNPs30	86.01±2.34***	6.41±0.67**	3.27 ±0.07***	210.36 ±3.12***	178.16 ±3.65***	267.61±5.01***	31.69±2.38***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

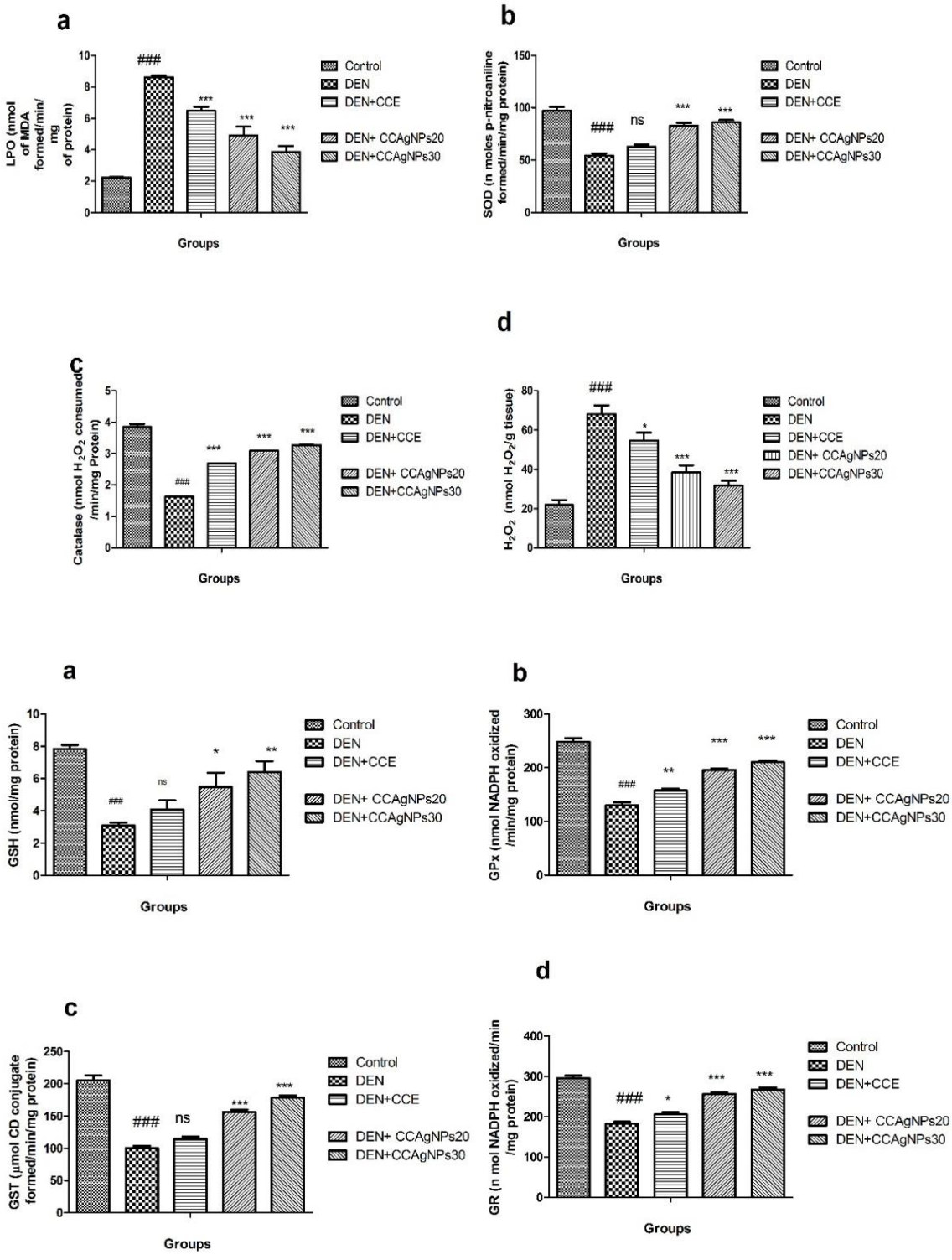


Figure 4.62 Effect of CCAgNPs on the levels of antioxidant enzymes in kidney of rats

4.24.6 Effect of CCAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Alteration in the level of Proinflammatory cytokine and inflammatory mediators, namely, TNF-, IL-6, NF- is enlightened in Table 4.63 and figure 4.63. DEN intoxication noticeably enhanced the proinflammatory cytokine and inflammatory mediators status of renal tissue when compare to normal control. The current study revealed that plant extract silver nanoparticles attenuated the noxious effect of DEN by reducing the activity of enhanced inflammatory mediators. Dose dependent downregulation was found in the activity, when treated with the different doses of silver nanoparticles.

4.24.7 Impact of CCAgNPs on tumour promotion markers in kidney of rats

Wistar rats when subjected to DEN treatment show significant enhancement in thymidine activity as depicted in table 4.64 and figure 4.64. Treatment with biofabricated silver nanoparticles of *M longifolia* leaf aqueous extract caused mark reduction in the raise level of ODC and thymidine in a dose dependent manner till the end of experiment. No significant difference were observed in the in ODC and thymidine of DEN + MLAgNPs (30 mg/kg BW) and normal control.

4.24.8 Histopathology of CCAgNPs on renal tissue

Figure 4.65 represented the histopathological studies of renal tissue in DEN induced hepatic cancer. Control groups showed no sign of diseases in renal tissue with normal tubular architecture having normal convoluted tubules and presence of glomeruli within the cortex. Den induced renal carcinoma group shows abundant minor foci of multiplication which is composed of cells and round nucleus emigrant to periphery of the cell which is termed as multicentric clear cell carcinoma. When plant extract is administered to DEN induced group, the cortex region of kidney displayed a formation of large cyst which is surrounded by flattened epithelium. Aggregates of clear cell were not seen and patchy lymphoid aggregates were found

on stroma. Further treatment with CCAgNPs pointedly attenuated this histopathological changes in both treated groups. Sections of renal at a higher dose showed a normal renal structure which was similar to control group rats.

Table 4.62 Effect of CCAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Treatment	**TNF-α**	**IL-6**	**IL-1β**	**NF-κB**
Control	350.20 ±8.23	923.19±14.29	211.3±26.78	538.30±10.29
DEN	732.561±1.98$^{###}$	2100.20±25.78$^{###}$	745.09±9.76$^{###}$	1637.38 ±27.90$^{###}$
DEN+CCE	448.78±9.30***	1645.29±21.27***	478.20±7.34***	1189.31 ±21.32***
DEN + CCAgNPs20	398.20±9.12***	1209.58±17.30***	345.2±16.13***	835.22± 16.78***
DEN + CCAgNPs30	310.11±9.12***	1179.23±15.23***	298.28±6.49***	721.89 ±13.40***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

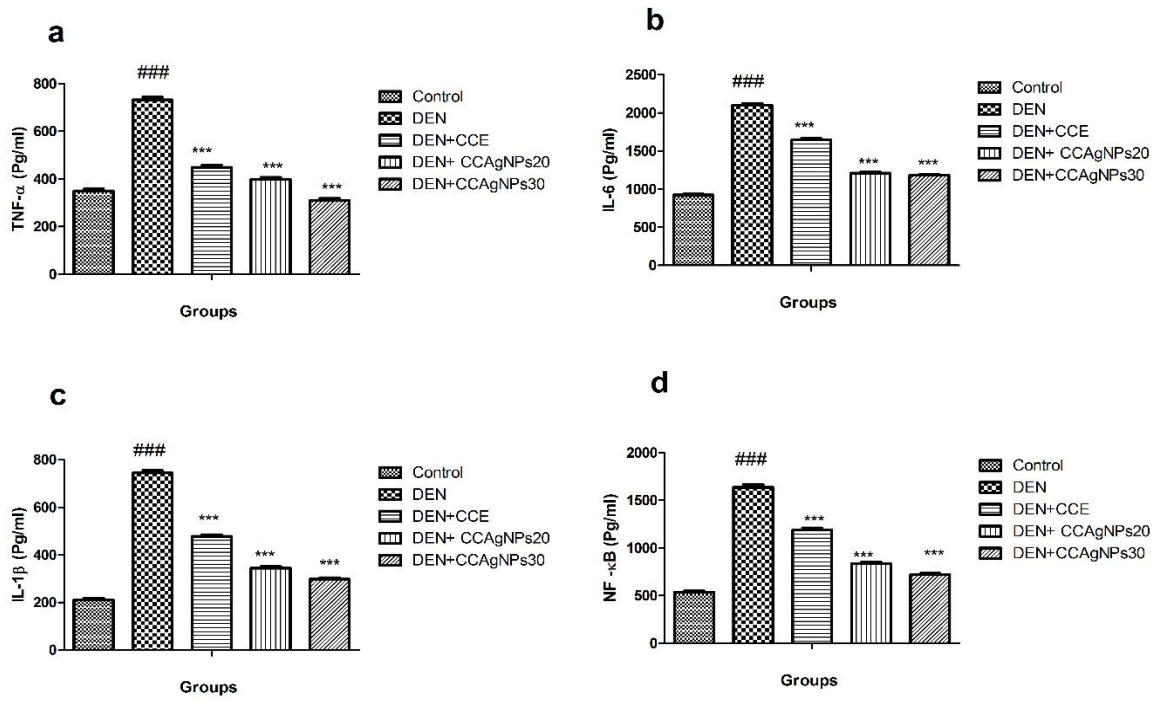


Figure 4.63 Effect of CCAgNPs on the proinflammatory cytokines and inflammatory mediators in kidney of control and DEN- induced RCC in rats

Table 4.63 Impact of CCAgNPs on tumour promotion markers in kidney of control and DEN-induced RCC in rats

Treatment	ODC	Thymidine incorporation
Control	850.23±21.34	1839.34±34.20
DEN	2045.66±32.12$^{\#\#\#}$	3209.30±40.29$^{\#\#\#}$
DEN+CCE	1690.21±27.12***	2478.49±36.29***
DEN + CCAgNPs20	1314.45±21.46***	2165.30±35.22***
DEN + CCAgNPs30	1011.00±22.34***	2021.87±34.12***

Results were represented as mean ± SEM of six animals in each group. It was analysed statistically significantly ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$) groups compared to normal control; ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) groups compared to DEN control; ns -not significant

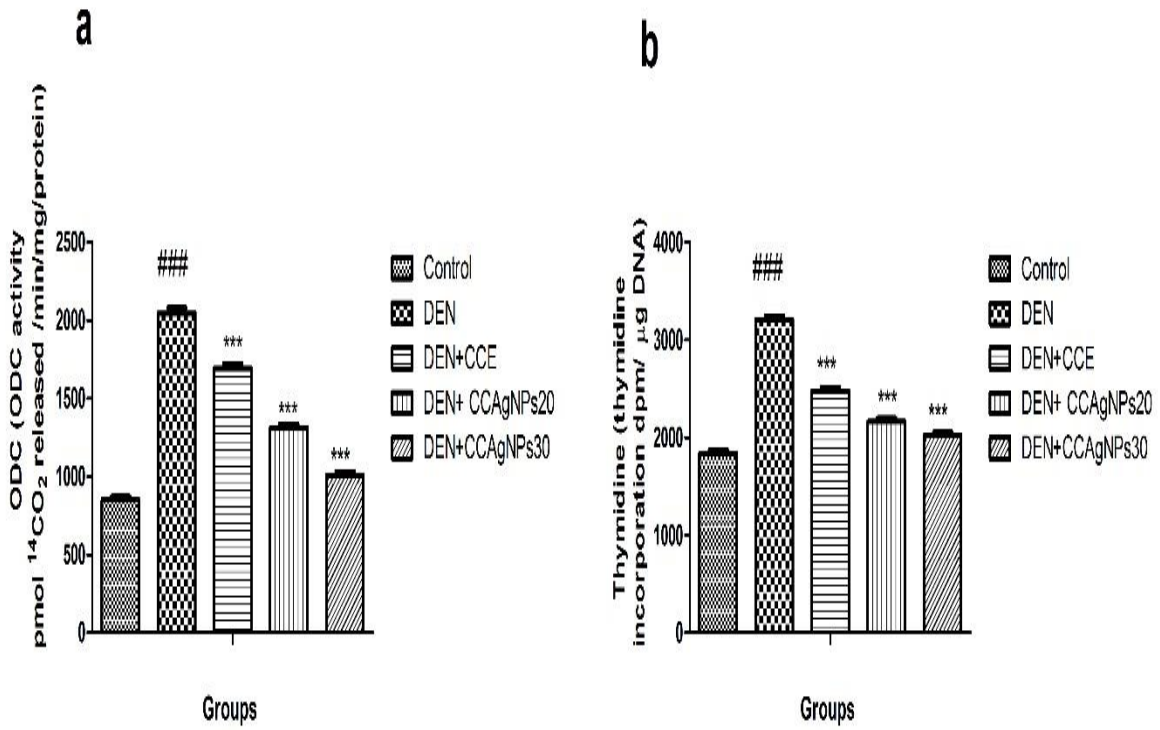


Figure 4.64 Impact of CCAgNPs on tumour promotion markers in kidney of control and DEN-induced RCC in rats

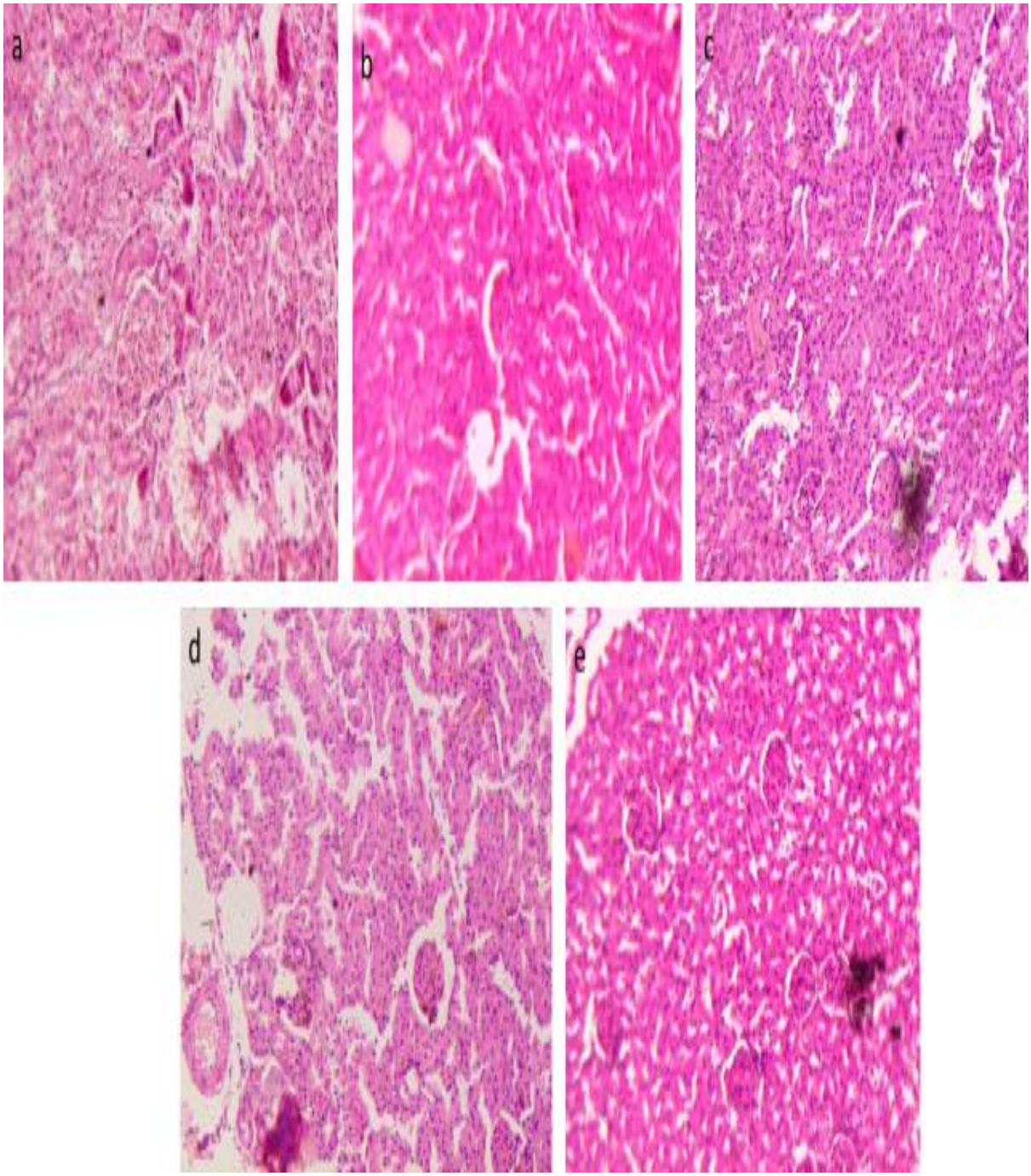


Figure 4.65 Photomicrograph of eosin-haematoxylin stained histological liver section area for (a) Normal Control group, (b) DEN induced liver cancer group (c) DEN+ CCE (d) DEN +CCAgNPs (20mg/kg bw) (e) DEN +CCAgNPs (30mg/kg bw)

Chapter-5

Discussion

HCC is one of the deadliest diseases and leading cause of decease due to inadequate and poor treatment choices in developing countries. It is common type of cancer which have poor prognosis. Though, several exogenous and endogenous factors are identified which affect the normal shape of cell development, through which cell converts into cancer cell. DEN is knowns as cancer causing agent amongst nitrosamines and responsible for causing hepatic cancer. Liver is consider as largest organs of the human body. Liver plays a pivotal role in the excretion of endotoxins and exotoxins. From past few years, literature studies show a new hopes for the treatment of such terrible disease and provide the knowledge of relation between free radical production and liver cancer. Therapeutic treatment such as tumour resection and liver transplantation are not achievable in progressive phases of Hepatocellular carcinoma (HCC). Consequently, effective chemotherapeutic agents is still searching to progress the survival rate of beings who is having an advanced HCC.

As we all know that Hepatic cancer is recognized for multi-drug resistance and bad response for chemotherapeutic agents. However, in ayurveda, well known traditional system of medicine on the basis of traditional knowledge used in the treatment of hepatic cancer. Herbal drugs plays an important role in managing various hepatic diseases. However, *Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas,* a traditional plants having a therapeutic property and useful in the treatment of various diseases and hepatic cancer. Therefore it is necessary to seriously determine the chemotherapeutic agent which is currently in the clinical setting and development and evaluation of new moieties derived from plant source is required which have less side-effects, ecofriendly and combat against hepatic cancer.

Plants contains a chemical constituents i.e. phenols possess an extensive antimutagenic and anticarcinogenic effects against different types of carcinogens. From past few years, researchers focussed on the plants bioactive molecules which have a protective property against

various ailments. Medicinal Plants which are rich in antioxidants and other nutrients protects the body against cancer causing agents, mutagenic, damage the DNA and lipid peroxidation.

In traditional system of medicine, a variety of drugs are available in for liver healing and cureness. Nowadays, allopathic medicine which is used in the treatment of liver ailments has been shifted towards the herbal products with the utilization of scientific tools and traditional knowledge. Simple and accurate herbal medicine is still a fascinating challenge for the management of liver problem. With the expanding and depth knowledge of the pathologic processes in the liver and a changes occur in hepatic transcriptome and proteome advances, novel medicine and therapy will develop in the future for acute and chronic liver ailment.

From very long time, plant derived products have been used in the treatment of various disease namely, cancer, heart attack, malaria, fever, and etc. Natural products has been used as ayurvedic medicine worldwide and consider for the development of anti-cancer drugs. In vitro and in vivo screening models are mentioned in the literature for anticancer activity. So, an effort has been made to examine a novel herbal based drug anticancer agent from plants i.e. silver nanoparticles and its systematically validated experimental animal models viz. *Phyllanthus Emblica*, *Madhuca Longifolia* and *Carissa carandas* against hepatic cancer and renal cancer induced by DEN in experimental rodents.

Phyllanthus Emblica, Madhuca Longifolia and *Carissa carandas* used as a folklore plants and utilize in treating different ailment but no study has been available in the literature about treating hepatic cancer and renal cancer in the form of silver nanoparticles. Hence, an attempt are had been done in our scientific laboratories to optimize its activity and safety profile for the preparation of aqueous extract, and further extract is used for the formulation of silver nanoparticles. In the current investigation hepatic and renal cancer evaluation of *Phyllanthus Emblica, Madhuca Longifolia* and *Carissa carandas* were performed.

Phyllanthus emblica

Prepared AgNPs were observed visually through colour change from brown to dark brown. UV study is done to check the absorbance of prepared nanoparticles as it excited due to surface plasma resonance and maximum absorption peak with the wavelength max at 418 nm. It was reported from other research paper that silver nanoparticles shows absorbance between 320 and 520 nm depend upon the particle size. FTIR studies were done to determine the interaction between the functional group of extract and synthesized nanoparticles. It was observed from FTIR that biomolecule played an important role for capping and stabilizing agent in silver nanoparticles. XRD studies reveal that silver nanoparticles were purely crystalline in nature and structure was face centered cubic.

Evaluations of prepared nanoparticles were done through FESEM and TEM to determine the surface morphology, shape and size respectively. It was observed that synthesis nanoparticles were quasi spherical in shape, look like a cluster of particles with rough surface and size ranges from 15 to 30 nm. The elemental analysis was done through EDX and indicates the presence of various elements along with Ag.

The effective dose of PEAgNPs at low concentration shows no lysis of cell. Apoptosis of cell occur at maximum concentration (50µg/ml). The results show morphological changes like shrinkage of cell, oxidative stress and biochemical changes. The IC_{50} value confirms the promising efficacy of PEAgNPs against hepatic cancer. Cytotoxic effect can be confirmed by the dose and time dependent manner of bioengineered silver nanoparticles (Dziedzic, 2016). Liver performs a various physiological function such as metabolism, secretion, storage, detoxifies chemicals and makes proteins for blood clotting. It is affected and targeted by various external toxic agents. Diethylnitrosamine (DEN) is supposed to be one of environmental carcinogens, and hepatotoxic which induce cancer in hepatic cell and generate free radicals in liver. DEN induced hepatocarcinogenesis was initiated by early preneoplastic foci appeared on liver and develop a high rate of cell proliferation.

In the present study, the phytoconstituents polyphenols present in *Phyllanthus emblica* leaf extract and biofabricated silver nanoparticles was used to shows its effect against DEN induced liver cancer toxicity in the liver of rats. It was seen that administration of plant extract silver nanoparticles reduced the development of nodules of hepatocytes on the liver and also decreased the foci of altered hepatocytes. Administration of single dose of DEN reduced the body weight as compare to normal rat it might be due to loss from skeletal muscle and adipose tissue. After the administration of biogenic silver nanoparticle, it gained the weight back close to normal rats and it reduces the formation of nodular hepatocytes on liver.

Human body have an abundance source of transaminase enzyme (AST and ALT) which catalyses the transfer of amino group. ALP is one of important marker enzyme which is located in the bile canaliculi lipid membrane. The most sensitive serum and liver markers enzymes such as ALT, AST, ALP and total bilirubin are involved in the diagnosis of normal and altered function of liver. Increased level of ALT, ALP, AST and total bilirubin was found in DEN group when compare to control group. Enhanced level of serum marker enzyme might be due to cell necrosis or hepatocellular damage by DEN, which altered the functional integrity of liver cell membrane. But when treated with silver nanoparticles, it showed a significant reduction in all serum marker enzymes over untreated group. Results proved the hepatoprotective effect of biofabricated silver nanoparticles in the inhibition of cell proliferation, maintain the functional integrity of cell and reduced enzyme leakage. Alpha feto protein (AFP) is termed as the gold marker for the estimation of hepatic carcinoma along with the determination of other hepatic parameters.

It is type of protein and on the basis of composition of size and shape, similar to the albumins. Up-regulation in the level of AFP suggested the expansion of tumour growth and further confirms the development of HCC. The level of AFP increased more than 10 times during the HCC in serum as comparison to NC. PEAgNPs treatment significantly down-

regulated the AFP content dose dependently and suggested the anticancer effect. The current hypothesis confirmed by the inhibition of hepatic nodules formation in the PEAgNPs treated group rats as compared to DEN control groups (table 1).

Total proteins play an important role in transport of substances, inflammation, and blood clotting and useful to determine the body disorder in liver and kidney. Synthesis of albumin take place in liver and transfers the fatty acids from adipose tissue to muscle tissue. Globulins is a type of proteins which transport the various substance in the blood and play a major role in defence mechanism of body (Kumar, 2017). Reduction in the level of globulins might be due to neoplastic growth by the prolonged treatment with DEN. Excessive dose of DEN causes breakdown of polyribosome which are situated in the endoplasmic reticulum and suppress the protein formation (Yim SH, 2010).

Excessive triggering of cytokines cascade in DEN induced group rats produce hepatocellular damage and leads to cause necrosis of cell. Treatment with standard drug, PEE and PEAgNPs significantly reduce the elevated levels of TNF-α, IL-1β, IL-6 as compare to DEN induced group and consistent effect seen on leukocyte migration. Reduction in proinflammatory cytokine levels by PEAgNPs in HCC proved its anti-inflammatory activity. The low levels of TNF-α and IL-1β is responsible for the reduction of leukocyte migration. TNF-α acts as chemotactic agent and triggers the expression of cling molecule on endothelial cells. So from the result, it was observed that silver nanoparticles is a most potent inflammatory agent which suppressed the levels of cytokines and effective in HCC (Verma, 2017).

NF-**k**B is a heterodimeric protein and is the member of five closely related protein. It is present in the cytoplasm and bind with DNA of IκB proteins. When initiate, it get unbound from IκB and triggers transcription. The level of NF-**k**B was raised via free radicals, carcinogen, and inflammatory reaction. In the current experimental study, DEN induced rats showed elevated levels of pro-inflammatory cytokine which in turn, activates NF-**k**B, free

radical species. It show acute inflammation and excessive expression of gene by increasing the frequency of transcription of TNF-α. PEAgNPs normalize the levels of NF-**k**B via mitigating the inflammatory process. The hepatoprotective effects of standard drug were comparable to the control drug (Verma, 2016).

Free radical scavenging enzymes such as CAT, GPX and SOD is considered to be a most important part of antioxidant defence mechanism. CAT and SOD are also consider as the first line antioxidant and play a significant role in the scavenging of various free radicals. Superoxide catalyses the dismutase of the superoxide radical into oxygen or hydrogen peroxides. It is a by-product which is produced during oxygen metabolism and if not controlled, it damage the cell (Xu, 2013).

Silver nanoparticles showed significant increase in SOD enzyme level when compared to DEN induced group and in turn, decreases reactive oxygen species generation and production and oxidative stress at cellular level (Blokhina, 2003). Catalase is tetramer enzyme, present in the peroxisomes of cells containing a heme group in centre and NADPH. It removes H_2O_2 by forming H_2O and O_2 (catalytic reaction), when the concentration of H_2O_2 is high and acts as peroxidic agent (peroxidatic reaction), removing H_2O_2 when concentration is low (Kinnula, 1993). Glutathione peroxidase (GPx) belongs to the family of peroxidase and dependent on the micronutrient selenium (Se), and play a significant role in the lipid hydroperoxides diminution to their corresponding free H_2O_2 and H_2O (Lubos, 2011). It protects the tissue from oxidative damage. DEN induced group show a reduction in all of these enzymes due to increase in ROS level during DEN metabolism.

GSH regulate the thiol-disulfide cycle of cell and synthesis take place on the availability of precursor cysteine and its biological function is to detoxify chemicals. In oxidative stress condition, the liver oxidized the glutathione and send into bile (Kaplowitz, 1981**).** Due to increased demand of lipid hydroperoxides, GSH level decline which is metabolism by GPx.

PEAgNPs showed high range of GSH level as compare to DEN induced group. This might be due to polyphenolic compound which is present inside the plant leaf which enhanced the level of GSH by inhibiting the GSR activity (Khan, 2012).

Glucose-6-phosphate dehydrogenase (G6PD) function in the reduction of NADPH and ribose 5-phosphate which is useful in the synthesis of nucleic acid. G6PD provide NADPH after the reduction of $NADP^+$ to maintain the sulfhydryl groups of cellular proteins and reduced the level of free radicals and peroxides (Kuo, 2000). The level of G6PD was low in DEN induced group as compared to control group, because of regulation of G6PD expression which altered and it involved in apoptosis. Vitamin C is a very strong antioxidant which has a capability of scavenging all free radicals physiologically. Synthesized silver nanoparticles showed an increased level of Vitamin C as compare to DEN induced group. It lowers the level of mitochondrial ROS formation and enhanced the activity of SOD and GPx (Skalska, 2016). Lipid peroxidation is responsible for tissue damage which is caused by free radicals. DEN induced group is likely to be generate more LPO product like malondialdehyde, that why they show elevated rate of LPO due to increased production or destruction of ROS. Biosynthesized silver nanoparticles significantly reduced the level of LPO and might be due to antioxidant property (Bahramikia, 2009).

Membrane bound enzymes such as Na^+/K^+ATPase and Ca^{2+}ATPase utilizes the energy in the form of ATP and transport the ion across the cell membrane by the process of hydrolysis. Na^+/K^+ATPase are the enzyme which transfer the active sodium/ potassium across the cell membrane but it activity is inhibited by hydroperoxides (Reinhard, 2013). Biofabricated silver nanodrugs significantly revert back all the value of membrane bound enzymes as compare to control group. It might be suggested that formulation prevent the ATPases by maintain the oxidation and it's by-products and control the level of ROS. It maintains the functional and

structural integrity of cell membrane by controlling the level of membrane bound enzyme (López-Revuelta, 2006)

***Phyllanthus emblica* extract and PEAgNPs activity on renal cancer**

The kidney is considered as intricate organs of the body which is comprises of precise components that perform all function in well-coordinated manner. Various medications, chemicals, and overwhelming metals have been appeared to have dangerous impacts on kidney and on its function (Do, 2014) Current study elucidates the antioxidant property of PEAgNPs by *in-vitro* method. It is well demonstrated that this formulation having a phenol and flavonoid content and oxidative processes.

Raised level of uric acid, BUN and serum creatinine is connected with drug initiated nephrotoxity. The determination of such serum parameters is utilized to diagnose drug induced renal tumour (Adeneye, 2008).

The outcomes uncovered that PEE and PEAgNPs improved the damage of kidney which was initiated by DEN administration. PEAgNPs and PEE showed a significantly reduction in the raised serum level of BUN, Uric acid and creatinine when contrasted with the DEN induced group. The raised serum level of Uric acid, BUN and creatinine is a marker of deteriorate kidney. BUN is derivative of protein metabolism and is discharged in the urine. Creatinine is obtained from endogenous sources and metabolite of creatinine and discharge through glomerular filtration of kidney via urine. It is clearly indicated from fig and table that raised level is responsible for the destruction of renal tissue. After administration of DEN and it’s directly interact with cell membrane and change the permeability and functional integrity of kidney cell membrane (Siham, 2008).

Uric acid can work as an important antioxidant but has been hypothesized to assume a vital part in the pathological process of renal disease. Oxidation of xanthine and hypoxanthine by XOR is a derivative of uric acid (Waring, 2001).

The pharmacological hindrance of XOR has been utilized broadly for the management of renal tumour. In such case, lifted serum urate, the prevailing monosodium type of UA at physiological pH, was found to display solid factual relationship with expanded untimely cancer death. Increase in uric acid level lead to hyperuricemia, and concluded as indicator of renal diseases instead of risk factor for procession (Ichida, 2012).

LDH is found in all tissue of body and is an iso-enzyme which is gathered in a homo- or hetero-tetramer structure by two protein subunits: LDHA and LDHB. LDLDHA and LDHB portray five subtypes of (LDH-1 to LDH-5) and LDH-6 frames a particular subtype which is a third protein subunit. LDH play a vital role in tumour metabolism and transform the pyruvate into lactate under glycolysis process (Fantin, 2006)

. In a few years, a large number of studies were conducted and reported that LDH levels are connected to the prediction of renal tumour. The overwhelming capacity of LDH is the catalyzation of the reversible reaction of pyruvate to lactate. When high concentration of intracellular LDH was discharged into blood, enhancing the serum LDH level, degeneration of kidney tissue take place (Shen J, 2016) Xanthine oxidase metabolised DNA & RNA into purine nucleotides and bases, again breakdown into xanthine and afterward uric acid. Uric acid is a product of endogenously incorporated purines. Xanthine oxidase is prevalently present in kidney, brain, liver and GIT. Transformation of xanthine oxidase in presence of xanthine dehydrogenase and responsible for change to NADH. The activity of these catalysts yields hydroxyl free radicals and hydrogen peroxide which can add to or start oxidative stress (Zhang Z,1998) Gamma-glutamyl transpeptidase is a key chemical in glutathione (GSH) liberation, digestion of endogenous stimulators, for example, leukotrienes and prostaglandins,

detoxification of xenobiotics including naturally essential mixes and cancer-causing agents, and cell forms reliant on the oxidation/lessening of glutathione. Due to various stimulus to renal tissue, these process is regularly take place and the catalyst is broadly conveyed to renal tissue. It was assume that gamma-glutamyl transpeptidase perform a major role in hepatic and renal tissue when response to any damage (Lieberman, 1995). Elevated level of GGT expression itself involved in neoplastic transformation and it supply GSH in cell. And it is one of major tool in cellular defensive mechanism. Metabolism of GSH by GST provides pro-oxidant effect along with modulatory effects.

GSH is considering as low molecular weight antioxidative tripeptide. GSH detoxify the chemotherapeutic medications; break down of nutrients, chemicals substances, and maintenance of homeostasis. It was seen from study that DEN administration to rats show a depletion in GSH levels and stop the activity of all antioxidant enzymes viz. GSH, GR, GST, SOD, Catalase, GPx. Biosynthesized Silver nanoparticles significantly revert back all the enzymatic and non-enzymatic enzymes. Therefore PEAgNPs exhibited potential nephroprotective activity against DEN induced groups by attenuated the level of all antioxidant enzymes in renal tissue (Ali F, 2012)

Lipid peroxidation affect the division of cell by damaging the amino acid sulfhydryl groups of RNA, DNA and proteins. It reacts with free oxygen species and form lipid hydroperoxides and again converts into peroxy and alkoxy radicals. The alkoxy radicals further metabolized into cyclic endoperoxides and MDA-like products (Catala A, 2009)

LPO products believed in repairing of DNA and triggers polymerase and reduce the level of NAD and ATP. DEN intoxicant increase the formation of ROS in renal cell due to toxic nature of LPO and its metabolites. It show a significant increase in level of MDA of toxic control group when contrast to normal control group. The current study favours the

chemoprotective effect of PEAgNPs in dose dependent manner by diminishing the activity of LPO.

Formation of ROS is a main reason behind anticancer drugs to combat against cancer cells. But important role is played by lipid peroxidation products which are induced by ROS. Various evident demonstrate that plant extract and plant mediated silver nanoparticles can sharpen the carcinoma cells particularly if there should be an occurrence of RCC. The activity against cancer is vanished before by over production of intracellular aldehydes of LPO and induction of apoptosis (Circu M, 2010)

Using Fenton reaction, hydrogen peroxide give highly reactive species $^{\bullet}OH$ radical and induce DNA to break inside the intact cell. PEAgNPs upgraded H_2O_2 activity in dose dependent manner of DEN induced renal cancer group. H_2O_2 causes change in nucleosome and chromosome of DNA. The protective effect of PEAgNPs against H_2O_2 activity prevents such changes in RCC due to presence of polyphenolic antioxidants.

It was proved from the study that dose dependent manner of PEAgNPs also possess an antiapoptotic activity and stop the destruction of DNA, membrane lipids, proteins and enzymes present in the cell.

Various evidence related to inflammation proposed that it was nearly connected with the pathological changes of DEN induced renal tumour. There are several inflammatory mediators which is totally linked with renal damage and raised the level of mediators and cytokines during DEN administration. But it was assumed that TNF-α is main factor behind this toxicity. It was revealed that TNF-α was prompted by ROS and generated after DEN administration. It was suggested from the study that DEN administration provoked increment of TNF-α and IL-6 levels in renal tissues and diminished by PEAgNPs treatment in dose dependent way. These studies demonstrated that PEAgNPs cure renal cancer through repressing inflammation **responses (rashid S, 2013)**

Many investigations have uncovered oxidative property which is main reason behind inflammation activation of a few proinflammatory cytokines. It additionally assumes a noteworthy part in the expansion of renal tumour. Redox reaction has additionally been appeared to impact NF-κB control and henceforth a few qualities associated with cell change, expansion, and angiogenesis. Despite the fact that, connection between ROS and NF-κB is mind boggling, ROS are believed to be ensnared as second emissaries in the enactment of NF-κB via TNF-α and other proinflammatory cytokines. Hindrance of NF-κB is a better procedure to control carcinogenesis and tumour advancement (Hoesel B, 2013)

ODC is consider as rate limiting enzyme during polyamine biosynthesised de novo. Polyamine is present in normal and cancerous tissue and changes the polyamine synthesis by alter ODC content. It instigate when response to tumour promoters and carcinogens and important tool for cancer investigation. ODC decarboxylates ornithine to putrescine. But in plants and microbes, arginine decarboxylase catalyses arginine to putrescine. ODC act as indicator during multiplication of cell and play a major role in cancerous tissue. In this model, DEN are having renal tumour promoter potency which affects the DNA synthesis directly. It increase ROS and decrease the activity of antioxidant after administration of tumour promoter agent which directly related to the maximum ODC induction (Snapir Z, 2009)

Madhuca longifolia

Carcinogenesis is a general term which means advancement of neoplasia. DEN is an outstanding hepatocarcinogen typically used to initiate liver malignancy in living models. It has been demonstrated that on essential metabolic enactment, DEN delivers the promutagenic adducts, O- 6 - ethyl deoxyguanosine and O- 4 and O-6 - ethyl deoxythymidine that generate DNA chain harm, depurination or attached to DNA and regularly producing a miscoding in DNA, initiation of liver carcinogenesis takes place. A rat model is used to examine diethylnitrosamine initiated carcinogenesis in rat is built up in our research facility. The

neurotic changes within the movement of tumours and its hindrance are relied upon to be reflected in the natural and histological parameters of the host framework (**Fridovich, H, 1972**).

Silver nanoparticles were prepared by aqueous leaves extract of *M. Longifolia*. The development and stability of the prepared silver nanoparticles in the colloidal solution were characterized by the UV– Vis spectrophotometer, FTIR, X-beam diffractometer (XRD), FESEM with EDX and TEM.

Silver nanoparticles plays a noteworthy part in the field of nanotechnology and nanomedicine The AgNPs prepared in this procedure are found to be effective anticancer agents against DEN induced hepatic cancer animal models.

One of the manifestations related with hepatocellular carcinoma is weight reduction and tissue destruction. There was a sharp abatement in the body weight and an expanded in the liver weights at 16 weeks in animals. Despite the fact that it was normal that treatment with the MLE, MLAgNPs and Silymarin would alter the trend.

In vitro cytotoxic study confirmed that the silver nanoparticles shows its effect on hepatic cancer cell line in a dose dependent way. It might be due to interaction of nanoparticles with the proteins of cell and produce some alteration due to its low stability and high reactive nature of silver ions (Omaye ST, 1979).

A significant enhancement was observed in the levels of all serum markers enzyme of DEN induced group when comparison to those normal group. MLAgNPs treated group restored all values of serum enzymes backs to that of normal level. These serum markers enzymes are used to detect the hepatic damage with HCC injuries where all these enzymes are discharged from the injured hepatocytes into the circulatory system since these are essentially restricted in the liver. Serum bilirubin is an intracellular enzyme available in the liver and is the indicator for hepatic damage. MLAgNPs shows the stability of membrane or repair the liver damage by

maintaining the functional integrity of the membrane in liver. They reduce the spillage of marker enzymes through the layers of membrane and giving hepatoprotective activity and hindrance of carcinogenesis (Zaheer N,)

In DEN induced group, defective protein synthesis takes place which cause hepatic cell damages in turn, HCC. Silver nanoparticles subsequently stop the deleterious effect of these non-hepatic enzymes and initiate the synthesis of protein. AFP is a typical gold marker diagnosed in the serum of HCC patient and germ cell malignancies. Elevated level of AFP is seen in the wistar rats when presented to hepatocarcinogens. It is a glycoprotein whose moleculer weight is 70 kD, situated at chromosome 4q11-q13, showing allelic losses in liver cancer. There are a few reports which demonstrate that AFP assumes a fundamental part in the control of tumor development, cell separation and mediate multiplication of human hepatoma cells conceivably through the AFP receptors. Elevated level of AFP was seen in DEN induced group demonstrating the existence of hepatic cell carcinoma. The plausible clarifications for the expansion of AFP synthesis by neoplastic hepatocytes are either expanded interpretation of AFP gene or post-translational change influencing AFP formation. MLAgNPs fundamentally lessened the AFP level when contrasted with DEN initiated liver cancer group.

A few studies demonstrated that liver is the main centre for the production of proinflammatory cytokines (IL-1β, TNF-α and IL-6) through blood (lymphocytes and monocytes) along with Kuffer cells, human and murine hepatocytes. Kuffer cell assume a vital part in the arrival of IL-6 through modification of MyD88-subordinate NF-kB triggering, which additionally stimulate the IL-1a from hepatocytes. DEN demonstrates the impact on the Kuffer cells and also mediate the NF-kB triggering MyD88-subordinate; which initiate the discharge of IL-6 and induced the hepatic cancer. Our investigation affirms the restraint of IL-6 and furthermore decreases the secretion of IL-6. Another proinflammatory cytokines, TNF-α which is consider as NF-kB activator, produced during inflammatory response assumes a noteworthy

part in inflammation and termed as cell multiplication accelerator (Tolba R, 2015). The outcome demonstrated the lessened substance of TNF-α in DEN induced hepatic cancer wistar rat group, which was down regulated by MLAgNPs, affirmed the improved level of TNF-α close to control group rats. Advance investigation for the affirmation of mode of action needs a broad research by the researcher to build up its known mechanism.

LPO is regarded as the traditional marker of oxidative stress. A few occurrences affirmed the conceivable system for destruction of the corpuscle in LPO, which is generated by the free radicals. Free radicals assimilate the electron from the plasma membrane and begin harming the cells with lipid destruction. Steady production of oxygen responds with the unsteady unsaturated fat, produces peroxy unsaturated fatty radical, this radical keeps on responding with the another free radical and generate eccentric chain of lipid peroxide during the oxidative stress. During DEN initiated hepatic cancer, MDA respond to different free radicals and start the oxidative stress, which are responsible for carcinogenesis (Mansour HH, 2006). The upgraded level of LPO after DEN administration enhanced the production of free radicals, which was affirmed by decrease level of endogenous antioxidant. Enhanced level of LPO concentration was deduced by the MLAgNPs dose dependently and ascribed to their free radical scavenging impact.

ROS and oxidative stress are the main factor responsible for the hepatic tissue damage which develop tumour, in turn cause HCC. The reason behind the free radical is GSH and its metabolizing enzymes. The antioxidant enzymes are the first-line defence mechanism against such damage and thus provide protection against the deteriorating outcome. The enzymatic and non-enzymatic antioxidants, which were researched, are known to diminish the oxidative stress by decreasing the production and assembling of superoxide radicals (O_2). Protection was seen by the *M.longifolia* embedded silver nanoparticles in dose dependent manner and revert back the DEN induced deduction in all antioxidant. The protective effect was shown by the

flavonoids present in the silver nanoparticles on the cell which modulate the gene expression and all enzymes level activity which directly involved in antioxidant defence and glutathione effect (Pradeep K, 2007). It might be possible that the different phytoconstituents of the plant are associated with the free scavenging radicals from the tissues, consequently, decreasing oxidative stress.

A few exploratory studies affirms that membrane bound agents such as Na^+/K^+ ATPase and Ca^{2+}ATPase transfers the ions over the cell membrane by utilizing the energy in the form of ATP. Production of lipid peroxidation change the basic structure of cell membrane which further specifically influences the membrane bound ATPase action. DEN induced liver cancer rats affirmed the lessening of the membrane bound enzyme and MLAgNPs treatment raised the Na^+/K^+ ATPase and Ca^{2+}ATPase by means of balancing out the intrusion in the potassium and calcium digestion (**Selvendiran, K. 2004**). So, we officially affirmed that the MLAgNPs administration maintains the concentration of the lipid peroxidation in DEN induced HCC groups, protects the hepatocytes from damage and responsible for cell membrane integrity.

Renal cancer

Carcinogenesis (development of cancer) in which proliferation of cell takes place from normal cells and this process is also known as oncogenesis or tumorigenesis. These changes occur at cellular, genetic, epigenetic levels and it is a multi-step process. Free radicals species are accounted for to be associated with advancement of carcinogenesis and oxidative stress. In our daily life, we continuously come in the contact of number of chemicals which demonstrate as free radical generators. These free radicals are capable for number of occasions that prompt renal cancer. These toxicant raises the levels of redox ion, which actuate the formation of ROS that can promptly assault the cellular mechanism. It was observed in literature that DEN induced renal cancer and altered the physiology of nephron and its lethal quality exposure is

well known process (Kundu JK, 2012). DEN is retained inside the portal vein through mesothelium after I.P treatment and goes into circulation by means of liver.

This outcomes in the formation of superoxide radicals (O2•-) to deliver hydroxyl radical (OH•), bringing about the generation of lipid peroxidation and affect the oxidative DNA in the body. From the study, it was proved that the introduction of DEN to damage the proximal tubular of kidney and resulting production of renal tumour in wistar rats at high rate and also induce renal cell carcinoma (Iqbal M, 2010).

This harmful chemical triggered oxidative stress and tissue damage was decreased by few free radical scavengers and antioxidant. Bioactive silver nanoparticles of plant extract gave a somewhat huge assurance to reduce the renal tumour which was induce by DEN consumption and this assurance can be ascribed to its antioxidant and free radical scavenging activity. Impact of MLAgNPs was also examined on other biochemical parameter and renal tumour promotion markers instigated by DEN. This induction show noteworthy exhaustion in the renal GSH substances and its related enzymes. This toxicant caused reduction in the levels of GSH and its metabolizing enzymes which is a natural antioxidant present in the cell.

Elevated levels of BUN, serum creatinine and uric acid in toxic induced wistar rats group represent cellular leakage and loss of cell membrane integrity. It associates with the same outcomes of down regulation of renal GSH is noted with associative diminish in GR, GPx, QR, and GST levels on DEN administration. When the level of GSH decreased, GR level maintain while GPx utilizes it for decomposition of lipid peroxides or hydro peroxides and ROS. After the administration of MLAgNPs to DEN induced group in a dose dependent manner, it recuperate the drained GR, GPx, GSH, and GST levels. Renal dysfunction occurred due to the enhanced levels of LPO and the related damage of membrane embroiled in the pathophysiology of renal cell carcinoma. Renal dysfunction is trailed by the raised levels of the serum enzymes showing leakage of cell and renal cell membrane functional integrity (Prabha SP,2012)

Toxic induced group animals show elevated level of LPO by the presence of mild inflammation in the renal tissue. MLAgNPs ameliorate the malicious impact of DEN on the renal tissue. This impact was correlated with oxidative stress and renal tumour promotion markers because other biochemical parameter also affected.

The ODC determine as a rate restricting enzyme utilize in the biogenesis of polyamines, spermidine, spermine, and putrecines. Raised level of ODC activity has been reliably recognized in renal cell and assumes a huge part in tumour induction. ODC activity and [^{3}H] thymidine incorporation have been generally utilized as biochemical marker to assess tumour inducing capability of a hazardous chemical substance (Wu D, 2015) The activity of ODC and thymidine incorporation were restrained by the biofabricated *Madhuca longifolia* silver nanoparticles in a dose dependent manner which reveals that it has anti-cancer and anti-proliferative property. Hindrance of ODC action and DNA synthesis uncovers that MLAgNPs may block tumour progression and altered the mechanism of polyamine biosynthesis and arachidonic acid digestion.

When this toxicant (DEN) administered to wistar rat it incited the expression of proinflammatory cytokines IL-6, TNF-α and IL-1β which are said to be direct transcriptional regulation of NF-κB. These cytokines and mediators have an imperative part in irritation, vascular permeability (capillary and microvascular permeability) and additionally multiplication of cell in cancer (Eda H, 2011). In this manner, MLAgNPs play a vital role by restraint their secretion and show its defensive impact against renal carcinogenesis. NF-κB known to manage articulation of various genes that assumes a vital part in aggravation and tumorigenic process, for example, cell expansion and angiogenesis. Different oxidants produced by DEN introduction are known to direct NF-κB expression. In understanding with the prior reports, DEN was found to enact NF-κB articulation in the kidney tissue prompting renal irritation (Nagel D, 2014) Administration of MLAgNPs found to repress NF-κB

expression in the kidney in this manner, recommending that silver nanoparticles prevent renal cell carcinoma. Consequently, hindrance of proinflammatory cytokines by MLAgNPs treatment may be currently acknowledged up 'till another important technique is available to control the cancer formation.

Carissa carandas

The present study was an endeavour to biosynthesize the silver nanoparticles of plant extract with a result to control the development of hepatic cancer in wistar rats. It was suggested that the silver nanoparticles delivers high amount of biomolecule in the liver and is exceptionally effective in impeding the DEN induced liver cancer. The nanoparticles synthesized by green synthesis are biodegradable, non-toxic and economic in nature, and provide therapeutic efficacy.

DEN initiated hepatocarcinogenesis in rat, is a standout amongst the most acknowledged animal models for hepatic cancer, which permits to determine the anticancer agents on various periods of neoplastic change and development. So, we have investigated the protective impact of silver nanoparticles of *Carissa carandas* on DEN initiated liver cancer in animals in dose dependent manner and attempted to interpret the mode of action of silver nanoparticles in liver cancer.

It has been already established in various documents that hepatocytes identified with salient hyperplasia, cell multiplication, and enzymatic markers is responsible for preneoplastic knobs in liver tissue. It was mentioned in literature that the development of hepatocyte knobs assumes a vital part in the expansion of hepatic cancer (Kalinina OA, 2003). Over the span of study, the outcome suggested that the synthesized silver nanoparticles declined the expansion hepatic knobs and diminished the substance of hepatic serum marker enzymes in test animals. The outcome fulfilled the target of our theory by diminishing the formation of hepatic knobs and rejuvenating the serum marker enzymes against DEN induced hepatocarcinogenesis. It was

observed that the silver nanoparticles were effective against eradicating the liver toxicity which was produced by DEN chemical.

Alpha feto protein (AFP) is believed as the gold parameter, its size, shape and composition are somewhat like an albumin. The DEN induced group show higher level of AFP as compared to normal control group and significantly declined by biofabricated silver nanoparticles in dose dependent way. Diseased rats when treated with CCAgNPs declined the hepatic knobs and claim the chemoprotective impact; the present explanation is bolstered by the macroscopical and histopathological examination.

Cell destruction shows connection with the enzyme leakage of liver tissue. Serum AST, ALT and ALP is the most delicate markers utilized in the determination of liver damage. The enhancement in the level of hepatic serum enzymes and may be because of the spillage of these cytosolic enzymes and moving towards the circulatory system occurred due to hepatic destruction after DEN introduction. This is beginning of hepatocellular destruction because of hypofunction of liver and interruption in the biosynthesis of these serum markers enzymes, with changing the penetrability of liver cell membrane (Sharma V, 2012). Administration of CCAgNPs re-established the levels of these enzymes by changing the permeability and integrity of liver membrane.

Assessment of serum proteins, for example, albumin and globulin are the most important criteria for evaluating the secretory capacity of the liver. Moreover, the elevation in serum non-hepatic enzymes showed gentle haemolysis and hindrance in the discharge of bile. The raised level of globulin recommends increment in physiology of liver.

Bilirubin is produced as a metabolic product of haemoglobin, which involves in conjugation with glucuronic acid in hepatocytes. Estimation of serum bilirubin imparts a major role in evaluation of hepatic capacity, and any irregular increment in the levels of serum bilirubin demonstrates hepatobiliary illnesses and inflammation of liver function (**Kamisako,**

T, 2000) Treatment with silver nanoparticles in a dose dependent manner decreased the content of serum bilirubin and involves in regeneration of normal activity of liver.

Enzymatic antioxidant parameters are engaged with rummaging superoxide anion to give hydrogen peroxide, subsequently declined the dangerous impact caused by these radicals. SOD and Catalase are vital enzymes of the antioxidants parameter. Declines in these parameter may bring about various harmful impacts (Weydert CJ, 2010) In this investigation, we watched that CCAgNPs expanded hepatic SOD and catalase enzymes in DEN induced liver cancer. These demonstrated synthesized silver nanoparticles can lessen reactive oxygen species and diminishing oxidative destruction to the hepatic tissues other than enhancing the action of enzymatic antioxidant parameters.

Glutathione (GSH) is non-enzymatic antioxidant parameter displays in high amounts in the liver. It evacuates free radical species, for example, hydrogen peroxide, superoxide radicals, alkoxy radicals and acts as a substrate for glutathione peroxidase and glutathione transferase. Declined level of GSH is embroiled in the higher content of lipid peroxidation in DEN treated animals (Ganie SA, 2013) When DEN induced hepatocarcinogenesis was treated with CCAgNPs significantly raised the level of GPx and GSH in a dose dependent way, and depicting its ability to scavenge reactive free radical.

TNF-αand IL-6 are assumed as important inflammatory mediators of Proinflammatory cytokines which is induced by monocytes and macrophages during inflammation. TNF-α imparts a pivotal role in inflammatory responses and induction of apoptosis. It may empower the improvement of IL-6, IL-1β, PGE_2, collagenase, and adhesion molecules evoking the physiological activity. NF-κB is of two types i.e. hetero-or homo-dimer which is frequently characterize in five NF-κB subunits NFκB1, NFκB2, RelA (p65), RelB, and c-Rel. It control the cellular activity in cancer such as cell viability and inflammation. Initiation of hepatic cancer made by DEN showed its impacts on Kuffer cells of liver by the triggering of NF-κB

and controls the various inflammatory mediators (IL-6 and TNF-α) into the circulatory system (**Hussein, S. Z, 2012**) It was expected that synthesized AgNPs reduces inflammation which is associated with hepatic cancer. We trust that biosynthesized silver nanoparticles possess its activity due to presence of bioactive compounds which is present in the nanoparticles.

Renal cancer

DEN is considered as an important environmental carcinogen, which leads to generate ROS and resulting into cellular damage and oxidative stress. Cytochrome P450, metabolized the DEN in the body to produce highly reactive free radicals and starts of lipid peroxidation of cell and other organelles of the cells. The generated free radicals, cause the oxidative injury to DNA, proteins and lipids in the cells. So, the antioxidants agents present in the herbals drugs prevent against the oxidative stress and nephrotoxicity induced by DEN (Pisoschi A, 2015). In the present study, we test to hypothesis that plant extract and its biosynthesized silver nanoparticles, could prevent against DEN induced renal cancer through the antioxidant property which is retained by the chemical constituent of plant extract.

The current study revealed that the introduction of DEN has initiated the renal cancer in animals and this was proved by the enhanced levels of the serum markers such as urea, creatinine and uric acids of DEN induced renal carcinogenesis in rats. Rise in the levels of the serum creatinine is an important indicator of renal failure and its range narrates the glomerular function. Upgradation in the levels of uric acid in blood caused hyperuricemia and a sensitive marker of inflammation which takes places at various sites of the body. Simultaneous administration of plant extract and biosynthesize silver nanoparticles significantly reduced the levels of urea, creatinine and uric acids and theoretically protected the DEN induced alterations in the renal tissue.

The noticeable enhancement in the levels of serum LDH, XO and GGT is a indicator for the nephrotoxicity and renal damage. DEN induced kidney damage which is characterized

by the elevations in the activity of LDH, XO and GGT. The rises in the levels of these serum enzymes might be owing to spillage of these cystolic enzymes into the blood stream and responsible for renal damage. This is an indicator for the beginning of kidney damage owing to renal dysfunction, disruption in the biosynthesis of these serum enzymes and change the permeability of the membrane (**Yousef, M. I.,, 2015**). Treatment with the CCAgNPs prevented DEN induced renal illness, by decrease in serum enzymes and maintaining the cellular membrane of the kidney.

The plenty of the long chain polyunsaturated fatty acids in the composition of lipids in renal makes the kidney susceptible to impairment caused by free radicals. The breakdown of nitrosamines has been advocated to produce ROS. The generated ROs initiate cellular damage, fragmentation to DNA, degrade the protein and lipid peroxidation and change the antioxidant defence mechanism. We observed that the DEN induced animals unveiled a significant upregulation in the activity of MDA which shows a serious harm to renal tissue (Valko m, 2016). Daily treatment with plant extract and CCAgNPs markedly attenuated the increased levels of MDA indicating that either therapy have a potent free radical quenching property.

In our study DEN administration resulted in significant elevation ($p<0.001$) in the level of MDA with the reduction in the GSH (free thiols), GSH and its oxidized equivalent glutathione disulfide which suggest a major redox reaction of the cell. GSH play either as a non-enzymatic antioxidant by reacting directly with thiols groups of reactive oxygen species or involved as a cofactor or coenzyme in the detoxification reaction for reactive oxygen species. The diminution of GSH content may be accredited to the direct conjugation of DEN and its metabolites with free or protein bound-SH groups and markedly reduction in the GPx with contrast to the controls. It was suggested that DEN treatment caused inflammation and disturbed the renal redox cycle. DEN treatment were also reduce the levels of other antioxidant enzymes such as GR and GST (Puiggros F, 2005). CCAgNPs treatment at both the dose levels

increased the content of the all renal antioxidant enzymes close to normal. Therefore we undertake that the nephroprotective tool of CCE and CCAgNPs against DEN induced oxidative stress is partly intervened by protecting GSH reduction and potentiation of the enzymatic antioxidant defences.

Exposure to DEN prompted the expression of proinflammatory cytokines TNF-α, IL-6, IL-1β under direct transcriptional directive of NF-κB. These cytokines have a crucial role in the vascular permeability, cell multiplication and inflammation. Oxidative stress is also main reason behind the inflammation mediated the production of many proinflammatory cytokines. It also plays an important role in the advancement of renal cancer. Redox system also affect the NF-κB regulation and several genes directly involved in the transformation, multiplication and angiogenesis of cell. There is a complex relationship between a reactive oxygen species and NF-κB. Inhibition of NF-κB is a good approach to regulate tumourgenesis and tumour advancement (Hassa PO, 2008).NF-κB is very delicate to ROS and stimulated by strong carcinogens such as DEN. CCAgNPs inhibits the secretion of the cytokines and plays a major role in its preventives effects against renal carcinogenesis. Therefore, the toxicity of DEN to a certain extent controlled by the inhibition of NF-κB.

Oxidative stress and inflammation are linked with the tumour promotion during renal nephropathy. ODC is considered as a first and rate limiting enzyme in the biosynthesis of polyamines, involved in DNA synthesis and multiplication of cell. Elevated levels of ODC activity was observed in transformed cell lines and is closely associated with tumour promotion and tumourgenesis. ODC activity and ^{3}[H] thymidine incorporation is important biochemical marker tumour promotion and to evaluate potential of an agent by targeting these (**Keough, M. P, 2011**). Treatment with DEN in the current study directed to a significant elevation in ODC activity and ^{3}[H] thymidine incorporation in the renal tissue, which is significantly improved by the treatment with CCAgNPs. Inhibition of ODC activity and synthesis of DNA discloses

that CCAgNPs may interrupt tumour promoting and dangerous functions of polyamine biosynthesis.

Morphologic examination of renal tissue showed the occurrence of tumour necrosis ad proteinous casts and further established by renal tubular damage. These alteration in the histopathological study of kidneys were effectively restored by higher doses of CCAgNPs.

This study reveals the chemoprotective effect of CCAgNPs against DEN induced nephrotoxicity and renal cancer. In short, our proposed study provides suggestions that treatment with CCAgNPs ameliorated DEN induced oxidative stress and inflammation in rats. Future examination focused on molecular pathways which involved in the modulatory mechanism of CCAgNPs on DEN induced renal tumourgenesis are prerequisite.

Chapter-6

Summary & Conclusion

6.1 Summary

Cancer is main reason for death in developed and developing countries and equivalent to myocardial infarction. Environmental factors (about 70-80%) is responsible behind for causing causer in humans. Now a day's surgery, chemotherapy and radiotherapy lowered the mortality rate of cancer but leads to cause side effects and adverse hazards. It is become challenging task for the researcher to replace the chemotherapeutic medicine with the economical drug for the treatment of cancer. Scientist focus in the field of natural products which reduces the side effects and supress the effect of carcinogenesis. They isolates a different bioactive compounds which have potential to prevent from hepatic cancer and renal cancer. There are more than 100 plants available worldwide which have a tendency to prevent or fight with different types of cancer. Therefore Exploration have been introduced to examine the efficacy of the silver nanoparticles from leaf extract of plant *Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas* on human hepatic cell line *in-vitro* and *in vivo* DEN induced hepatic cancer in rats.

Standardization were done by evaluating pharmacognostical and phytochemical study were performed to identify the biological active compounds present in the plant extract. The phytochemical screening of the leaves of *Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas* shows the presence of secondary metabolites, which play an important role in therapeutic efficacy of medicine. The extract obtained by extraction of *Phyllanthus Emblica leaves* revealed the presence of alkaloids, flavonoids and glycosides. The extract obtained by extraction *Madhuca Longifolia leaves* revealed the presence of alkaloids, phenolic compounds, tannins, proteins, glycosides and flavonoids. The extract obtained by extraction *Carissa carandas leaves* revealed the presence of alkaloids phenolic compounds, tannins, glycosides and flavonoids.

Facile green synthesis of Silver nanoparticles was carried out by using *Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas leaves*. UV-Vis spectra were carried out for the

synthesized nanoparticles. The achieved absorption spectrum shown the presence of silver nanoparticles under the range of 200-800 nm. FTIR analysis were performed on the leaves extract and prepared silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*). The obtained frequencies in the spectrum confirmed the presence of different functional group which act as a capping and reducing agent in the silver nanoparticles. XRD studies reveals the crystalline plane and structure of silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*). The size and surface morphology of prepared silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) were confirmed by FESEM and TEM images. The purity and elemental composition of silver peaks were seen in EDX spectra.

From the study, it is concluded that plant have phytopharmaceutical importance. So, further studies were are required to carry out its biological activity. The plant mediated silver nanoparticles display anticancer property and effective in renal and hepatic cancer. The plant biosynthesized silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) show a strong cytotoxic effect on human hepatic (HUH-7) cell lines and evaluated by MTT assay. The IC_{50} value of silver nanoparticles were. The therapeutic property of does not drug depends on its clinical Value, but also to reduce side effects. The plant extract alone not produce any significant effect, so it is formulated into silver nanoparticles and reduce the toxic side effects.

Diethylnitrosamine (DEN) is a highly poisonous and toxic carcinogen which is present in our environment and responsible for the production reactive oxygen species (ROS), subsequently causing oxidative stress and cellular injury. In the present study, a single i.p. Injection of DEN in wistar rats causes a noticeable upgradation in the activities of serum AST, ALT, ALP and AFP. They are main indicator of hepatic damage and help in the diagnosis of hepatic diseases because after cellular damage, these enzymes are discharged to blood. The prepared silver

nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) reduced the level of serum enzymes and prevent liver damage by reducing free radical induced oxidative injury in hepatic.

There was marked increment in the concentration of α-fetoprotein (AFP) of DEN induced group and which was brought back to near value of normal by silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) in a dose graded system.

In the level of lipid peroxidation, there was signification elevation was observed in hepatoma bearing rats. Significant reduction was found in activities of lipid peroxidation by biosynthesized silver nanoparticles dose dependently may be due to scavenging the hydroxyl and peroxide radicals.

After the administration of biosynthesized silver nanoparticles from plant leaves (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*), an significant enhancement was observed in endogenous antioxidant enzymes which was lowered in DEN induced liver cancer rats.

There was alteration found in the membrane bound ATPase in liver of DEN induced hepatocarcinogenesis and altered parameter was increased by plant mediated silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) dose dependently. This clearly shows the anticancerous impact of silver nanoparticles

Histopathological studies also favours the outcomes of above findings. DEN induced group show loss of liver architecture, whereas, groups when treated with plant extract and silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) recovered the overall tissue architecture dose dependently. From the results, it must be conclude that biosynthesized silver nanoparticles useful in term of pharmaceutical product and can be use in the clinical treatment of hepatic cancer.

DEN is a well-known renal carcinogen. In the present study, aqueous extract of *Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas* and bio synthesized silver nanoparticles of all extract ameliorates the DEN induced renal oxidative stress, hyperproliferative reaction and renal cancer DEN enhances blood urea nitrogen, serum creatinine and uric acid in the animals when compared to normal control rats. A significant reduction was observed in the activities of all serum enzymes when treated with silver nanoparticles of different plant extract at the both levels.

An upregulation was observed in the level of LDH, Y-GGT and xanthine oxidase of all DEN induced renal carcinogenesis, which was downregulated by the silver nanoparticles of plant extract (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) at different dose levels.

Biofabricated silver nanoparticles of plant extract (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) at the both dose levels attenuates the MDA formation in the renal tissue of animals which was increased after DEN introduction in all rats except normal control group. Introduction of DEN also enhances the hydrogen peroxide generation with reduction in renal glutathione content, antioxidant enzymes, viz., glutathione peroxidase, glutathione reductase, catalase, glucose-6-phosphate dehydrogenase and phase-II metabolizing enzymes such as glutathione-S transferase. Oral introduction of rats with silver nanoparticles of plant extract (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) resulted in significant downregulation in H_2O_2 production, and incidence of tumours. The content of renal glutathione content, its metabolizing enzymes and antioxidant enzymes were also improved to significant level ($p < 0.001$).

DEN also lead to enhance in the levels of inflammatory markers and some proinflammatory cytokines viz. TNF-, IL-6, NF-. Proinflammatory cytokines and inflammatory markers were

also recuperated to normal level by silver nanoparticles (*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas*) at both levels treatment.

Tumor promotion markers viz., ornithine decarboxylase (ODC) and [^{3}H] thymidine incorporation into renal DNA were also significantly increased in DEN induced renal carcinogenesis. Treatment of animals orally with silver nanoparticles body resulted in a significant downregulation in ODC activity and [^{3}H] thymidine incorporation. Further all these alteration were also supported by histopathological observations.

Thus, current study favours silver nanoparticles of all plant extract ()*Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas* at both acts as potent Chemopreventive agent and suppresses DEN induced renal carcinogenesis and oxidative and inflammatory reaction in Wistar rat.

6.2 Conclusion

So in the same apprehension, it can concluded that the leaves of *Phyllanthus Emblica, Madhuca Longifolia and Carissa carandas* selected for the hepatic cancer activity and studied in the animal model. In animal model, it was observed that the silver nanoparticles of the aqueous extract of all plants showed significant effects for treating the hepatic cancer.

When we discussed about the renal cancer, similar results were also observed in the renal carcinogen treatment in in normal healthy rats (animal model). The biosynthesized silver nanoparticles of aqueous extract of *Phyllanthus emblica, Madhuca longifolia and Carissa carandas* showed the significant recovery effects on the DEN induced renal cancer animals. Because of the presence of the bioactive molecules in all the plants, we can concluded that the plant possess a anticancer property and biofabricated silver nanoparticles of plants extract consider for a hepatic and renal cancer and can be used for the therapeutic activity.

6.3 Scope for the future work

The bioactive molecules present in the plant i.e. *Phyllanthus emblica, Madhuca longifolia and Carissa carandas* possessing an anticancer property (hepatic and renal cancer). The nano-based drug from plant source with potential advantages consider as a non-toxic and biodegradable carrier for the delivery of the drug in the cancer treatment. So after all the findings about the all the plants, the silver nanoparticles of the plant extracts having a property to cure the hepatic and renal cancer. So the study could be further explore in the future by the formulation of silver nanoparticles into some products and the efficacy and all parameters of the products in the form of novel drug delivery system for hepatic and renal cancer will check in all aspects.

Chapter-7

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