

**ANTICARCINOGENIC ACTIVITY OF
CINNAMALDEHYDE
AND
NANO CINNAMALDEHYDE AGAINST
MAMMARY CANCER IN RATS**

By

**Ravi Killi, M.V.Sc.,
GVD/13-002**

*Thesis submitted to the
Sri Venkateswara Veterinary University
in partial fulfilment of the requirements for the award of the
degree of
Doctor of Philosophy in the faculty of Veterinary Science*



**Department of Veterinary Pharmacology & Toxicology
NTR College of Veterinary Science
Gannavaram-521102
May-2018**

CERTIFICATE

Dr. Ravi Killi has satisfactorily prosecuted the course of research and that the thesis entitled “**ANTICARCINOGENIC ACTIVITY OF CINNAMALDEHYDE AND NANO CINNAMALDEHYDE AGAINST MAMMARY CANCER IN RATS**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

(Dr. P. Ravi Kumar)

Date: 05-05-18

Place: Gannavaram

CERTIFICATE

This is to certify that the thesis entitled “**ANTICARCINOGENIC ACTIVITY OF CINNAMALDEHYDE AND NANO CINNAMALDEHYDE AGAINST MAMMARY CANCER IN RATS**” submitted in partial fulfilment of the requirements for the degree of ‘Doctor of Philosophy in Veterinary Science’ of Sri Venkateswara Veterinary University is a record of the bonafide research work carried out by **Dr. Ravi Killi** under my guidance and supervision. The student’s advisory committee has approved the subject of the thesis.

No part of the thesis has been submitted for any other degree or diploma. The author of the thesis has duly acknowledged all the assistance and help received during the course of investigation.

External Examiner

Dr. P. Ravi Kumar
(Chairman of the Advisory Committee)

Thesis approved by the Student Advisory Committee

Chairman: Dr. P. Ravi Kumar

Professor & University Head
Dept. of Vet. Pharmacology & Toxicology
NTR College of Veterinary Science
Gannavaram-521102

Member: Dr. K. Bharavi

Professor
Dept. of Vet. Pharmacology & Toxicology
NTR College of Veterinary Science
Gannavaram-521102

Member: Dr. V. Rama Devi

Professor & Head
Dept. of Vet. Pathology
NTR College of Veterinary Science
Gannavarm-521102

Member: Dr. R. Vinoo

Professor & Head
Dept. of Animal Genetics and Breeding
NTR College of Veterinary Science
Gannavaram-521102

CONTENTS

Chapter No.	Title	Page No.
I	INTRODUCTION	1 - 5
II	REVIEW OF LITERATURE	6 - 31
III	MATERIALS & METHODS	32 - 44
IV	RESULTS	45 - 116
V	DISCUSSION	117 - 136
VI	SUMMARY	137 - 140
	LITERATURE CITED	141 - 175

LIST OF FIGURES

Figure No.	Title	Page No.
1	Chemical structure of cinnamaldehyde	19
2	Transmission electron microscopic image of synthesized cinnamaldehyde mediated zinc nano particles (x 40K)	46
3	Scanning electron microscopic image of synthesised cinnamaldehyde mediated zinc nano particles (x 3500K)	46
4	Zeta potential and average size of the cinnamaldehyde zinc nano particles (CZN)-DLS method - Nanopartica SZ-100 (HORIBA)	47
5	Acute oral toxicity - cinnamaldehyde treated rat liver - showing congestion and mild degenerative changes (H&E x10)	51
6	Acute oral toxicity - nano zinc cinnamaldehyde treated rat liver - showing degenerative changes (H&E x10)	51
7	Acute oral toxicity - cinnamaldehyde treated rat myocardium - showing congestion and haemorrhage (H&E x10)	52
8	Acute oral toxicity - nano zinc cinnamaldehyde treated rat myocardium - showing congestion and hemorrhage (H&E x10)	52
9	Acute oral toxicity - cinnamaldehyde treated rat stomach - showing mild degenerative changes (H&E x10)	53
10	Acute oral toxicity - nano zinc cinnamaldehyde treated rat stomach - showing degenerative changes and infiltration in submucosa (H&E x10)	53
11	Acute oral toxicity - cinnamaldehyde treated rat kidney - showing congestion and mild degenerative changes (H&E x10)	54
12	Acute oral toxicity - nano zinc cinnamaldehyde treated rat kidney - showing congestion and mild degenerative changes (H&E x10)	54
13	Acute oral toxicity - cinnamaldehyde treated rat brain - showing congestion in cerebellum (H&E x10)	55
14	Acute oral toxicity - nano zinc cinnamaldehyde treated rat brain - showing congestion (H&E x10)	55
15	Chromatogram of cinnamaldehyde and cinnamic acid (0.01 to 0.1 µg/ml) in acetonitrile : 0.1% glacial acetic acid : water (50:25: 25)	56
16	Response linearity of cinnamaldehyde in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)	58

17	Response linearity of cinnamic acid in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)	58
18	Representative chromatogram of cinnamaldehyde and cinnamic acid showing recovery from spiked plasma samples in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)	59
19	Cinnamaldehyde mean plasma concentration versus time curve in rats (n=8) dosed with cinnamaldehyde at the rate of 500 mg/kg b.wt., orally	61
20	Cinnamic acid mean plasma concentration versus time curve in rats (n=8) dosed with cinnamaldehyde at the rate of 500 mg/kg b.wt., orally	61
21	Cinnamaldehyde (CNMA) and cinnamic acid (CA) mean plasma concentration versus time curve in rats (n=8) dosed with cinnamaldehyde at the rate of 500 mg/kg b.wt., orally	63
22	Effect of prophylactic cinnamaldehyde treatment on tumor latency period in rats administered with DMBA for mammary tumor production	65
23	Normal distribution pattern of mammary glands in rats	66
24	Mammary tumors in DMBA administered rats	66
25	Mammary gland tumor in thoracic and inguinal region in DMBA administered rat	67
26	Mammary gland tumor mass excised from DMBA administered rat	67
27	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on tumor volume (cm ³) in rats administered with DMBA	68
28	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on tumor burden (cm ³) in rats administered with DMBA	69
29	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on body weight (g) in rats	70
30	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma total sialic acid (µg/ml) levels in rats	72
31	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the liver of rats	75
32	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the lung tissue of rats	75

33	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the heart tissue of rats	76
34	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the kidney of rats	76
35	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the brain tissue of rats	77
36	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the mammary gland tissue of rats	77
37	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the plasma of rats	78
38	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the liver of rats	81
39	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the lung tissue of rats	81
40	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the heart tissue of rats	82
41	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the kidney of rats	82
42	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the brain tissue of rats	83
43	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the mammary gland tissue of rats	83
44	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the plasma of rats	84
45	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol}/\text{mg}$ protein) in the liver of rats	87
46	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol}/\text{mg}$ protein) in the lung tissue of rats	87
47	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol}/\text{mg}$ protein) in the heart tissue of rats	88

48	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ protein) in the kidney of rats	88
49	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ protein) in the brain tissue of rats	89
50	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ protein) in the mammary gland tissue of rats	89
51	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ protein) in the plasma of rats	90
52	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma total protein (T.P) and albumin (Alb) levels (g/dl) in rats	94
53	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma blood urea nitrogen (BUN) and cholesterol levels (mg/dl) in rats	94
54	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma creatinine and total bilirubin (T.B) (mg/dl) in rats	95
55	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma glucose (mg/dl) in rats	95
56	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma AST and ALT levels (IU/L) in rats	96
57	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma ALP levels (IU/L) in rats	97
58	Normal control - rat mammary tissue - normal architecture of mammary gland (H&E $\times 10$)	100
59	DMBA control - rat mammary tumor - showing tubular adenocarcinoma - proliferation of tubular structures separated by thin connective tissue (H&E $\times 10$)	100
60	DMBA control - rat mammary tumor - showing invasive tubular adenocarcinoma (H&E $\times 10$)	101
61	DMBA control - rat mammary tumor - showing tubular adenocarcinoma - alveoli lined by multiple layers of epithelial cells (H&E $\times 40$)	101
62	DMBA control - rat mammary tumor - showing papillary adenocarcinoma (H&E $\times 4$)	102
63	DMBA control - rat mammary tumor - showing papillary adenocarcinoma - proliferation of neoplastic cells giving finger like projections in to the lumen (H&E $\times 10$)	102

64	DMBA control - rat mammary tumor - showing comedo carcinoma with accumulation of necrotic cellular debris in the distended ducts (H&E x10)	103
65	DMBA control - rat mammary tumor - showing cribriform carcinoma with irregular shaped secondary lumina within the lobule showing sieve like pattern (H&E x10)	103
66	DMBA control - rat mammary tumor - showing cribriform carcinoma with neoplastic cells in the lobule forming secondary lumina (H&E x40)	104
67	Tamoxifen control - rat mammary tumor - showing area with regressive changes (H&E x4)	104
68	Tamoxifen control - rat mammary tumor - showing desquamation, less cellularity, connective tissue hyalinization and presence of multi nucleated cells (H&E x10)	105
69	Tamoxifen control - rat mammary tumor - showing regressive changes and desquamation of epithelium (H&E x10)	105
70	Tamoxifen control - rat mammary tumor - showing desquamation of epithelium (H&E x10)	106
71	Tamoxifen control - rat mammary tumor - area showing regressive changes with desquamated epithelium (H&E x10)	106
72	Tamoxifen control – rat mammary tumor - showing alveoli lined with single layer of epithelium, presence of cellular debris and connective tissue proliferation in the stroma (H&E x40)	107
73	CNMA prophylactic – rat mammary tumor - showing most of the alveoli were contain single layer of epithelia cells and stroma filled with abundant connective tissue (H&E x4)	107
74	CNMA prophylactic - rat mammary tumor - showing few alveoli were uniformly lined by single layer of epithelial cells and areas of epithelial desquamation (H&E x10)	108
75	Nano zinc cinnamaldehyde treatment-rat mammary tumor - showing different sizes of alveoli lined by single layer of epithelial cells and also alveoli were increased in number (H&E x4)	108
76	Nano zinc cinnamaldehyde treatment - rat mammary tumor - showing alveoli lined with single layer of epithelium and few alveoli were dilated (H&E x10)	109
77	Cinnamaldehyde treatment - rat mammary tumor - adenocarcinoma (H&E x4)	109
78	Mammary tumor, rat - DMBA control - TEM showing nuclear pleomorphism and nuclear folding (x3.5 K)	110

79	Mammary tumor, rat - DMBA control - TEM showing nuclear indentation and pleomorphism (x3 K)	110
80	Mammary tumor, rat - CNMA prophylactic - TEM showing a desquamated epithelial cell with an eccentrically placed indented nucleus and electron dense vesicles in cytoplasm (x3.5 K)	111
81	Mammary tumor, rat - CNMA prophylactic - TEM showing marked pleomorphism of nuclei (x3.5 K)	111
82	Mammary tumor, rat - Tamoxifen control - TEM showing connective tissue proliferation (x2.5 K)	112
83	Mammary tumor, rat - Tamoxifen control - TEM showing numerous centrally placed electron translucent bodies, cellular components were lost, large vesicles are present. Pleomorphic cells showing different stages of nuclei (x1.8 K)	112
84	Mammary tumor, rat - Tamoxifen control - TEM showing cells with various stages of nuclei showing severe to moderate swelling (x5 K)	113
85	Mammary tumor, rat - CNMA treatment - TEM showing perinuclear vesicles in cytoplasm of a cell (x3 K)	113
86	Mammary tumor, rat - CNMA treatment - TEM showing perivascular fibrous tissue proliferation (x2 K)	114
87	Mammary tumor, rat - CZN treatment - TEM showing pericellular fibroblast proliferation (x3 K)	114
88	Mammary tumor, rat - CZN treatment - TEM showing nuclear pleomorphism and a dividing nucleus in one of the cells (x1.8 K)	115
89	DMBA induced rat mammary gland adenocarcinoma showing abundant ER alpha expression in the nuclei of the glandular epithelium. Immunoperoxidase/ DAB substrate/ Mayer's haematoxylin counter stain (x400)	116
90	DMBA induced rat mammary gland adenocarcinoma showing abundant PR expression in the nuclei of the glandular epithelium. Immunoperoxidase/ DAB substrate/ Mayer's haematoxylin counter stain (x400)	116

LIST OF TABLES

Table No.	Title	Page No.
1	Treatment protocols in various experimental groups (n=7)	37
2	Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on mean body weight gain (g) in rats (n=5)	49
3	Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on relative organ weights in rats (n=5)	49
4	Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on various haematological parameters in rats (n=5)	49
5	Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on various plasma biochemical parameters in rats (n=5)	50
6	Cinnamaldehyde standard concentrations versus area under curve in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)	57
7	Cinnamic acid standard concentration versus area under curve in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)	57
8	Per cent recovery of cinnamaldehyde from spiked plasma samples with extraction solvent containing acetonitrile and 0.1% glacial acetic acid (90:10)	60
9	Per cent recovery of cinnamic acid from spiked plasma samples with extraction solvent containing acetonitrile and 1% glacial acetic acid (90:10)	60
10	Calculated noncompartmental kinetic model parameters of cinnamaldehyde (dosed with CNMA @ 500 mg/kg b.wt, orally) in rats	62
11	Calculated noncompartmental kinetic model parameters of cinnamic acid (dosed with CNMA @ 500 mg/kg b.wt, orally) in rats	62
12	Effect of cinnamaldehyde prophylactic treatment on tumor latency period in rats administered with DMBA	65
13	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on tumor morphometrics	68
14	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on body weight in rats	70
15	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma total sialic acid ($\mu\text{g/ml}$) levels in rats	71

16	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (SOD, U/mg protein) in various tissues and plasma of rats	74
17	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione (GSH) activity ($\mu\text{g}/\text{mg}$ of protein) in various tissues and plasma of rats	80
18	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS activity ($\mu\text{mol}/\text{mg}$ protein) in various tissues and plasma of rats	86
19	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma biochemical profile-I	93
20	Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma biochemical profile-II	96

Acknowledgements

I am thankful to, Dr. P. Ravi Kumar, Professor and University Head, Dept. of Vet. Pharmacology and Toxicology, NTR College of Veterinary Science, Gannavaram, for everything. He is more than a guide for me. I learned so many lessons which are necessary in life from him. He is a good teacher, gentleman, good administrator and kind person with a helping hand always. It is my pleasure to be associated with him.

I am grateful to, Dr. K. Bharavi, Professor, Dept. of Vet. Pharmacology and Toxicology, NTR College of Veterinary Science, Gannavaram, for his support, guidance and suggestions in my work and carrier.

I am indebted to, Dr. V. Rama Devi, Professor and Head, Dept. of Vet. Pathology, NTR College of Veterinary Science, Gannavaram, for providing everything to do the pathology part of my work and timely guidance.

I am thankful to, Dr. R. Vinoo, Professor and Head, Dept. of Animal Genetics and Breeding, NTR College of Veterinary Science, Gannavaram for his support and guidance in completion of this work.

I am very much thankful to, Dr. G.S. Rao, Associate Dean, NTR College of Veterinary Science, Gannavaram who made me to learn by his lectures and sharing of personal experiences. He was particularly of help in kinetic part of the study.

I am gratified to, Dr. TNVKV Prasad, Senior Scientist, Institute of Frontier Technology, Regional Agricultural Research Station, ANGRAU, Tirupati for the help in preparing nano material used in my work.

I am highly thankful to, Dr. P. Annapurna, Assoc. Professor and Dr. Ch. Sudha Rani Chowdary Asst. Professor, Dept. of Vet. Pathology for their constant motivation and generous help.

I sincerely thank to, Dr. K. Sudhakar and Dr. M. Muralidhar, Dept. of Animal Genetics and Breeding, for making me to learn the basics in molecular biology and their helping hand in collection of literature.

I am thankful to, Dr. P.V.S. Kishore, Professor and Head, Dept. of Veterinary Anatomy for providing facilities in carrying out histopathology work.

I am thankful to Dr. K. Aswini Kumar, Dr. T.V Chaitanya, Dr. M. Naveen Swaroop Dr. S. Gangu Naidu and Mrs. A. Sri Venkata Kanaka Durga, Dept. of Biochemistry, for extending the lab facility to carryout biochemical analyses.

I sincerely thank to, Dr. G. Sri Vidya and Dr. K.V. Venkat Rao Asst. Professors, Dept. of Vet. Pharmacology and Toxicology, for their concern and moral support in early preparation of the thesis.

My sincere thanks to, Dr. K.S.R Vithal, Professor, Library and Information Sc., for his receptive nature and timely help.

I am thankful to, Abdul Khuddus, Smt. Pavani Ganga Devi, Ranga rao, Smt. Ch. Syamala Devi, Rama Krishna, Venkateswaramma, Anji Babu, Vijayamma, Samuel, Ashok and Nageswar Rao, supporting staff of Dept. of Vet. Pharmacology and Toxicology, Dept. of Vet. Pathology and Dept. of Biochemistry, for their valuable helping hand.

I place on record my gratitude to, my beloved senior Dr.Ch.Venkat Satish Kumar, for his help during my PG days and his constant guidance, suggestions and

encouragement in completion of this work. My sincere gratitude to Dr. V. R. Dhyana, who helped me in histopathology work with patience.

I am very much thankful to, my colleagues and juniors Badhrayya, Senthil Nathan, Gunupuru Bhanu Prasad Rao, Shaan, Sri Ram, Jonathan James Moonnumackal, Manroop, Afroz Jahan, Usha, Sai Mahesh Reddy, Nagarjuna, Jissa, and Phanidra and also PG colleagues from other departments Dr. Anupama, Nanda Kishore, Paila Suresh, Paras Satish, Tarak, Madhesh, Pandu Bava, Srikanth, Sri Vidya, Bhavya, Bassu, Chinna, Rossee, China Sateesh, Shireesha, Krupa, Daniel, Bhargav, Shiva Ramkrishna, Achyuth Rao, Vasu, Raghu Ram, Arun, and many others for their all-out help and pleasant company.

I sincerely thank the office staff Smt. Geetha, Smt. Surya Kumari, Miss. Lakshmi Prasanna, Shiva Rama Krishna, senior Nagaraju and Junior Nagaraju and library staff members Smt. Prasanna, Shiva, Bosu, Nageswar Rao and others.

I am very much thankful to, my UG batch mates and sponsors, when I am in need of money during my Ph.D, Dr. T. Hari Krishna, VAS VD Battili, Dr. S. Anil Kumar, VAS, VD Kesupalli, Dr. B. Srinivasulu, VAS, VD, Kurupam and Dr. R. Pratap Kumar for their concern.

It is my pleasure to thank my 2k5 B.V.Sc., batch mates of C.V.Sc., Tirupati for their company during my UG days.

I am grateful to, Dr. Bala Krishna, VAS, Dr. Satyanarayana and V. Shivaprasad Rao, JVO, who played a critical role in the climax of my Ph.D and helped a lot in submitting the thesis at an early time.

I wish to express my thanks to, college watchmen Vijay Kumar, Nageswar Rao, Shiva Sankar, Rajesh and Kiran, for their timely help, patient listening of my feelings when I got bored during late hours in the lab.

I am thankful to, my house owners Smt. Sri Lakshmi and Annae Rama Rao, for their indirect support during my three year study.

I am very much thankful to, my Mamayya and Atthayya Sri Bobba Ajoy Kumar and Smt. Bodisree and my Bavmaridhi Srinivas Reddy and Smt. Priya, who always concerned about my well being and career development.

I am very much thankful to, my uncles and aunts Sri M. Chandhra Shekar Reddy and Smt. Vijaya Gowri, Dr. S. Uma Maheswar Reddy and Smt. Subhashni, Smt. Raghu Bala, Smt. Latha and their family members, for their affection.

I am thankful to, Krishna Reddy mamayya, for his blessings and constant encouragement in completing my work and preparation of thesis.

I feel happy to, express my gratitude to Murali anna and Pasi for their help when I am in need and their children brilliant Parineeth and cute Sreehan alias the 10 Seconds, for being a part in my life.

I am very much grateful to, my mamayya and attha Sri B. Kesava Rao and B. Alivealu Mangamma, for their helping hand and caring during my post intermediate days and B. Raja Shekar and Dr. Bala Bargav, for their concern on me.

My sincere regards to, my grandparents Late Killi Chelliah - Bharathamma and late Rajulu - Achamma. I am very much grateful to my uncles and aunts Sri Narendhra Dev and Smt. Nagamani, Sri Surya Prakash Dev and Smt. Vijaya Lakshmi, Sri Chandra Prakash Dev and Smt. Shoba Rani, Sri Yadhu Bhusan Dev and Smt. Vara Lakshmi and

Sri Ganesh Dev and Smt. Naga Mani, for their company and making me to understand the lessons of life.

It is my pleasure to, thank my younger brother Praveen Killi and sisters, Neeraja, Meena, Manasa, Dharani, Sindhu, Sravanthi, Sowmya, Mithra Vinda, Murali Manohar Sai, Bhumika, Shravika and Nikitha, for their love and affection.

My sincere thanks to, my sister-in-laws Parvathi, Saamma and Siri and brother in laws Sreenu and Rama, for their concern on my well being.

I whole heartedly thank my dearest friends Suneel and Dinesh and their families, for always being a part of my life.

I take this opportunity to profusely thank my Ph.D colleague Dr. Suresh N Nair, for being a God's gift all through my study period. He has been a continuous inspiration and pillar of support in all the needs. His elderly advices and technical skills have tremendously helped me to reach this stage. I am very much thank full to his beloved parents and his wife Mrs. Kavitha Nair and daughter Ms. Anakha for their indirect support.

I am highly thankful to, my uncle Sri. B. Chandra Shekar Rao and Smt. Dhamayanti, for their help and blessings.

I am thankful to, my brother-in-law Sri Jagadeesh and my sister Madhavi and their children Vinay and Lahari, for their affection and concern on my career and wellbeing.

All that I am or ever hope to be, I owe to my mother Smt. Lakshmi who sacrificed her entire life for her children and my daddy Late Sri. K. Jagganadha Rao, who taught me the basic values for leading a happy life.

I am very much grateful to, my wife, Dr. B. Vamsi Krishna, without whom I could not have thought of pursuing my Ph.D.

I am very much indebted to, my Peddamma Sri. Thavitamma, for sacrificing her major part of life for my family.

My brother and sister-in-law, Sri K. Raghu and Smt. B. Kumari and their children Chinmayee Sri and Sri Sai Sarvanan Adithya, have been a great support in my life and I am always indebted to them. Words are not enough to explain about my brother and sister-in-law for their constant support, playing a Father and Mother role and treating me as their elder son. My brother perfectly took his responsibility which is rare in these days by sacrificing his early life for the sake of me with the valuable support from my vadhinamma. I am very much thankful to God for making me a part in this family.

I thank the Sri Venkateswara Veterinary University for giving me the opportunity to pursue my Post Doctoral studies.

(Dr. Ravi Killi)

DECLARATION

I, **Dr. Ravi Killi**, hereby declare that the thesis entitled, “**ANTICARCINOGENIC ACTIVITY OF CINNAMALDEHYDE AND NANO CINNAMALDEHYDE AGAINST MAMMARY CANCER IN RATS**” submitted to Sri Venkateswara Veterinary University for the degree of Doctor of Philosophy is a result of original research work done by me. It is further declared that one research article from the thesis has been submitted for publication.

(Dr. Ravi Killi)

Date: 05-05-2018

Place: Gannavaram

Name of the Author	:	Ravi Killi
Title of the Thesis	:	Anticarcinogenic Activity of Cinnamaldehyde and Nano Cinnamaldehyde against Mammary Cancer in Rats
Degree to which it is submitted	:	Doctor of Philosophy
Faculty	:	Veterinary Science
Discipline	:	Veterinary Pharmacology and Toxicology
Major Advisor	:	Dr. P. Ravi Kumar
University	:	Sri Venkateswara Veterinary University
Year of submission	:	2018

ABSTRACT

Cancer is a growing health problem in both developing and developed countries. Cancer chemoprevention is defined as the use of natural or synthetic agents that reverse, suppress or arrest carcinogenic and/or malignant phenotypic progression towards invasive cancer. The effectiveness of many anticancer drugs is limited due to their inability to reach the target site in sufficient concentrations and efficiently exert the pharmacological effect on affected cells without harming the healthy cells. Therefore, the search for new anticancer agents with better efficacy and fewer side effects is an ongoing phenomenon.

Plant phenolic compounds are a group of secondary metabolites with wide pharmacological activities (Al-Rimawi *et al.*, 2016). Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of different oxidative associated diseases such as cancer (Dai and Mumper, 2010). *trans*- Cinnamaldehyde is a phenolic compound found naturally in

various species of the genus *Cinnamomum* and is used in preparing beverages, medicinal products, perfumes and cosmetics. Nanoparticles (NPs) are versatile agents with a variety of biomedical applications including drug delivery. Keeping this in view, the present study was undertaken to evaluate the anticancer potential of cinnamaldehyde (CNMA) and its nano preparation, the nano zinc cinnamaldehyde (CZN) in chemical induced rat breast cancer model. Further, cinnamaldehyde was also studied for its disposition kinetics in rats. For induction of mammary tumors, fifty days old virgin female Sprague-Dawley rats were administered with single oral dose of 20 mg dimethyl benz(a)anthracene (DMBA). Those rats positive for mammary tumors on 90th day were only selected for further studies. The study was carried out in seven experimental groups viz. normal control (C), DMBA control (DMBA-C), cinnamaldehyde prophylactic (CNMA-P), cinnamaldehyde treatment (CNMA-T), nano cinnamaldehyde control (CZN-C), nano cinnamaldehyde treatment (CZN-T) and tamoxifen (TAM). CNMA-P group rats received cinnamaldehyde (50 mg/kg b.wt) orally starting from the 45th day i.e. five days prior to the DMBA administration and continued to received the same until the end of the study on 120th day. CNMA-T, CZN-C and TAM treatment groups received the respective treatments for a period of 30 days beginning from the 90th day. All the animals were sacrificed on 120th day. Plasma concentration of cinnamaldehyde and cinnamic acid were estimated in rats administered with cinnamaldehyde at a dose rate of 500 mg/kg b.wt. From the pharmacokinetic study it was evident that cinnamaldehyde is rapidly converted into cinnamic acid (CA). Apparent volume of distribution (V_d) and plasma clearance (Cl) were high for CNMA but its AUC_{0-t} , MRT and $t_{1/2}$ values were low when compared to CA.

Tumor volume, tumor multiplicity, tumor burden, body weight, tissue antioxidant and peroxidation status and various plasma biochemical parameters including total sialic acid (TSA) levels were assessed in all experimental groups. In

addition tumor latency period was recorded in CNMA-P group. Mammary tumor tissues were subjected to light and electron microscopic examination for pathological changes and immunohistological studies for the presence of estrogen and progesterone receptors.

Prophylactic treatment with cinnamaldehyde increased the tumor latency period and decreased the tumor volume, tumor burden and tumor multiplicity. Compared to DMBA control group, cinnamaldehyde and its nano preparation showed favourable results in terms of tissue antioxidant profiles and plasma biochemical parameters. Mild to moderate regressive changes were observed in various treatment groups. However tamoxifen treated rats showed greater extent of favourable changes in terms of tumor histology.

The study revealed that, cinnamaldehyde (CNMA) and nano zinc cinnamaldehyde (CZN) have mild degree of anticancer effects, with CZN being little more effective than CNMA. However CNMA was more effective when used prophylactically than when used as a therapeutic agent. Hence, it is likely that CNMA can reduce the possibility of breast cancer occurrence and severity when used prophylactically.

List of symbols and abbreviations

%	: Percentage
@	: At the rate
±	: Plus or minus
®	: Trade mark
°C	: Degree centigrade
μ	: Micron
μg	: Micro gram
μL	: Micro litre
μm	: Micrometer
ALP	: Alkaline phosphatase
ALT	: Alanine aminotransferase
ANOVA	: Analysis of variance
AST	: Aspartate aminotransferase
AUC	: Area under curve
AUMC	: Area under moment curve
b.wt	: Body weight
BUN	: Blood urea nitrogen
CA	: Cinnamic acid
Cl	: Clearance
CNMA	: Cinnamaldehyde
CNZ	: Cinnamaldehyde nano zinc
CPCSEA	: Committee for the Purpose of Control and Supervision of Experiment in Animals
DAB	: 3,3- Diaminobenzidine tetrahydrochloride
DLC	: Dynamic light scattering
DMBA	: 7,12-Dimethylbenz(a)anthracene
DMSO	: Dimethyl sulfoxide
DTNB	: 5-5'-dithiobis(2-nitrobenzoic acid), Ellman's Reagent

EDTA	: Ethylenediaminetetraacetic acid
ELISA	: Enzyme-linked immunosorbent assay
ERBA	: Embarras river basin agency
ER	: Estrogen receptor
<i>et al</i>	: And others
g	: gram
g_n	: Gravitational force of earth
GSH	: Reduced glutathione (Glutathione)
HCl	: Hydrochloric acid
HPLC	: High pressure Liquid chromatography
IHC	: Immunohistochemistry
IP	: Intra peritoneal
Ke	: Elimination rate constant
Kg	: Kilogram
Ltd.,	: Limited
M	: molarity
M/s	: Messrs
MDA	: Malondialdehyde
mg	: milligram
Min.	: Minutes
ml	: Milli litre
mM	: Milli molar
mM	: Milli molar
MRT	: Mean residence time
NANA	: N- acetylneuraminic acid
NBT	: Nitro blue tetrazolium
NIN	: National Institute of Nutrition
nm	: Nano-meter
NPs	: Nanoparticles
OECD	: Organisation for economic co-operation

	and development
P.O.	: Per oral
PR	: Progesterone receptor
pH	: Negative log of hydrogen ion concentration
r	: Radius
ROS	: Reactive oxygen species
rpm	: Revolutions per minute
RT	: Room temperature
SD	: Sprague-Dawley
sec	: Seconds
SEM	: Scanning electron microscopy
SOD	: Superoxide dismutase
SRL	: Sisco Research Laboratories
T.P	: Total protein
$t_{1/2}$	: Half life
TAM	: Tamoxifen
TBA	: Thiobarbituric acid
TBARS	: Thiobarbituric acid reacting substances
TCI	: Tokyo Chemical Industry
TEM	: Transmission electron microscopy
TEP	: Tetraethoxypropane
T_{max}	: Time to reach to maximum concentration
<i>trans</i> -CNMA	: Trans cinnamaldehyde
TSA	: Total sialic acid
V	: Volume
V_d	: Volume of distribution
Wk(s)	: Week(s)
Wt	: Weight
Zn	: Zinc

α	:	Alpha
β	:	Beta
π	:	Pi

CHAPTER I

INTRODUCTION

Cancer is a major public health problem in many parts of the world. It is currently second leading cause of death and is expected to surpass heart diseases as the leading cause of death in the next few years (Siegel *et al.*, 2015). Cancer is a multifactorial disease arising from the accumulative effects of gene products of mutant proto-oncogenes, tumor suppressor genes and DNA repair genes, leading to the uncontrolled growth and spread of cancer cells (Klug *et al.*, 2010). Breast cancer represents the most common neoplastic disease and a major cause of cancer deaths in women. Apart from age (> 50) and a family history of breast cancer, many of the known risk factors for this disease are related to a woman's reproductive history, such as early menarche, nulliparity or a late pregnancy, and late menopause as well as prolonged oral contraceptive use and hormone replacement therapy (McPherson *et al.*, 2000). The failure in eradicating the disease is largely due to the lack of knowledge of specific etiologic agent, the precise time of initiation, and the molecular mechanisms responsible for cancer initiation and progression.

Breast is a hormone responsive organ by excellence. Its development is influenced by a myriad of hormones and growth factors, responding selectively to given hormonal stimuli with either cell proliferation or differentiation that permanently modify both the architecture and the biologic characteristics of the gland. Among all of

the complex hormonal influences, estrogens are considered to play a major role in promoting the proliferation of both the normal and the neoplastic breast epithelium. The presence of estrogen receptor alpha (ER α) in target tissue or cell is essential for their responsiveness to estrogen action. In fact, the expression levels of ER α in particular tissue have been used as an index of the degree of estrogen responsiveness (Katzenellenbogen, 1980). A vast majority of human breast carcinomas are initially positive for ER α , and their growth can be stimulated by estrogens and inhibited by antiestrogens (Russo *et al.*, 2000). Interaction of ERs with 17 β -estradiol (E2) leads to transcriptional activation of the associated genes *via* recruitment of co-activators and components of the basal transcriptional machinery. In addition to the nuclear ERs, plasma membrane-associated ERs mediate the non-genomic signalling pathway (Marino *et al.*, 2006).

Cancer is a multistage process defined by at least three stages: initiation, promotion, and progression (Ames and Gold, 1992; Guyton and Kensler, 1993; Schulte-Hermann *et al.*, 1990). It is generally believed that the initiation of breast cancer results from uncontrolled cellular proliferation and/or aberrant programmed cell death or apoptosis as a consequence of cumulative genetic damages that lead to genetic alterations that result in activation of proto-oncogenes and inactivation of tumor suppressor genes (Black, 1994; Van de Vijver, 1993).

Oxidative stress interacts with all three stages of cancer. During the initiation stage, reactive oxygen species (ROS) may produce DNA damage by introducing gene mutations and structural alterations into the DNA. In the promotion stage, ROS can contribute to abnormal gene expression, blockage of cell-to-cell communication, and modification of second-messenger systems, thus resulting in an increase in cell proliferation or a decrease in apoptosis of the initiated cell population. Finally, oxidative

stress may also participate in the progression stage of the cancer process by adding further DNA alterations to the initiated cell population (Klaunig, 1998).

The possibility of using bio-modulators of breast carcinogenesis, such as selective estrogen receptor modulators (SERMS), aromatase and cyclooxygenase inhibitors, and dietary factors is very promising (Howe and Dannenberg, 2002; Powles, 2003). There are no very effective drugs to completely cure most of the cancers. There is a general call for new drugs that are highly effective, possess low toxicity, and have a minor environment impact. In fact, natural products play a major role in cancer prevention and treatment. A considerable number of antitumor agents currently used are of natural origin. Dietary plant-such as fruits, vegetables, spices, cereals, and edible tubers/roots-which also contain significant levels of bioactive natural compounds, may provide human health benefits beyond basic nutrition to reduce the risk of many chronic diseases including cancer (Liu, 2003). Many epidemiological studies have concluded that a diet rich in fruits and vegetables reduce the incidence of cancer in humans (Dewanto *et al.*, 2002). The cancer-protective effects elicited by these dietary compounds are believed to be due to the induction of cellular defence systems including the detoxifying and antioxidant enzyme system as well as the inhibition of anti-inflammatory and anti-cell growth signalling pathways culminating in cell cycle arrest and/or cell-death (Kwon *et al.*, 2007).

The usefulness of most conventional cancer therapeutics is often limited due to insufficient delivery of therapeutic drug concentrations to the target tumor or due to severe and harmful toxic effects on normal tissues. It has been shown that drug delivery to various sites within the body is directly affected by particle size (Hughes, 2005; Kawashima, 2001). Local drug delivery or drug targeting results in increased local drug concentrations and provides strategies for more specific therapy. Nanoparticles have specific particles as tools to enable these strategies (De Jong and Borm 2008).

Nanotechnology is traditionally defined as submicron sized molecular devices or nanoparticles predominantly ranging from 5 to 500 nm in at least one dimension. Especially in the area of drug delivery relatively large (Size 100 nm) nanoparticles may be needed for loading a sufficient amount of drug onto the particles (De Jong and Borm, 2008). Nanotechnology is offering several advantages to phytochemical delivery and to drug delivery in general (De Jong and Borm, 2008). These include benefits such as their small size which allows penetration of cell membranes, binding and stabilisation of proteins and lysosomal escape after endocytosis. Nanotechnology offers the potential to improve drug solubility, prolong drug half-lives in plasma, minimize off target effects and concentrate drugs at a target site (Harris *et al.*, 2006).

Mammary carcinomas induced in rats by means of chemical carcinogens, mainly 7,12-dimethylbenz(a)anthracene (DMBA), seem to provide a good model for understanding of the mechanisms of susceptibility to carcinogens (Russo *et al.*, 1979). Since cancer is the most common cause of death in the world population, the possibility that readily available natural substances from plants, herbs, vegetables and spices may be beneficial in the prevention of cancer (Abdullaev, 2002). Many epidemiological studies have concluded that a diet rich in fruits and vegetables reduces the incidence of cancer in humans (Dewanto *et al.*, 2002).

Cinnamomum cassia is important not only as a spice but also for its antioxidant activity (Lin *et al.*, 2003; Shan *et al.*, 2005; Dugoua *et al.*, 2007). Cinnamic aldehyde and its derivative cinnamic acid are the main chemical ingredients of *C. cassia*, which has been reported to possess anti-oxidative (Foti *et al.*, 2004) and anti-inflammatory properties (Liao *et al.*, 2012). Cinnamaldehyde is another phytochemical found in cinnamon and exhibits several biological effects such as anti-tumoral, proapoptotic and anti-inflammatory activities (Chao *et al.*, 2008; Ka *et al.*, 2003).

It is well known that zinc is a powerful antioxidant metal. It is the core constituent of antioxidant enzymes such as superoxide dismutase (SOD) and a recognized protector of sulfahydryl (-SH) groups; it is also thought to impair lipid peroxidation by displacing transition metals such as iron and copper from catalytic cells (Aitken and Roman, 2008). ZnO NpS is able to protect cell membrane integrity against oxidative stress damage, increase antioxidant enzyme levels and decreased MDA level. It can improve antioxidant activity, enhance the activities of antioxidants and decrease the levels of free radicals (Badkoobeh *et al.*, 2013).

In view of the above the present study was undertaken with the following objectives,

1. To study the kinetics of cinnamaldehyde in rats.
2. Assessment of anticarcinogenic effect of cinnamaldehyde in rats with chemically induced mammary cancer.
3. Evaluation of anticarcinogenic effects of nano cinnamaldehyde in rats with chemically induced mammary cancer.

CHAPTER II

REVIEW OF LITERATURE

2. CANCER

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells, which if not controlled, can result in death. Cancer is caused by external factors, such as tobacco, infectious organisms, unhealthy diet, and internal factors such as inherited genetic mutations, hormones and immune conditions. These factors may act together or in sequence to cause cancer. In 2012 there were 14.1 million new cancer cases and 8.2 million cancer deaths worldwide (International Agency for Research on Cancer, IARC) and by 2030, the global burden is expected to grow to 21.7 million new cancer cases and 13 million cancer deaths (Bray *et al.*, 2012).

2.1 BREAST CANCER

Breast cancer is an uncontrolled growth of breast cells originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules as a result of mutations in the genes responsible for regulating the growth of cells and keeping them healthy.

Globally, breast cancer accounts for > 4,00,000 deaths (Jemal *et al.*, 2011). In India, the number of new breast cancer cases is about 1,00,000 per year and is expected

to rise to 2,50,000 new cases per year by 2015 (Yadav and Jaroli, 2010). Breast cancer projection for India during time period 2020 suggests the number to go as high as 17, 97,900 (Malvia *et al.*, 2017). Many factors are believed to contribute to this high burden of breast cancer, including lifestyle, environmental, genetic and biological factors (McTiernan, 2008; Cummings *et al.*, 2009).

2.1.1 Role of Estrogen in Breast Cancer

Breast cancer is a hormone dependent malignancy. Sex steroid hormones have a major role in the growth and development of target tissues including the mammary gland where they interact with other hormones, growth factors and cytokines in the precise regulation of proliferation and differentiation. The risk of developing breast cancer has been traditionally linked to exposure to estrogen, mainly because, a majority of breast cancers contain receptors for this hormone (Russo *et al.*, 2000).

Clinical-epidemiological studies have shown a strong correlation between the actions of ovarian steroid hormones, particularly 17 β -estradiol, and carcinogenesis in the mammary gland and uterus (Pike *et al.*, 1993; Boyd *et al.*, 1996). The biological effects of estrogens are mediated by estrogen receptor (ER), a member of the superfamily of nuclear receptors that function as ligand-inducible transcription factors (Tsai and O'Malley, 1994). The presence of estrogen receptors (ERs) in cells is a useful marker indicating their potential regulation by estrogenic stimuli. ERs are expressed in the epithelial cells of the mammary gland, a classic target tissue in which estrogens play a central role during development and growth. It has only recently been clearly shown that estrogens induce cell proliferation in the normal human mammary gland (Laidlaw *et al.*, 1995). About 60-70% of human breast cancers are ER-positive and estrogen dependent (Masood, 1992) and ER and progesterone receptor (PR) expression is an important indicator of potential response to hormonal therapy (Trichopoulos, 1972). Despite higher or lower levels of ER expression, most tumors positive for ER respond

to anti-estrogen therapies. However, approximately 30% of the ER-positive tumors fail to respond to anti-estrogen therapies, and thus carry a poor prognosis (Allred *et al.*, 2004). The factors that control ER expression in tumor cells are unknown, and it is not well understood why a small fraction of ER-positive breast cancers do not respond to anti-hormone therapies.

The mechanism of estrogen activation *via* ERs is a very complex process. ERs are transcription factors that are specifically activated by estrogens. Hormone, estrogen on binding to ER protein, several events occur, including conformational changes, receptor dimerization, liberation of chaperones, phosphorylation, exposure of zinc fingers, interaction with accessory proteins, and binding of estrogen-ER complex to the estrogen-responsive elements present in the promoter region of the target genes and recruitment of various co-activators to mediate transcriptional regulation (Landel *et al.*, 1994; Beato and Sanchez-Pacheco, 1996; McDonnell and Norris, 2002).

Although estrogens may favour DNA damage through free radicals and thus be involved in tumour initiation (Han and Liehr, 1994), they have been mainly implicated in tumor promotion because of their mitogenic activity. When the cells are under pressure for continuous cell cycling, it is more probable that errors at the DNA level (endogenously and exogenously produced) are not repaired and hence transmitted to the daughter cells. Such errors may involve inactivation of antioncogenes or of DNA repair mechanisms and activation of oncogenes.

Estrogen administration induces increased cell proliferation with a delay (the maximal effect at 7 days), (Laidlaw *et al.*, 1995). This is clearly seen *in vitro*, when estrogens-deprived cultured conditions, and *in vivo*, when oestrogens are required to induce the growth of ER-positive human breast cancer cells implanted into athymic nude mice (Brunner *et al.*, 1989). In addition, a study on mammary gland tissues from mice treated with implants containing pure anti-estrogens revealed that estrogens act

directly on mammary cells and not primarily through the stimulation of pituitary mammogens (Silberstein *et al.*, 1994).

Estrogens are mitogenic as a result of accelerated progression through G₁ phase of the cell cycle (Lippman *et al.*, 1976; Leung and Potter, 1987). Recruiting non-cycling, G₀, cells into the cell cycle has been documented for estrogen. Another target of estrogen in the control of cell cycle progression is the proto-oncogene *c-myc*. *c-myc* is apparently directly transcriptionally regulated by estrogen via an atypical estrogen response element (Dubik and Shiu, 1992). The mitogenic, apoptotic and oncogenic functions of c-Myc depend upon dimerization with the heterologous protein Max, DNA-binding and transactivation, suggesting that c-Myc transforms cells by activating genes involved in cell proliferation and/or apoptosis (Amati and Land, 1994). c-Myc is rapidly upregulated by estrogen in breast cancer cells, suggesting a similar importance in estrogen-regulated cell cycle progression. Mitogenic effects of estrogen appear to be mediated by at least two apparently distinct pathways, of which c-Myc and cyclin-D1, respectively, are the key regulators for DNA synthesis and progression from G₁ to S phase of the cell cycle (Doisneau-Sixou *et al.*, 2003).

Estrogens also inhibit apoptosis, by disruption of estrogen-dependent signalling (Wang and Phang, 1995). Increasing evidence indicates that 17 β -estradiol (E₂) induces nongenomic effects (often immediate and transient) *via* signalling pathways more commonly associated with growth factor activation of cell surface receptors (Cato *et al.*, 2002).

Majority of breast cancers (~ 70%) express estrogen receptors and in these cases estrogen is considered to promote tumor growth. Hence, treatments subsequent to surgery, radiopathy, and/or chemotherapy are directed towards reducing oestrogenic effects within the breast tissue (Smith and Chua, 2006). The ER α is an important target

to develop drugs for the treatment and prevention of breast cancer (Jensen and Jordan, 2003).

Estrogens would be expected to exert opposite effects on the same targets given that antiestrogens are competitive inhibitors of the ER-mediated actions of estrogens (Jordan and Murphy, 1990). Studies have demonstrated that in breast cancer cells growth was arrested in G₁ phase by pre-treatment with antiestrogens (Prall *et al.*, 1998). Antiestrogen treatment of MCF-7 cells leads to an acute decrease of c-Myc expression, a subsequent decline in cyclin D1, and ultimately arrest of cells in a state with features characteristic of quiescence (Doisneau-Sixou *et al.*, 2003).

2.1.2 Oxidative Stress - Cancer

Oxidative stress is the condition where the balance between oxidising and reducing compounds is disrupted. Excess radicals, is the result of this disruption, may interact with biomaterials and thus induce cellular oxidative damage. This oxidative damage may cause adverse modifications of DNA, proteins, and lipids (Morrissey and O'Brien, 1998). Oxidative damage might be involved in several pathological conditions such as atherosclerosis, cancer and chronic inflammation (Haliliwell, 1994). Environmental carcinogens form another cause of risk to human health. By causing changes to DNA they initiate a multistep process ultimately leading to cancer. The human body possesses a solid defence mechanism to minimise carcinogenic risks. This defence mechanism is active on different levels, and in different stages of the process of carcinogenesis.

Excessive production of reactive oxygen species (ROS) in oxidative stress exhausts antioxidant defences. These stress conditions cause DNA damage, resulting in the production of mutated tumor-suppressor genes (Kang *et al.*, 1997; Hussein, 2005). Current developments in cancer research suggest that a number of apoptotic stimuli share common mechanistic pathways characterized by the generation of ROS through

oxidative stress. ROS typically include the superoxide radical ($O^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$) which cause damage to cellular components including DNA and proteins (Ott *et al.*, 2007).

Also, oxidative stress has a role in the initiation and progression of breast cancer (Mobley and Brueggemeier, 2004), several pro-inflammatory mediators, such as cytokines, chemokines, prostaglandins (PGs), nitric oxide (NO), and leukotrienes disrupt normal signalling cascades within cells which contributes to the development of neoplasms (Surh *et al.*, 2005). A depression in inflammation-associated pathways, especially cyclooxygenase (COX), has been associated with a reduced risk for breast, colon, lung, pancreatic, and head and neck cancers. Cancer is generally characterized by unusually high iNOS expression and NO production; thus, the potential for elimination of NO by NO scavengers and/or compounds which inactivate iNOS by food components may be a viable prevention strategy. NO is an inflammatory mediator implicated in cancer development, which has been shown to be inhibited by bioactive components present in herbs and spices (Tsai *et al.*, 2007). Tsai (2007) reported that rosemary was superior in blocking NO formation, followed by decreasing efficacy with tarragon, cinnamon, oregano, basil, marjoram, allspice, and thyme. Thus in addition to endogenous antioxidant systems, a diet rich in antioxidant food products also protects DNA and increases resistance against oxidative stress (Jayakumar and Kanthimathi, 2012).

Although measurement of markers of oxidative stress relevant to carcinogenesis is at an early stage of development they have the potential to become an integral part of early diagnostics and the assessment of tumor metabolism. For comprehensive understanding of endogenous carcinogenesis, oxidative markers of protein and lipid damage are also necessary. Free radicals are difficult to detect, identify and monitor because of their short life, particularly in living organisms. Therefore, detection and

monitoring of free radical process can be achieved only by detecting and measuring the products of their reactions with endogenous bio components or exogenous compounds selectively added to a biosystem. In biosystems these products are called molecular markers, which are a subclass of biomarkers (Gledhill and Mauro, 1991).

Compounds that are high in antioxidant potential are efficient interceptors of DNA-reactive elements (Malone, 1991). Generally this refers to the electrophilic nature of chemical carcinogens. Strong evidence exists that one class in particular, the plant phenolics, can prevent DNA addiction, presumably by presenting alternative targets for attack by carcinogens (Newmark, 1985; Teel and Castonguay, 1992).

2.1.3 Metabolism and Cancer

Phase I enzymes have an important role in the activation of procarcinogens; equally important in disease prevention are the phase II enzymes which are involved in the body's natural detoxification process and in drug metabolism and excretion. Plant components can prevent cancer in early phases of carcinogenesis through modulation of carcinogen metabolism. Extensive research in the metabolism of these compounds indicates that they are efficient modulators of carcinogen activation and detoxification. They are also highly reactive biologically, influencing both phase I (carcinogen activation) and phase II (carcinogen detoxification) enzymes. Compounds in garlic, pepper, rosemary, turmeric, and cinnamon appear to influence phase I and phase II enzymes (Debersac *et al.*, 2001; Van Erk *et al.*, 2004).

2.1.4 Apoptosis and p53 in Cancer

Apoptosis is a very tightly programmed cell death with distinct biochemical and genetic pathways that play a critical role in the development and homeostasis in normal tissues (Lockshin and Williams, 1965). Apoptosis is characterized by distinct morphological features such as cell shrinkage, chromatin condensation, plasma membrane blebbing, oligonucleosomal DNA fragmentation, and finally the breakdown

of the cell into smaller units (apoptotic bodies) (Earnshaw, 1995). Defects in the mechanism of apoptosis might lead to various abnormalities ranging from autoimmune diseases to cancer (Lorenzo and Susin, 2004). Apoptosis is a key process in cancer development and progression. The ability of cancer cells to avoid apoptosis and continue to propagate is one of the fundamental hallmarks of cancer and is a major target of cancer treatment. On the other hand induction of apoptosis might act as a protective mechanism against cancer. Evidence indicates that insufficient apoptosis can manifest as cancer or autoimmunity. Under many stressful conditions like precancerous lesions, activation of the DNA damage checkpoint pathway can serve to remove potentially harmful DNA-damaged cells *via* apoptosis induction to block carcinogenesis. Thus, the apoptotic signals help to safeguard the genomic integrity, while deregulation of the apoptotic pathways may not only promote tumorigenesis but also render the cancer cell resistant to treatment. Thus, the evasion of apoptosis is a prominent hallmark of cancer. Cancer cells, in fact, harbour alterations that result in impaired apoptotic signalling, that facilitate tumor development and metastasis (Hassan *et al.*, 2014). Over the past decade, significant advances have been made in discovery and validation of several types of novel cancer therapeutics which tries to prime the apoptotic machinery to act as promising apoptosis-inducing agents, bearing high hopes for the management of cancers resistant to conventional treatment (Hassan *et al.*, 2014). Apoptosis is controlled by a large number of genes acting as death switches. The tumor suppressor gene p53 is regarded as the master guardian of the cell and is able to activate cell-cycle check-points, DNA repair and apoptosis to maintain genomic stability (Sherr, 2004). The bax/bcl-2 protein ratio determines the life or death of cells in response to an apoptotic stimulus. A higher level of bax/bcl-2 ratio decreases the resistance to apoptotic stimuli, leading to apoptosis (Chougule *et al.*, 2011). The bcl-2 protein has an antiapoptotic effect, whereas the bax is known for proapoptotic activity. It has also been

well documented that signalling pathway leading to apoptosis involves the sequential activation of cysteine proteases known as capsases (Tang *et al.*, 2010; Ahamed *et al.*, 2011). Under many types of stress, *p53* induces cell cycle arrest and apoptosis to maintain genomic integrity. Reactive oxygen species (ROS) generation and oxidative stress have been proposed as common mediators for apoptosis (Nel *et al.*, 2006; Ott *et al.*, 2007; Yip *et al.*, 2011). Successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal situation is achievable by inducing apoptosis in cancer cells (Hassan *et al.*, 2014). Thus, modulating apoptosis may be useful in the management and therapy or prevention of cancer. Certain products from plants are known to induce apoptosis in neoplastic cells but not in normal cells (Taraphdar *et al.*, 2001).

An important cell cycle regulator that plays a central role in the regulation of cell cycle arrest and cell death is the tumor suppressor, *p53* (Vogelstein *et al.*, 2000). Tumor suppressor *p53* maintains genome stability by regulating diverse cellular functions including cell cycle arrest, apoptosis, senescence and metabolic homeostasis. Stress signals, such as DNA damage, hypoxia, oncogene activation and ribosomal stress, activate the *p53* tumor suppressor mainly through posttranslational modifications. The *p53* gene which is strongly implicated in animal and human carcinogenesis is a significant regulator of the process of apoptosis (Storer *et al.*, 1997). In the presence of DNA damage, *p53* protein triggers cell-cycle arrest to provide time for the damage to be repaired or for self-mediated apoptosis (Ahamed *et al.*, 2008; Farnebo *et al.*, 2010). Therefore loss of *p53* leads to aberrant cell proliferation and cell death and eventually tumor formation. Indeed, *p53* inactivation occurs in almost all human cancers. Tumor cells have inactivated *p53*, 50% of this inactivation is achieved through mutations in its sequence and alterations in *p53* signalling pathway (Hassan *et al.*, 2014). However, *p53* mutation is not common in estrogen-responsive tumors. The two prototypical tumor

suppressor genes encode the RB (retinoblastoma-associated) and TP53 proteins; they operate as central control nodes within two key complementary cellular regulatory circuits that govern the decisions of cells to proliferate or, alternatively, activate senescence and apoptotic programs (Hanahan and Weinberg, 2011). TP53 receives inputs from stress and abnormality sensors that function within the cell's intracellular operating systems: if the degree of damage to the genome is excessive, or if the levels of nucleotide pools, growth-promoting signals, glucose, or oxygenation are suboptimal, TP53 can call a halt to further cell-cycle progression until these conditions have been normalized. Alternatively, in the face of alarm signals indicating overwhelming or irreparable damage to such cellular subsystems, TP53 can trigger apoptosis. Notably, the various effects of activated TP53 are complex and highly context dependent, varying by cell type as well as by the severity and persistence of conditions of cell stress and genomic damage (Hanahan and Weinberg, 2011).

2.2 THERAPEUTICS

An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or in combination) to block the development of cancer in human beings. Cancer chemopreventive agents are divided into two principal categories: blocking agents that prevent the mutagenic initiation of the carcinogenic process and suppressing agents that prevent the further promotion or progression of lesions that have already been established (Mirvish, 1981).

2.2.1 Selective Estrogen Receptor Modulators (SERMs) – Breast Cancer

Selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifen can exhibit tissue-specific oestrogenic activity. Thus they are both antagonists in breast tissue but agonist in bone, while only raloxifen is a pure antagonist in the

uterus (Yamamoto *et al.*, 2003). Tamoxifen (TAM), a triphenylethylene, was introduced into clinical use on the basis of its now well-recognized estrogen antagonist activity in the breast by inhibiting the binding of estrogen-to-ER. TAM has been extensively used for decades to treat a wide range of cancers (Salami and Karami-Tehrani, 2003). In ER-positive human breast cancer, a selective estrogen receptor modulator (SERM), tamoxifen, is typically used in adjuvant therapy. The US Food and Drug Administration (FDA) has approved tamoxifen, and studies have shown that in ER-positive cancer, treatment for 5 years reduces recurrence by 47% and the risk of death by 26% (Lippman and Brown, 1999).

Unfortunately, tamoxifen therapy is associated with undesirable side effects, including endometrial cancer, thromboembolic events and liver cancer as well as hot flashes, insomnia, vaginal discharge and vaginal dryness (Carthew *et al.*, 2001; Howell *et al.*, 2005). Extended administration of tamoxifen evokes serious side effects and frequently results in gradual insensitivity to this treatment (Rajput *et al.*, 2013). In addition to its effects in the breast, tamoxifen has an estrogen agonist effect in bone, liver, and uterus that may explain the favourable effects on inhibiting bone loss, improving serum lipid concentrations, and its effect of increasing the incidence of uterine cancer. Tamoxifen was shown to induce regression of advanced breast malignancies.

Therefore, the search for other SERMs has continued. Recently, third-generation aromatase inhibitors, which block the conversion of androgens to estrogens, have been shown to be potentially more efficacious than Tamoxifen (Howell *et al.*, 2005), but recently reported side effects, including bone loss, joint pain and cardiac events have caused concern (Kalidas and Brown, 2005).

2.2.2 Phytotherapy - Cancer

Plants, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemopreventive drug discovery and development (Abdullaev, 2001). A large and increasing number of patients in the world use medicinal plants and herbs for health purposes. *In vitro* studies indicate herbs, spices, and their bioactive components can inhibit, and sometimes induce, pathways that regulate cell division, cell proliferation, and detoxification, in addition to the inflammatory and immune response (Hollman and Katan, 1999).

The concept of chemoprevention by dietary phytochemicals is gaining increasing attention because of their chemical diversity, structural complexity, inherent biological activity, affordability, easy availability, and lack of substantial toxic effects. Most importantly, phytochemicals protect against different types of cancer due to their ability to modulate a plethora of signal transduction pathways (Aggarwal and Shishodia, 2006; Deorukhkar *et al.*, 2007 and Khan *et al.*, 2008).

To date, several hundreds of compounds have been identified as potential modifiers of cancer, several of which are active ingredients in herbs and spices. Herbs and spices, or their bioactive components, may act alone or in concert to reduce cancer risk through their antimicrobial, antioxidant and antitumorigenic properties, as well as their direct suppressive effect on carcinogen bioactivation (Kaefer and Milner, 2008). Plant phenolics are ubiquitous in the plant kingdom. Several have been tested for anticarcinogenic potential. For example, caffeic and ferulic acids prevent lung cancers in mice (Wattenberg, 1992). Among the more potent of these agents is the compound ellagic acid, found abundantly in strawberries. Monoterpenes are a class of volatile chemicals found in many plants. Limonene, a monoterpene present abundantly in the citrus fruits, inhibits the development of mammary cancer when fed to rats (Elegbede *et*

al., 1984). More importantly, this monoterpene regresses mammary tumor after they have become established in the rat (Haag *et al.*, 1992).

Spices are traditionally thought to have beneficial effects on health and specific spices are used in traditional medicine as part of therapeutic formulations (Jayakumar and Kanthimathi, 2012). Ethnopharmacological studies on spices revealed a wide range of biological activities including antioxidant, anti-inflammatory, anti-tumor, immunomodulatory and antiradical activities (Jayakumar and Kanthimathi, 2012). Oregano, sage, peppermint and thyme contain the greatest antioxidant capacity for fresh herbs while cloves, allspice and cinnamon contain the highest levels of antioxidant activity among dried spices (Dragland *et al.*, 2003). Many natural compounds in plants have been reported to have potential antimutagenic or anticarcinogenic effects (Edenharder *et al.*, 1998).

Natural compounds may play a role in scavenging free radicals such as hydroxyl radicals generated by chemical hazardous agents. Basic research has illuminated four potential mechanisms by which components in plants act to prevent cancer. In terms of preventing cancer, two concepts are important at the early phase of carcinogenic process: interception of DNA – reactive elements and activation and detoxification of potential carcinogens. In later stages, food components may interfere with the proliferation of clonal altered cells or disrupt the biology of the tumor in such a way that further growth or metastasis is impeded (Wargovich, 2000).

2.3 CINNAMALDEHYDE

Cinnamaldehyde (CNMA) is an organic compound with the chemical formula $C_6H_5CH=CHCHO$ (Figure 1). The trans isomer of cinnamaldehyde predominates in nature. It gives cinnamon its flavour and odour (Pubchem, CID 637511). It is a flavonoid that is naturally synthesized by the shikimate pathway (Gutzeit and

Ludwig-Muller, 2014). This occurs as a pale yellow and viscous liquid in the bark of cinnamon trees and other species of the genus *Cinnamomum*. The essential oil of cinnamon bark is about 50% cinnamaldehyde (Singh *et al.*, 2007).

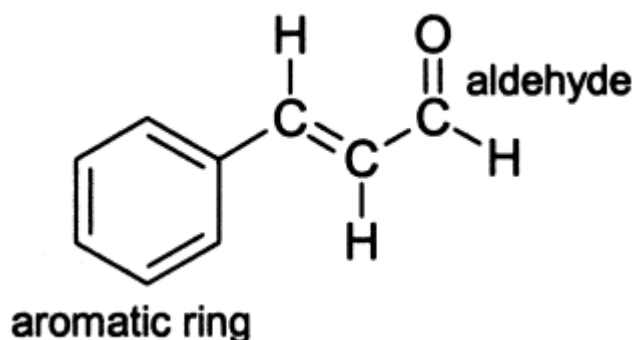


Figure 1. Chemical structure of cinnamaldehyde

2.3.1 Pharmacokinetics

In vitro tests using human skin homogenates show that cinnamaldehyde is metabolized by aldehyde dehydrogenase (ALDH) to produce cinnamic acid (CA). Another known metabolite cinnamic alcohol was found to be converted back into cinnamaldehyde by the action of alcohol dehydrogenase (ADH) (Cheung *et al.*, 2003). Pharmacokinetic studies in F344 rats revealed, after oral administration cinnamaldehyde was rapidly oxidized to cinnamic acid and excreted mainly as hippuric acid in the urine (Cocchiara *et al.*, 2005).

There two proposed pathways for CNMA administered both orally and intravenously: one leads to the formation of cinnamic acid and the second pathway leads to the synthesis of conjugated protein products followed by regeneration of CNMA by certain enzyme activities. The first pathway was obvious, since cinnamic acid was immediately detected in rats after administration of CNMA. The existence of second pathway is supported by the fact that the terminal elimination half-life of CNMA in rats after i/v administration is much longer than the half-life of CNMA, obtained *in vitro* after spiking fresh rat blood with CNMA (Yuan *et al.*, 1992). Since the oxidation

of CNMA to cinnamic acid is not considered to be reversible, the large difference between the two half-lives could only be explained by the regeneration of CNMA from protein conjugates *in vivo*. Since CNMA is a lipophilic liquid, its gastrointestinal absorption was relatively rapid, which is confirmed by the rate of excretion of hippuric acid in urine. The conjugation of CNMA with the gastro-intestinal proteins could explain the fact that even after 24 hrs of dosing, CNMA was still detectable in blood despite its rapid gastro-intestinal absorption and elimination. The regeneration of CNMA would be a slow process since protein conjugates are generally more stable than free aldehydes (Yuan *et al.*, 1992).

In a pharmacokinetic study on cinnamaldehyde in rats, using gas chromatography-mass spectrometry (GC-MS) conducted by Zhao *et al.* (2014) revealed that the bioavailability of CNMA was 20% after oral administration, and the elimination half-lives of cinnamaldehyde were 6.7 ± 1.5 and 1.7 ± 0.3 hr for oral and IV administration respectively.

2.3.2 Pharmacological Properties

trans –cinnamaldehyde contains an α , β - unsaturated carbonyl moiety known as Michael acceptor, in its structure. Compounds containing Michael acceptor display chemotherapeutic and chemopreventive properties. Notably numerous naturally occurring phytochemicals containing Michael acceptor such as curcumin, quercetin and myricetin have been found to exert antiproliferative activity through thioredoxin reductase (TrxR) inhibition (Chew *et al.*, 2010).

In vitro studies on various cancer cell lines demonstrated anti-proliferative and pro-apoptotic properties for cinnamaldehyde (Moon and Pack, 1983; Kwon *et al.*, 1998; Jeong *et al.*, 2003; Lee *et al.*, 2007).

The anticancer effect of cinnamaldehyde in human promyelocytic leukemia HL-60 cells was due to induction of apoptosis by ROS-mediated mitochondrial permeability transition and subsequent release of cytochrome *c* (Ka *et al.*, 2003).

From the study of Fang *et al.* (2004) it was evident that cinnamaldehyde showed selective toxicity to Jurkat and U937 cancer cell lines without being cytotoxic to peripheral blood mononuclear cells.

2'-Hydroxycinnamaldehyde and 2'-benzoyloxy-cinnamaldehyde inhibited tumor growth by inducing G₂/M phase arrest and apoptosis through accumulation of ROS and activation of caspase-3 in a p53-independent manner (Han *et al.*, 2004).

Wu *et al.* (2005) reported that cinnamaldehyde treatment in PLC/PRF/5 cells results in inhibition of cell proliferation, cell cycle arrest and also induce apoptosis through activation of proapoptotic Bcl-2 family proteins and mitogen activated protein kinase (MAPK) pathway. Wu and Ng (2007) opined that induction of p53 gene and MAPK signalling pathways are essential for cinnamaldehyde mediated apoptosis in human hepatoma PLC/PRF/5 (CD95-negative) cells.

Hydroxyl cinnamaldehyde, a derivative of cinnamaldehyde isolated from *C. cassia*, induced apoptotic cell death in SW620 human cancer cells, through promoting the expression of capase-3 (apoptosis regulator) gene and inhibiting the bcl-2 (anti-apoptotic regulating) gene in a dose dependent manner. It also inhibited the cell growth by acting on AP-1 (oncogene transcription factor) (Lee *et al.*, 2007).

In a study conducted on human A375 melanoma cells cinnamaldehyde inhibited NF-kB (redox-sensitive oncogenic transcription factor) transcriptional activity and TNF- α induced IL-8 production (Cabello *et al.*, 2009).

Ng and Wu (2011) reported that out of three major constituents of *Cinnamomum cassia*, cinnamaldehyde demonstrated most potent antiproliferative activity in human hepatoma Hep G2 cells as good as 5-flurouracil. Cinnamaldehyde downregulated the

expression of antiapoptotic protein Bcl-x_l and up regulated the apoptotic proteins CD95 (APO-1), p53 and Bax, including cleaving the poly (ADP-ribose) polymerase (PARP) in a time dependent manner. Yu *et al.* (2014) opined that cinnamaldehyde may have a promising role as an adjuvant in combination therapy with 5-fluorouracil and oxaliplatin in colorectal cancer treatment.

Cinnamaldehyde treatment, through feed containing 5000 ppm, significantly reduced the lung tumor occurrence (adenomas and carcinomas), and multiplicity induced by 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) in rasH2 transgenic male and non transgenic female mice, but no significant effect was noticed in rasH2 transgenic female mice (Imai *et al.*, 2002).

Chao *et al.* (2010) observed that cinnamaldehyde treatment reduced the high glucose induced proliferation and hypertrophy in renal interstitial fibroblasts by inactivating the ERK/JNK/p38 MAPK pathway.

In a study conducted by Babu *et al.* (2007) oral administration of cinnamaldehyde produced significant antihyperglycemic effect, along with lowered total cholesterol and triglyceride levels and increased HDL-cholesterol in streptozotocin (STZ) induced diabetes in rats. Cinnamaldehyde showed protective effect against streptozotocin-induced pancreatic beta (β) cell damage by decreasing the production of lipid peroxidation products and increased the activities of antioxidant enzymes (Subash babu *et al.*, 2014).

Cinnamaldehyde prevented development of hypertension in insulin deficiency and insulin resistance through normalization of vascular contractility in addition to its insulinotropic effect in insulin deficiency (El-Bassossy *et al.*, 2011). In fructose-fed hypertensive rats cinnamaldehyde attenuated cataractogenesis due to its antihypertensive, anti-oxidant and aldose reductase inhibition activities (Singh *et al.*, 2016).

Youn *et al.* (2008) reported that anti-inflammatory action of cinnamaldehyde was mediated through suppressing the activation of toll-like receptors (TLRs) by inhibiting receptor oligomerization. Chao *et al.* (2008) opined that cinnamaldehyde mediated inhibition of pro-inflammatory cytokines production in lipopolysaccharide (LPS) stimulated macrophages and monocytes, might be partly due to reduction of ROS release and suppression of the JNK1/2 (c-Jun NH₂-terminal kinase) and ERK1/2 (extracellular signal regulated kinase) activation. Cinnamaldehyde inhibited the adhesion of TNF α -induced monocytes to endothelial cells and suppressed the expression of all adhesion molecules, VCAM-1 and ICAM-1, at the transcription level and also abolished the downstream signals of VCAM-1, ICAM-1 and NF- κ B in a time dependent manner. Pretreatment with cinnamaldehyde exerted its anti-inflammatory effects by blocking the degradation of the inhibitory protein I κ B- α , in TNF α treated endothelial cells (Liao *et al.*, 2008).

Cinnamaldehyde modulates the monocyte/macrophage- mediated inflammatory and immune responses by suppressing, the production of nitric oxide (NO) and inhibiting the upregulation of surface levels of co-stimulatory molecules (CD80 and CD69), pattern recognition receptor (toll-like receptors), complement receptors (CR3) and cell-cell adhesion (Kim *et al.*, 2010).

Chen *et al.* (2016) observed that in LPS-challenged murine BV-2 microglial cells cinnamaldehyde inhibited NO production, reduced protein expression levels of iNOS, COX-2, but increased expression of p53 gene. In C57BL/6 mice ischemia/reperfusion (I/R) induced brain injury model trans-cinnamaldehyde significantly ameliorated the infarct volume in brain and neurological deficit score. In addition CNMA markedly attenuated expression levels of inflammatory associated proteins iNOS and COX-2 in ischemic brain tissue.

Wang *et al.* (2005) reported that cinnamaldehyde, the major compound in *Cinnamomum osmophloeum* leaf essential oils, possessed the strongest antifungal activity against *Coriolus versicolor* and *Laetiporus sulphureus* with a MIC value of 50 ppm and 75 ppm respectively. Yen and Chang (2008) reported that combination of cinnamaldehyde with eugenol showed synergistic effect against wood decay fungi due to the interference of fungal cell wall synthesis and cell wall destruction plus antioxidant effect.

Cinnamomum burmannii stick and its major components, *trans* (E) – cinnamaldehyde and proanthocyanidins possessed significant *in vitro* antibacterial activity against five foodborne pathogens or fecal indicator bacteria, *B. Cereus*, *L. Monocytogenes*, *S. aureus*, *E. coli* ATCC25922 and *S. anatum* (Shan *et al.*, 2007). Cinnamaldehyde inhibited bacterial cell division by binding to FtsZ protein thereby disrupting cytokinetic Z-ring formation (Domadia *et al.*, 2007). In a study on leaf essential oils and their constituents from *C. osmophloeum* the strongest antibacterial activity against gram positive and gram negative bacteria was exhibited by cinnamaldehyde (Chang *et al.*, 2001). Cinnamaldehyde was also believed to kill bacteria by inhibiting energy generation and glucose uptake (Gill and Holley, 2004). According to Nostro *et al.* (2012) cinnamaldehyde and carvacrol polymeric films had a significant bactericidal effect against *Staphylococcus aureus* and *Escherichia coli* and a bacteriostatic effect against *Staphylococcus epidermidis* and *Listeria monocytogenes* and also reduced the biofilm formation.

Ali *et al.* (2005) conducted an experiment to evaluate the antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. The data obtained in this study revealed that both the compounds produce significant decrease in the viability of the organism at 2 µg/ml strength in acidic pH.

Cinnamaldehyde exhibited protective effect against coxsackievirus B₃ (CVB₃)-induced viral myocarditis (VMC) through its metabolite, cinnamic acid and also directly reduced the inflammation associated with VMC by directly inhibiting the TLR4-NF- κ B signal transduction pathway (Ding *et al.*, 2010). Growth of various strains of influenza such as A/USSR/92/77 (H1N1), A/Aichi/2/68, A/Urdon/77 (H3N2) and B (Lee/40 and Singapore/64) was inhibited by *trans*-cinnamaldehyde with equal efficacy (Hayashi *et al.*, 2007). Cinnamaldehyde directly killed and damaged the adenovirus (ADV) type 3 in addition to inhibiting the apoptosis pathway by lowering the expression of caspase-3, 8 and 9 protein (Liu *et al.*, 2009).

2.3.3 Toxicity

From the studies conducted on Osborne-Mendel rats the mean oral lethal dose for cinnamaldehyde was 2.22g/kg (Jenner *et al.*, 1964), where as in white rats, white mice and guinea pigs the LD₅₀ for all these three species was reported to be 3.4 g/kg body weight (Zaitsev and Rakhmanina, 1974). Sporn *et al.* (1965) reported that the oral LD₅₀ in rats was 3.35 g/kg. Minimal to moderate forestomach hyperplasia in males was observed at doses of 470 mg/kg and above and body weights were slightly decreased in females dosed at the rate of 940 mg/kg (Hebert *et al.*, 1994).

In a three months study conducted on male and female F344/N rats and B6C3F₁ mice, fed with microencapsulated *trans*- cinnamaldehyde, the fore stomach appeared to be the primary target organ. In mice, olfactory epithelial degeneration of the nasal cavity was also observed. In a two year dietary study, exposure to *trans*-cinnamaldehyde resulted in olfactory epithelial pigmentation in male and female mice and it was not carcinogenic (Hooth *et al.*, 2004).

Gowder and Devaraj (2006) observed a time and dose dependent effect on the antioxidant status in the kidneys of rats fed with cinnamaldehyde. Rats fed with cinnamaldehyde at a dose rate of 73.5 mg/kg b.wt for a period of 90 days, decreased the

activity of antioxidant enzyme, catalase and increased thiobarbituric acid-reactive substances in the kidney.

Suzi and El-Makaway (2007) reported that cinnamaldehyde significantly elevated liver enzymes, total bilirubin and reduced hepatic glutathione levels in a dose dependent manner in mice fed with three different dose levels over a period of 30 days.

2.4 NANO-THERAPY IN CANCER PREVENTION

Nanotechnology allows the manipulation of materials at nanoscale level (1-100 nm), which enables precision engineering to control nanoparticles (NPs) physicochemical properties, as well as their interactions with biological systems (Lee *et al.*, 2007; Wang, 2008). The underlying rationale is that reduction of materials to nanoscale can sometimes lead to the development of new structural, physico-chemical, electronic and magnetic properties that are not present in large bulk-sized particles consisting of the same material systems. The principle factors believed to cause properties of nanomaterials to differ from their larger micrometer-sized bulk counterparts include an increase in relative surface area, a greater percentage of atoms at the materials surface, quantum effects that can affect chemical reactivity, and other physical and chemical properties (Lanone and Boczkowski, 2006; Nel *et al.*, 2006). Nanoparticles (NPs) are composed of an inorganic core and an organic shell. Most inorganic materials such as metals, semiconductors and their complex compounds can be the core through the chemical or physical synthesis. The shell also known as stabiliser uses various organic chemicals from small molecules to polymers in order to make NPs confine their sizes and structures during synthesis steps as well as to make NPs stable in external environment against colloidal aggregation. Regardless of the core materials, the stabiliser is critical to synthesize biocompatible NPs due to its inherited toxicity and high electrostatic properties (Lee *et al.*, 2014). The size of nanoparticles can facilitate their entry into tumor tissues and their subsequent retention, by a process

recognized as the enhanced permeation and retention (EPR) effect. Therapeutic approaches making use of the EPR effects are now recognized as the ‘gold-standard’ in the design of new anticancer agents. The EPR phenomena can be described as a combination of ‘leaky’ tumor blood vessels resulting from alterations in angiogenic regulators, enlarged gap junctions between endothelial cells and compromising lymphatic drainage in the tumor microenvironment (Rasmussen *et al.*, 2010). In fact, the EPR effect typically operates in the range of 100-400 nm (Bagchi *et al.*, 2012). This localized imbalance allows nanoparticles of certain sizes (Cho *et al.*, 2008) to enter readily, but to be passively retained within the tumor interstitial space, thereby improving therapeutic potential. In a recent report, particles of 100-200 nm size showed a fourfold higher rate of tumor uptake compared with particles > 300 nm, or < 50 nm in size (Li and Huang, 2008). Although smaller nanoparticles do not readily make use of EPR effect, they typically show more nanotoxicity related to their larger surface area/volume ratio (Cho *et al.*, 2008; Li and Huang, 2008). Nanoparticles of differing physicochemical properties such as size, shape and surface charge can exert different cytotoxic effects on cells (Singh *et al.*, 2009).

The electrostatic nature of nanoparticles is another important consideration as electrostatic interactions between positively charged nonmaterial's and target cells are believed to play an important part in cellular adhesion and uptake (Ohgaki *et al.*, 2001). Compared with normal healthy cells whose outer surface possess of neutral charged zwitterionic phospholipids (Leroueil *et al.*, 2007), cancer cells frequently maintain a high concentration of anionic phospholipids on their outer leaflet and large membrane potentials (Abercrombie and Ambrose, 1962, Papo *et al.*, 2003), and overexpress specific groups of charged proteins and carbohydrates (Panchal, 1998). In addition, studies have shown that intracellular pH increases with cell cycle progression and proliferation (Shrode *et al.*, 1997, Rich *et al.*, 2000), which could affect

electrostatically-driven interactions with charged particles at cell membrane. Whereas nanoparticles with higher positive charge may be desirable for higher toxicity to cancer cells, very high positive charge may not be suitable for *in vivo* cancer treatment owing to rapid clearance (Xu *et al.*, 2007). Thus, tailoring the surface charge of nanoparticles is expected to influence their cytotoxicity and will probably be an important parameter for developing cancer therapies.

Recent *in vitro* observations indicating that certain types of metal oxide nanoparticle can preferentially kill cancer cells, with strikingly less toxicity against normal cells (Hanley *et al.*, 2008; Wang *et al.*, 2009). Inorganic NPs, including metaloxides, are promising materials for applications in medicine and cancer therapy (Visaria *et al.*, 2006; Wang *et al.*, 2011; Wu *et al.*, 2011).

Many natural products are considered as candidates for non-toxic reducing agents or particle-surface stabilizers. Generally, phytochemicals consist of unsaturated carbon atoms comprising of ethylene, aromatic, carboxylic and carbonyl groups, and the bioactivity of phytochemicals vary with the chemical structure (RiceEvans *et al.*, 1996; Silva *et al.*, 2002; Modak *et al.*, 2005 and Amaral *et al.*, 2009).

2.4.1 Zinc Oxide Nanoparticles

Inorganic nanoparticles (NPs), including metal oxides, are promising materials for application in medicine, such as cell imaging, biosensing, drug/gene delivery, and cancer therapy (Visaria *et al.*, 2006; Wang *et al.*, 2011; Wu *et al.*, 2011).

Zinc oxide (ZnO) NPs belonging to a group of metal oxides, are characterized by their photocatalytic and photo-oxidizing ability against chemical and biological species. Zinc oxide nanoparticles are one of the most abundantly used nanomaterials in cosmetics and sunscreens as they efficiently absorb UV light and also do not scatter visible light (Sharma *et al.*, 2012). Also ZnO NPs are being used in the food industry as

additives and in packaging due to their antimicrobial properties (Gerloff *et al.*, 2009; Jin *et al.*, 2009).

In recent times, ZnO NPs have received much attention for their use in cancer therapy (Zhang *et al.*, 2011). Recent studies have shown that ZnO nanoparticles show a high degree of cancer cell selectivity with the ability to surpass the therapeutic indices of some commonly used chemotherapeutic agents in similar *ex vivo* studies (Hanley *et al.*, 2008; Wang *et al.*, 2008).

ZnO NPs induce cytotoxicity in a cell specific and proliferation-dependent manner, with rapidly dividing cancer cells being the most susceptible, and quiescent cells being the least sensitive (Hanley *et al.*, 2008; Ostrovsky *et al.*, 2009; Premanathan *et al.*, 2011). There was upregulation both in mRNA and protein levels of tumor suppressor gene p53 and apoptotic gene bax while the antiapoptotic gene bcl-2 was downregulated in ZnO NP treated human liver cancer cells HepG2 cells (Akhtar *et al.*, 2012). ZnO NPs have distinct effects on cell viability killing cancer cells without effecting normal cells (Akhtar *et al.*, 2012).

According to Ahamed *et al.* (2011) ZnO nanorods, induce apoptosis in human alveolar adenocarcinoma (A549) cells through ROS and oxidative stress and via p53, survivin, bax/bcl-2 and caspase pathways.

However, there are also reports on the cytotoxic effects of ZnO NPs *in vitro* (Heng *et al.*, 2010; Xia *et al.*, 2008; Yuan *et al.*, 2010) and concerns with respect to the possible genotoxic effects (Sharma *et al.*, 2009; Yin *et al.*, 2010), due to their potential devastating long term damage caused to humans.

Several studies in isolated mitochondria have shown that Zn^{+2} inhibit cellular respiration by interfering with cytochrome *bc1* in complex III as well as with α -ketoglutarate dehydrogenase in complex I. Zn^{+2} may exert extra-mitochondrial effects contributing to ROS generation, including activation of NADPH oxidase via

protein kinase C as well as NO production and generation of peroxynitrite through the induction of nitric oxide synthase activity. Zinc could also inhibit key enzymes in the glycolytic pathway, leading to ATP depletion in cells. High levels of Zn^{+2} could contribute directly to mitochondrial permeability transition (MPT) pore opening and cytochrome *c* release, resulting in apoptosis/necrosis in target cells (Xia *et al.*, 2008).

The toxicological evaluation of zinc oxide as reported by SCCNFP (Scientific Committee on Cosmetic Products and Non-food products 2003) revealed that the LD₅₀ (single oral ingestion) of normal ZnO for rats was more than 5 g/kg b.wt and belonged to non-toxic chemicals category. From an acute oral toxicity study in mice, Wang *et al.* (2008) reported that ZnO nano particles (20 nm and 120 nm) are relatively nontoxic. Mice treated with 120 nm size ZnO NPs showed dose dependent pathological effects in stomach, liver, heart and spleen (Wang *et al.*, 2008). Exposure to 20 nm and 120 nm ZnO NPs produced toxic effects in various organs including the stomach, liver, kidney, pancreas and spleen.

Ko *et al.* (2015) reported that 14-day repeated oral dosing of 100 nm size ZnO NPs at a dose rate of ≥ 500 mg/kg/day in rats, resulted in significant alterations in clinical signs, body weight, haematology, serum biochemistry and histopathology.

2.5 EXPERIMENTAL MAMMARY CANCER MODELS

The typical development of new cancer chemotherapy includes *in vivo* experiments conducted on animal models to define the efficacy (potential activity determination), the ADME pharmacological parameters (drug absorption, distribution, metabolism and elimination) and the toxicological profile (Venkatesh and Lipper, 2000). Hence animal models form the crucial prerequisites for cancer therapy development.

Currently available animal models of breast cancer can be categorized into 1. Chemo-induced models, 2. Transgenic animals and 3. Grafted cancers (Mollard *et al.*, 2011)

2.5.1 Chemically Induced Mammary Cancer Models

Mammary glands of several rat strains, mainly Sprague-Dawley and Wistar-Furth, are susceptible to transformation induced by chemical carcinogens, and the two most widely used active chemical inductors of mammary carcinogenesis are 7,12-dimethylbenz(a)anthracene (DMBA) and N-methylnitrosurea (NMU) (Dias *et al.*, 2000; Giullino *et al.*, 1975). DMBA induced animal model closely mimics human breast cancer (Dias *et al.*, 2000; Rajkumar *et al.*, 2006 and Kaur *et al.*, 2010). Chemically induced rat mammary tumors are generally hormone-dependent adenocarcinomas (Russo and Russo, 2000). DMBA is highly lipophilic and requires metabolic activation for its carcinogenicity. Several tissues are capable of activating DMBA, and these include the mammary gland. In the breast, DMBA is converted to epoxides, active metabolites with a capacity for damaging the DNA molecule, the main event in carcinogenesis initiation (Barros *et al.*, 2004).

CHAPTER III

MATERIALS & METHODS

3.1 EXPERIMENTAL ANIMALS

Virgin female Sprague-Dawley rats (n=90) weighing 80-100 g and aged 40 days were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition (ICMR), Hyderabad, India. All the experimental protocols used in this study were approved by the Institutional Animal Ethics Committee of NTR College of Veterinary Science (2/IAEC/NTR CVSc./2015). The animals were caged group wise and maintained under constant environmental conditions with a room temperature of 23 ± 2 °C and relative humidity between 55-65% with 12 h light/12 h dark cycle. Animals were provided with reverse osmosed water and standard rat pellet diet *ad libitum* throughout the experimental period.

3.2 MATERIALS

3.2.1 Drugs and Chemicals

trans-Cinnamaldehyde (99% purity) procured from Sigma-Aldrich, Tamoxifen citrate obtained from TCI chemicals, India, and all the chemicals used in pharmacokinetic analysis were of HPLC grade, acquired from Sigma-Aldrich and Merck.

3.3 PREPARATION OF DRUGS

3.3.1 Preparation of *trans* - Cinnamaldehyde

Appropriate quantity of cinnamaldehyde (CNMA) was dissolved in 0.1% tween-20 in water to make 500 mg/ml solution for pharmacokinetic study, whereas CNMA was dissolved in corn oil at the rate of 50 mg/kg for anti-carcinogenic studies.

Pharmacokinetic studies: 96.20 μ l of *trans*-cinnamaldehyde (99%) was dissolved and makeup to 100 ml with acetonitrile (HPLC grade) to prepare 1 mg/ml concentration solution. Cinnamic acid (98%) 102.04 mg was dissolved in 100 ml of acetonitrile to give a final concentration of 1 mg/ml stock solution. From the stock solution subsequent dilutions were made in acetonitrile.

3.3.2 Preparation of Cinnamaldehyde Mediated Zinc Nano Particles (CNZ)

Zinc oxide solution, 0.1 M, was prepared and 90 ml of which was added slowly to 9.7 ml of 99% *trans*-cinnamaldehyde with vigorous stirring at a heating temperature of 40-45°C until the contents turned pale yellow to brown colour, which indicates synthesis of CNMA mediated zinc nanoparticles (CNZ) or nano cinnamaldehyde. The prepared solution was cooled to the room temperature and the particles were allowed to stabilise for 24hrs.

3.3.3 Characterisation of Nanoparticles

Particle size and zeta potential (surface potential) was determined using Nanaopartica SZ-100 (HORIBA) at frontier technology laboratory, Regional Agriculture Research Station, Tirupati. Scanning electron microscopy (SEM, model: JEOL JFC-1600) and transmission electron microscopy (TEM, model: Hitachi, H-7500) studies were carried out as per the protocol given by Bozzola and Russell (1999) at Ruska labs, College of Veterinary Science, Hyderabad.

3.3.4 Preparation of Tamoxifen drug solution

Appropriate quantity of tamoxifen citrate was dissolved in sesame oil and used at the rate of 10 mg/kg for anti-carcinogenic studies.

3.4. PREPARATION OF REAGENTS

1. Biuret reagent was made of sodium hydroxide (1.6g) and hydrated copper (II) sulphate (1.5 g), together with potassium sodium tartrate (4.5g) in 200 ml of water.
2. 0.1 M phosphate buffer was prepared by dissolving 3.1 g of sodium dihydrogen phosphate and 10.9 g of disodium hydrogen phosphate in distilled water and adjusted the pH to 7.4 with sodium hydroxide/potassium hydroxide.
3. Sodium carbonate (5 mM) - EDTA (0.1 mM) buffer: 530 mg of Na_2CO_3 and 37.2 mg of EDTA was dissolved in 1 L of distilled water and the pH was adjusted to 10.8 with sodium hydroxide
4. 96 μM Nitroblue tetrazolium (NBT): 78.49 mg of NBT was dissolved in 1 litre of distilled water
5. 0.6% (w/v) Triton-X: 0.12 g (1.06 g/ml) of triton X - 100 was dissolved in 20 ml of distilled water.
6. 20 mM Hydroxylamine hydrochloride: 1.389 g of reagent was dissolved in 1 litre of distilled water.
7. 4% Sulfosalicylic acid: 4 gm of sulfosalicylic acid was dissolved in 100 ml of double distilled water
8. 3 mM DTNB (Ellman's reagent): 1.189 g of 5, 5-dithio-bis-(2)-nitrobenzoic acid was dissolved in 1 L distilled water.
9. 5% Trichloro acetic acid (TCA): 5 gm of TCA was dissolved in 100 ml of double distilled water.
10. 0.67% TBA: 0.67 gm of thiobarbituric acid was dissolved in 10% dimethyl sulfoxide in water
11. 1 mM TEP solution: 249.8 μl of TEP reagent was taken into a volumetric flask and made up to 1 litre with distilled water to prepare 1 mM stock solution and required working standards were prepared from the stock using 1% H_2SO_4 distilled water.

12. 10% Neutral buffered (NBF) formalin: 100 ml of 37% formaldehyde, 6.5 gm of disodium hydrogen ortho phosphate and 4 gm of sodium dihydrogen phosphate were dissolved in distilled water to make 1 litre final solution.
13. Citrate buffer for IHC studies: 10 Mm sodium citrate buffer was prepared by dissolving trisodium citrate dehydrate in 1 litre distilled water and adjusted the pH to 6.0.

3.5 METHODS

3.5.1 Acute Oral Toxicity Studies of Cinnamaldehyde and Nano Cinnamaldehyde

Acute oral toxicity studies of *trans* CNMA and nano CNMA were conducted according to OECD guidelines 425 (up- and down- method) limit test. Five female rats were used for study of each compound. The animals were fasted overnight and provided with water *ad lib*. The drugs were administered orally at a dose rate of 2000 mg/kg (limit dose) orally once using oral feeding needle. The animals were monitored daily over a period of 14 days for any signs of toxicity. Body weight, water and feed consumption were recorded daily and mean body weight gain was calculated at the end of the study. Blood samples were collected on 13th day, whole blood was used for haematological analysis and plasma was separated for biochemical analysis. At the end of the study animals were sacrificed and relative organ weights of liver, lung, heart, kidney, brain and spleen was calculated. Histopathological studies were performed in liver, heart, stomach, kidney and brain tissues.

3.5.2 Pharmacokinetic Studies of Cinnamaldehyde

3.5.2.1 Standardization of HPLC Conditions. HPLC equipped with C₁₈ reverse phase column (particle size 5 µm, 4.6 mm x 250 mm) as the stationary phase and photo diode array was used for detection. Column oven temperature was set to 30°C and detector wavelength at 285 nm.

3.5.2.2 Standard Calibration Curve - Response Linearity. Response linearity was measured using 0.005, 0.01, 0.05, 0.1, 0.25, 0.5 and 1.0 µg/ml of CNMA and 0.05, 0.1, 0.25, 0.5 and 1.0 µg/ml CA solutions respectively.

3.5.2.3 Standardization of Recovery of Cinnamaldehyde and Cinnamic acid.

Acetonitrile, methanol, glacial acetic acid and water were tried in different combination and proportions for extraction of CNMA and CA simultaneously from plasma spiked samples. Four hundred micro litre of selected/suitable extraction solvent was added to 200 µl of plasma sample and vortexed and centrifuged for 20 min at 17000 rpm. The supernatant was separated and filtered using 0.22 µm nylon membrane syringe filter. 20 µl of filtrate was injected into HPLC system.

3.5.2.4 Pharmacokinetic Protocol in Rats. Eight adult healthy female rats were fasted overnight with *ad libitum* water and cinnamaldehyde was given orally at the dose rate of 500 mg/kg body weight in 0.1% Tween-20 in water.

Blood (approximately 500 µl) was collected from retro-orbital plexus at 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 240, 360, 720, 1080 and 1440 min into heparinised containers and plasma was separated by centrifugation at 5000 rpm for 15 min and stored in -20°C till analysis.

3.5.2.5 Pharmacokinetic Analysis. The plasma concentration versus time data obtained for cinnamaldehyde and its major metabolite cinnamic acid in the present study were used for calculating various pharmacokinetic parameters by non-compartmental method using PKSolver, version 2.0 (Zhang *et al.*, 2010).

- a) AUC_{0-t} and $AUMC_{0-t}$ was determined by trapezoidal method
- b) MRT was calculated by dividing $AUMC_{0-t}$ with AUC_{0-t}
- c) Half-life ($t_{1/2}$) was calculated by multiplying MRT with 0.693
- d) Elimination rate constant (K_e) was manually derived by dividing 0.693 with

$$t_{1/2}$$

- e) Plasma clearance (Cl) was calculated using formula $Cl = \text{Dose}/AUC$
- f) Volume of distribution (V_d) was calculated by multiplying clearance (Cl) with MRT

3.5.3 Anticarcinogenic Studies

Table 1. Treatment protocol in various experimental groups (n=7)

No.	Group	Treatment
I	Control (C)	Corn oil @ 1 ml, single dose , per os on 50 th day
II	DMBA control (DMBA-C)	DMBA in corn oil @ 20 mg/rat, per os, on 50 th day
III	Cinnamaldehyde prophylactic (CNMA-P)	CNMA in corn oil @ 50 mg/kg b.wt daily starting from 45 th day till the end of the experiment + DMBA in corn oil @ 20 mg/rat, per os, single dose on 50 th day.
IV	Cinnamaldehyde therapeutic (CNMA-T)	DMBA in corn oil @ 20 mg/rat, per os, single dose on 50 th day + CNMA in corn oil @ 50 mg/kg b.wt, one month after mammary tumor development for a period of one month (i.e about 170 th day to 200 th day)
V	Nano cinnamaldehyde control (CZN-C)	Nano zinc cinnamaldehyde @ 50mg/kg b.wt, per os from 50 th day onwards for 30 days
VI	Nano cinnamaldehyde therapeutic (CZN-T)	DMBA in corn oil @ 20 mg/rat, per os, single dose on 50 th day + CZN @ 50 mg/kg b.wt, one month after mammary tumor development for a period of one month (i.e about 170 th day to 200 th day)
VII	Tamoxifen (TAM)	DMBA in corn oil @ 20 mg/rat, per os, single dose on 50 th day + tamoxifen @ 10 mg/kg b.wt in sesame oil per week by I.P route, one month after mammary tumor development for a period of one month (i.e about 170 th day to 200 th day)

After DMBA administration, rats were daily observed for development, localization and size of growths. Ninety days post DMBA administration, 80% rats developed mammary tumors and only these rats were used for further studies.

Tumor latency period was/ tumor incidence with time was recorded in DMBA administered and cinnamaldehyde pre-treated groups (or the pre-treatment effect of cinnamaldehyde on tumor occurrence with time was recorded). Number of tumors in individual rats and total number of tumors in a group were recorded. Tumor morphometrics viz. mean tumor volume, tumor burden and tumor multiplicity were calculated. Body weights were recorded at weekly intervals and mean body weights were calculated. Blood samples were drawn from the retro orbital plexus. Whole blood was used for haematological analysis and plasma samples were separated from the blood for the estimation of total sialic acid (TSA), total protein, albumin, glucose, cholesterol, blood urea nitrogen (BUN), creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Antioxidant marker, SOD, non enzymatic antioxidant marker, GSH and peroxidation marker, TBARS were estimated in mammary tissue, liver, lung, heart, kidney, brain and plasma at the end of the study.

Mammary tissues were subjected to histopathologic (H&E), transmission electron microscopic and immunohistochemistry studies.

3.5.4 Tumor Latency period

The onset of tumors with time after DMBA administration was recorded and compared between DMBA-C and CNMA-C groups.

3.5.5 Tumor Morphometrics

3.5.5.1 Tumor Volume. The volume of tumor was calculated by using the formula, $V = \frac{4}{3} \pi r^3$, where 'r' was the mean of r1 and r2 which are two independent radii of the tumor mass (Mollard *et al.*, 2011).

3.5.5.2 Tumor Multiplicity. Tumor multiplicity was calculated as the average number of total tumors per rat in the group.

3.5.5.3 Tumor Burden. Tumor burden was calculated by multiplying the mean tumor volume with the mean number of tumors.

3.5.6 Tumorigenesis Marker

3.5.6.1 Estimation of Total Sialic Acid (TSA) levels (Plucinsky *et al.*, 1986).

Principle

Total sialic acid reacts with resorcinol to give a blue coloured complex, which is extracted by butyl acetate and n-butanol and read at 580 nm.

Procedure

Hundred micro litre of sample (plasma) and 900 µl of distilled water were taken in screw-capped tubes, which were vortexed and placed on ice. 1.0 ml of resorcinol reagent (10 ml of 2% (w/v) stock resorcinol in water, 9.75 ml of water, 0.25 ml of 0.1 M copper sulphate brought to a final volume of 100 ml with concentrated HCl) was added to each tube and vortexed and placed in a boiling water bath (100°C) for exactly 15 min followed by 10 min. in an ice bath. 2 ml of butyl acetate/ n-butanol (85:15, v/v) was added to each tube, and then the tubes were centrifuged at 1200 g_n for 10 min. at room temperature (RT). The extracted chromophore (blue coloured) was read on spectrophotometer at 580 nm against a standard curve of 0, 20, 40, 50, 60, 80 and 100 µg of N- acetylneuraminicacid (NANA), prepared by treating NANA tubes as TSA assay tubes.

3.5.7 Relative Organ Weights

Weights of vital organs viz. liver, lung, heart, kidney, brain and spleen of rats were noted at the time of sacrifice. Relative organ weight was expressed as % of body weight.

$$\text{Relative organ weight} = (\text{Organ weight}/\text{Body weight}) \times 100$$

3.5.8 Plasma and Tissue Homogenate Preparation

Plasma was separated by refrigerated centrifugation of citrated blood at 3000 g_n for 10 min and kept at -20°C for further analysis. The organs were collected and immediately 10% homogenates were prepared using 0.1 M PBS centrifuged under refrigerated conditions at 20000 g_n for 20 min. The supernatants were collected and preserved under -20°C till analysis.

3.5.9 Estimation of Total Protein – Biuret Method (Fenk *et al.* 2007)

Principle

The reaction of the cupric ions with the nitrogen atoms involved in peptide bonds leads to the development of peptide hydrogen atoms under the alkaline conditions, which is read at 550 nm.

Procedure

20 µl of test compound was mixed with 180 µl of biuret reagent and absorbance was measured at 540 nm. Concentration of protein in test compounds were estimated from the regression equation made with the standard curve.

Protein standard of 100 mg/ml was prepared with bovine serum albumin in phosphate buffer saline. Working standards were prepared with concentrations of 80, 60, 50, 40, 20, 10 and 5 mg/ml and standard curve was constructed with a regression line.

3.5.10 Antioxidant profile

3.5.10.1 Superoxide Dismutase (SOD) (Madesh and Balasubramanian, 1998).

Principle

The reaction involves generation superoxide by hydroxylamine hydrochloride auto-oxidation and the inhibition of superoxide dependent reduction of the nitro blue tetrazolium (NBT) to its blue colour formazan, which is read at 550 nm.

Procedure

Thousand two hundred micro litre of sodium bicarbonate (5 mM) – EDTA (0.1 mM) buffer, 500 µl of 96 µM NBT and 100 µl Triton-x were mixed. The reaction was started by adding 100 µl of 20 mM hydroxylamine hydrochloride. After one and half minute, 70 µl of test compound was added and the first absorbance was taken after 30 sec (zero minute). The second reading was taken after 3 minutes of first reading. A blank was simultaneously run along with the test.

Blank reading (B) was calculated as,

$$B = (\text{Absorbance}_{550} \text{ of blank at 3 minute} - \text{Absorbance}_{550} \text{ of blank at zero minute}) / 3$$

Test reading (T) was calculated as,

$$T = (\text{Absorbance}_{550} \text{ of test at 3 minute} - \text{Absorbance}_{550} \text{ of test at zero minute}) / 3$$

% inhibition is calculated as = $(B - T) \times 100/B$

From this, the quantity required for 50% inhibition is calculated and expressed as U/mg of protein (one unit of SOD is the amount in mg of protein required to inhibit the NBT reduction by 50%).

3.5.10.2 Reduced Glutathione (GSH) (Sedlak and Lindsay, 1968).

Principle

This method is based on a reaction of reduced glutathione (GSH) with 5-5' dithiobis-2-nitrobenzoic acid (DTNB) to give a compound that absorbs light at 412 nm.

Procedure

One hundred and fifty micro litre (150 µl) of test sample (serum/tissue homogenate) and 300 µl 4% sulfosalicylic acid in water were placed in centrifugation tubes vortexed and centrifuged at 15000 rpm for 15 min at 4°C. After deprotenisation the supernatant was taken and was added with equal volume of DTNB (3 mM). They were incubated for 5 minutes and the yellow colour developed was read at 412 nm. The values were expressed as µg of GSH/mg of protein.

Standard curve was plotted using 10, 20, 40, 50, 60, 80, 90 and 100 µg/ml working standards of GSH and by plotting the absorbance value against their respective absorbance values. This was used to calculate the concentration of the test samples.

3.5.11 Lipid Peroxidation Profile

3.5.11.1 Thiobarbituric Acid Reacting Substances (TBARS) (Balasubramanian *et al.*, 1988).

Principle

Malondialdehyde (MDA), formed from the breakdown of polyunsaturated fatty acids, reacts with thiobarbituric acid to give a pink - red colour absorbing light maximally at 532 nm.

Procedure

One fifty micro litre of test sample was deproteinised by adding 300 µl of 5% trichloroacetic acid and the mixture was kept on ice for 15 min. After incubation the samples were centrifuged at 2200 g_n for 15 min under refrigerated conditions. The supernatant was separated and equal volume of TBA was added and kept in a boiling water bath for 10 min. The contents were then allowed to cool and the absorbance was recorded at 532 nm.

Concentrations of test TBARS were estimated from the standard curve plotted with standard solutions of 40, 20, 10, 5, 2.5, 1.25 and 0.625 µM TEP and the results were expressed as µmol/mg of protein.

3.5.12 Plasma Biochemical Profile

Plasma was separated from blood and various biochemical profiles *viz.* Total protein (Biuret method), albumin (BCG dye method), glucose (Trinder's method, end point/fixed time), cholesterol (cholesterol oxidase/peroxidise method), blood urea nitrogen (GLDH – urease method, initial rate), creatinine (Jaffe's method, initial rate), total bilirubin (TB)(modified method of Pearlman Lee), alanine aminotransferase

(ALT) (IFCC method), aspartate aminotransferase (AST) (IFCC method) and alkaline phosphatase (ALP) (IFCC method) were determined as per the protocol given by kit manufacturer.

3.5.13 Gross Examination and Histopathology

All the major internal organs of the animals were grossly examined at the time of sacrifice and liver, lung, heart, kidney, brain and mammary tissues were collected from rats used for acute oral toxicity study. Mammary tumors and mammary tissues were collected from different groups of rats used in the anticarcinogenic study. Collected tissue samples were fixed in 10% NBF and processed by routine paraffin embedding technique. Sections of 4-5 μm thick were cut and stained by Harris haematoxylin and eosin for microscopic studies (Luna, 1968).

3.5.14 Transmission Electron Microscopy (TEM) (Bozzola and Russell 1999).

Samples were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hr at 4°C and washed with PBS for 2 times each 45 min. then post fixed in 1% aqueous osmium tetroxide for 2 hr. Later washed with deionized distilled water for 4 times each 45 min., dehydrated in series of graded alcohols, infiltrated and embedded in araldite 6005 resin or spur resin (Spurr, 1969) and incubated at 80°C for 48 hr for complete polymerization. Ultra thin (60 nm) sections were made with a glass knife on ultra microtome (Leica Ultra cut UCT-GA-D/E-1/100), mounted on copper grids and stained with saturated aqueous uranyl acetate and counter stained with Reynolds lead acetate. The stained sections were viewed under TEM (Hitachi, H-7500 from Japan) at required magnifications as per the standard protocol practicing at RUSKA Lab's, College of Veterinary Science, PVNRTVU, Rajendranagar., Hyderabad, India.

3.5.15 Immunohistochemistry (IHC) Studies

Four micron thick sections were subjected to immunohistochemistry (IHC) for estrogen receptor alpha (ER alpha) and progesterone receptor (PR). Antigen retrieval

was performed at 95°C in citrate buffer (pH:6) for 20 minutes using an antigen retriever (EZ retriever, Biogenex). The ready to use rabbit monoclonal antibody to ER-alpha and mouse monoclonal antibody to PR with a polymer HRP (horseradish peroxidase) detection system (Biogenex, USA) were used. IHC procedure was carried out according to the protocol of the kit with DAB (3, 3'-diaminobenzidine tetrahydrochloride) as chromogen and Harris haematoxylin as counter stain.

3.5.16 Statistical Analysis

Results were analysed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests using GraphPad Prism 7.02. Data was expressed as mean $\pm$ SEM and the level of significance was $P < 0.05$. Pharmacokinetic results were analysed manually and the graphs were made using Microsoft Excel[®]-2010.

CHAPTER IV

RESULTS

An experimental study was conducted to evaluate the anticarcinogenic activity of cinnamaldehyde (CNMA) and nano zinc cinnamaldehyde (CZN) in chemically induced breast cancer in virgin female Sprague Dawley rats.

Nano zinc cinnamaldehyde particles was synthesized and characterized before proceeding for the study.

Acute oral toxicity and anticarcinogenic activity of cinnamaldehyde and nano zinc cinnamaldehyde were studied. Further cinnamaldehyde was studied for its pharmacokinetic parameters.

4.1 CHARACTERIZATION OF CINNAMALADHYDE ZINC NANO PARTICLES (CZN)

Synthesized cinnamaldehyde nano zinc particles were characterized using following techniques.

4.1.2 Electron Microscopic Analysis of Nanoparticles

The TEM image of CZN showed particles with different shape (pentagon or hexagon) and size, surrounded by a thin homogenous translucent sheath all around the electron dense particles (Figure 2). SEM studies revealed the presence of nano zinc CNMA as spherical shaped particles in electron dense matrix (Figure 3).

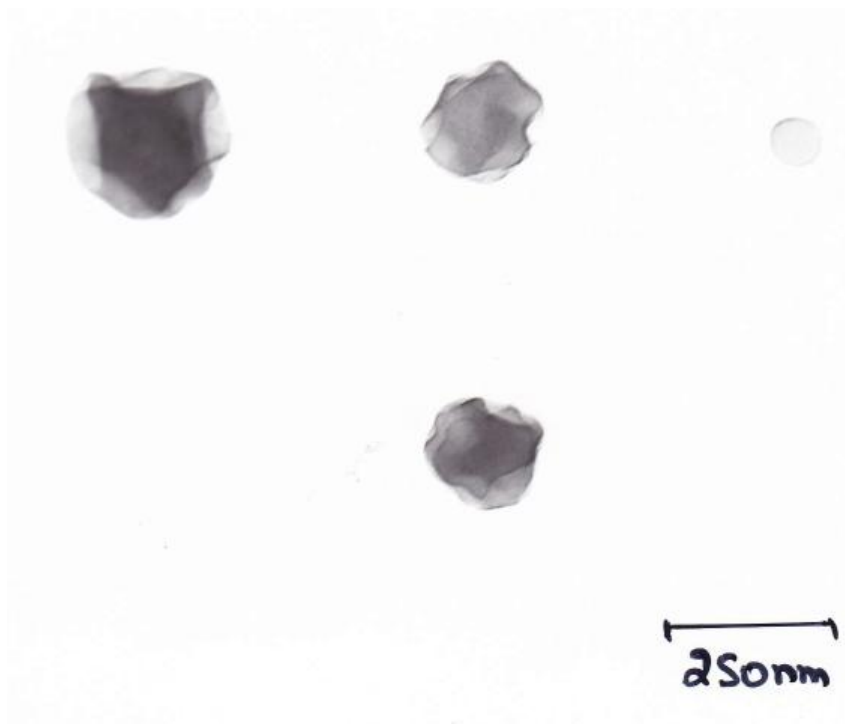


Figure 2. Transmission electron microscopic image of synthesised cinnamaldehyde mediated zinc nano particles (x 40K).

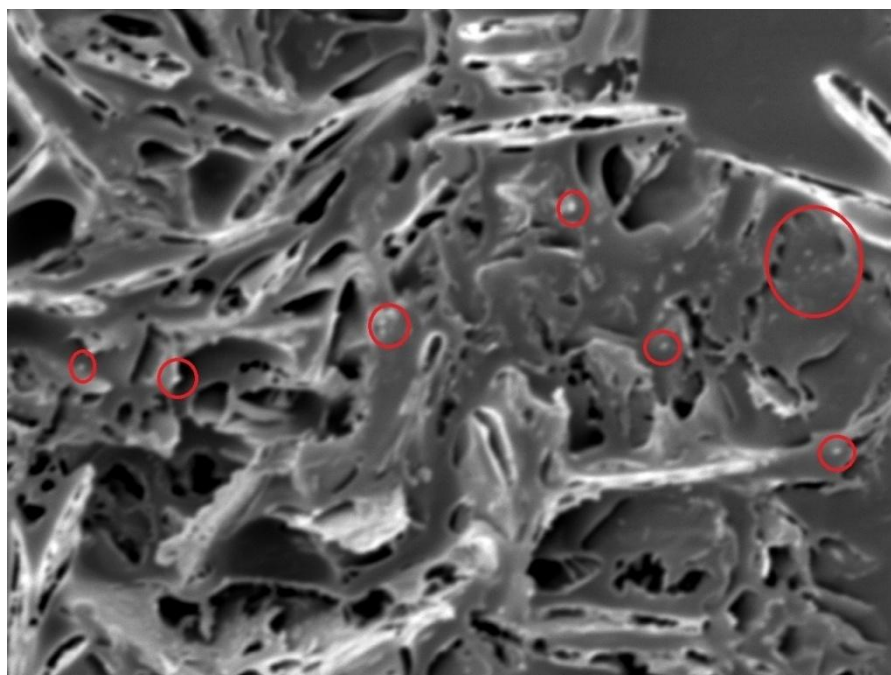


Figure 3. Scanning electron microscopic image of synthesised cinnamaldehyde mediated zinc nano particles (x 3500K)

4.1.3 Zeta Potential and Particle Size (Dynamic light scattering technique)

The mean surface potential of synthesized CZN particles was -6.6 mv and the average particle size recorded was 70 nm (Figure 4).

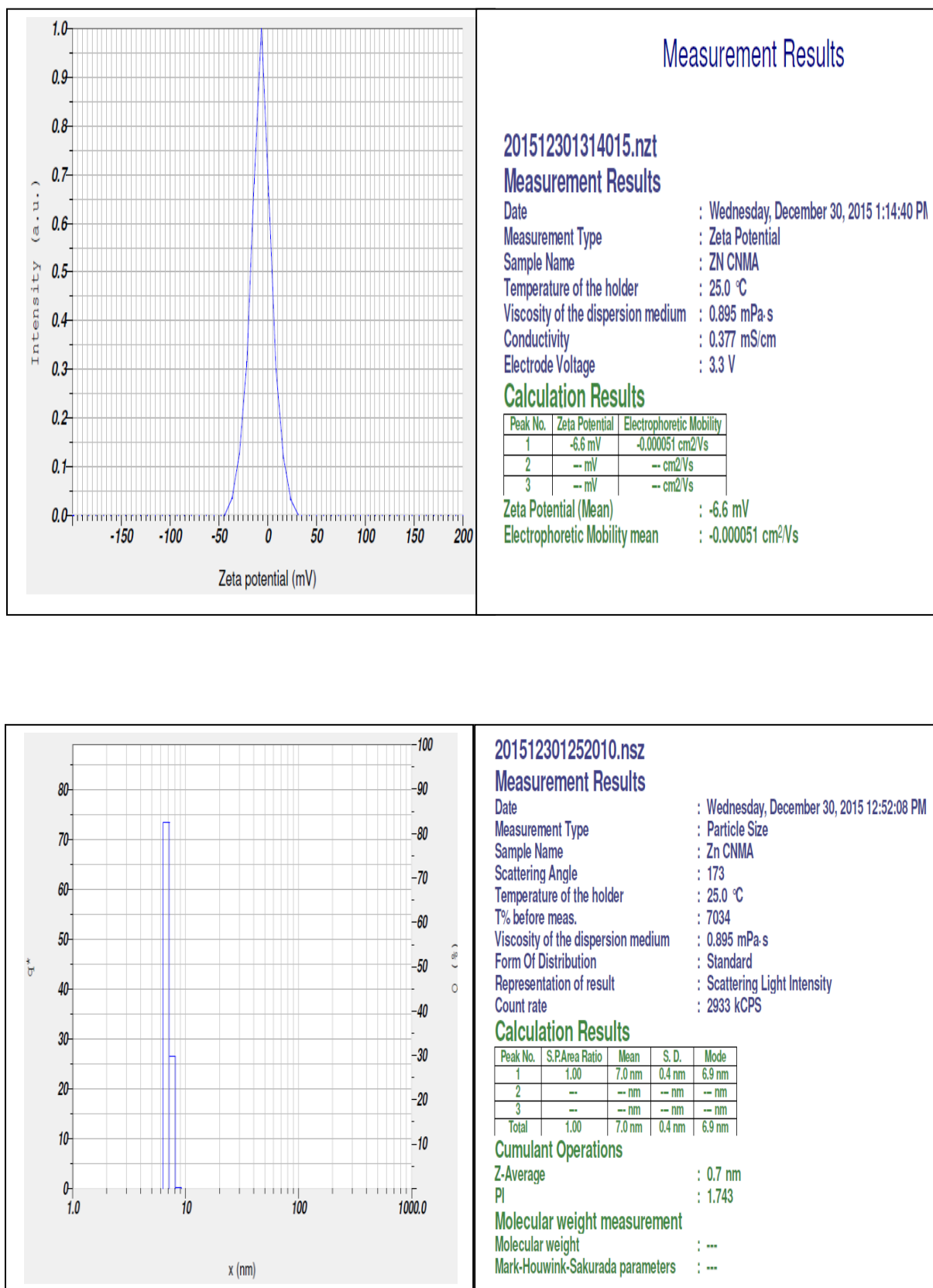


Figure 4. Zeta potential and average size of the cinnamaldehyde zinc nano particles (CZN) – DLS method- Nanopartica SZ-100 (HORIBA)

4.2. ACUTE ORAL TOXICITY

Toxicity study was conducted according to OECD, Test Guideline 425, Up-and-Down-procedure, for cinnamaldehyde (CNMA) and nano zinc cinnamaldehyde (CZN). Animals in both the treatment groups not displayed any signs of toxicity.

4.2.1 Mean Body Weight Gain

Body weight gain (g) of rats at the end of 14th day in control, cinnamaldehyde and nano cinnamaldehyde were 38.8 ± 2.90 , 36.8 ± 2.05 and 31.8 ± 1.49 respectively. A non significant ($P > 0.05$) decrease was observed in CNMA and CZN group when compared to control group (Table 2).

4.2.2 Relative Organ Weights

The relative weights of liver, lung, heart, kidney, brain and spleen did not show significant ($P > 0.05$) difference in CNMA and CZN treated rats when compared to control rats (Table 3).

4.2.3 Haematological Analysis

Various haematological parameters viz. haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte count, neutrophil count and monocyte count in cinnamaldehyde and nano cinnamaldehyde treated groups were comparable to those observed in control group (Table 4).

4.2.4 Plasma Biochemical Analysis

Biochemical analysis for plasma- total protein, albumin, creatinine, BUN, ALT, AST, ALP, glucose total cholesterol and total bilirubin revealed no significant ($P > 0.05$) difference between the control and CNMA group. Whereas BUN, AST and ALP levels in nano cinnamaldehyde group were significantly increased from control while the remaining parameters were statistically comparable (Table 5).

Table 2. Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on body weight gain (g) in rats (n=5)

Group	0 day	14 th day	Mean b.wt gain
Control	228 ± 2.55	266.8 ± 1.31	38.8 ± 2.90
CNMA	221.6 ± 2.65	256 ± 1.26	36.8 ± 2.05
CZN	227.4 ± 3.25	258.4 ± 1.96	31.8 ± 1.49

Values are mean ± SEM (n=5) one way ANOVA, GraphPad Prism (7.02)

Means with *superscripts in a row differ significantly from control (P < 0.05)

Table 3. Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on relative organ weights in rats (n=5)

Organ	Control	CNMA	CZN
Liver	3.52 ± 0.17	3.63 ± 0.02	3.67 ± 0.06
Lung	0.63 ± 0.04	0.67 ± 0.01	0.68 ± 0.01
Heart	0.43 ± 0.007	0.48 ± 0.01	0.44 ± 0.01
Kidney	0.68 ± 0.02	0.68 ± 0.01	0.69 ± 0.01
Brain	0.63 ± 0.01	0.61 ± 0.01	0.68 ± 0.01
Spleen	0.31 ± 0.02	0.28 ± 0.01	0.28 ± 0.01

Values are mean ± SEM (n=5) one way ANOVA, GraphPad Prism (7.02)

Means with *superscripts in a row differ significantly from control (P < 0.05)

Table 4. Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on various haematological parameters in rats (n=5)

	Control	CNMA	CZN
Hb (gm %)	12.2 ± 0.58	12.0 ± 0.71	12.0 ± 0.71
PCV (%)	34.8 ± 1.39	35.0 ± 1.00	35.8 ± 0.58
RBC (× 10 ⁶)	8.40 ± 0.51	8.0 ± 0.35	8.40 ± 0.53
WBC (× 10 ³)	9.80 ± 0.37	10.0 ± 1.00	10.6 ± 0.93
Lymphocyte	68.4 ± 2.25	68.6 ± 0.93	70.0 ± 1.93
Neutrophils	28.6 ± 1.03	33.0 ± 1.58	33.6 ± 1.57
Monocyte	1.00 ± 0.32	1.4 ± 0.51	1.60 ± 0.40

Values are mean ± SEM (n=5) one way ANOVA, GraphPad Prism (7.02)

Means with *superscripts in a row differ significantly from control (P < 0.05)

Table 5. Effect of cinnamaldehyde and nano zinc cinnamaldehyde (2000 mg/kg b.wt) on various plasma biochemical parameters in rats (n=5)

Parameter	Control	CNMA	CZN
Total protein (mg/dl)	7.15 ± 0.37	7.78 ± 0.34	7.76 ± 0.40
Albumin (mg/dl)	3.83 ± 0.37	4.19 ± 0.25	3.97 ± 0.31
BUN (mg/dl)	28.44 ± 0.63	32.16 ± 1.43	33.02 ± 1.12 [*]
Creatinine (mg/dl)	0.79 ± 0.04	0.85 ± 0.06	0.89 ± 0.05
ALT (IU/L)	38.93 ± 2.12	44.76 ± 2.28	45.26 ± 1.91
AST (IU/L)	70.09 ± 2.40	77.27 ± 1.58	78.55 ± 2.44 [*]
ALP (IU/L)	141.0 ± 2.49	147.9 ± 2.07	150.4 ± 1.30 [*]
Glucose (mg/dl)	65.16 ± 1.51	58.70 ± 1.95	59.17 ± 1.95
Cholestrol (mg/dl)	52.87 ± 2.19	51.9 ± 3.39	51.09 ± 1.45
Total bilirubin (mg/dl)	0.539 ± 0.02	0.494 ± 0.014	0.541 ± 0.03

Values are mean ± SEM (n=5) one way ANOVA, GraphPad Prism (7.02)

Means with *superscripts in a row differ significantly from control (P < 0.05)

4.2.5 Histopathology

At the end of the study liver, heart, kidney, brain and stomach of rats from different treatment groups were subjected to histopathological examination. The liver and kidney sections of CNMA treated rats showed congestion and mild degenerative changes. Rats in CZN group also revealed similar changes along with mild neutrophilic infiltration. Areas of mild congestion and haemorrhages were observed in the myocardium of CNMA and CZN rats. Stomach sections of CZN rats revealed presence of neutrophils in submucosa whereas CNMA group showed mild degenerative changes. Brain tissue evinced few areas of mild congestion in both the treatment groups (Figure 5 to 14).

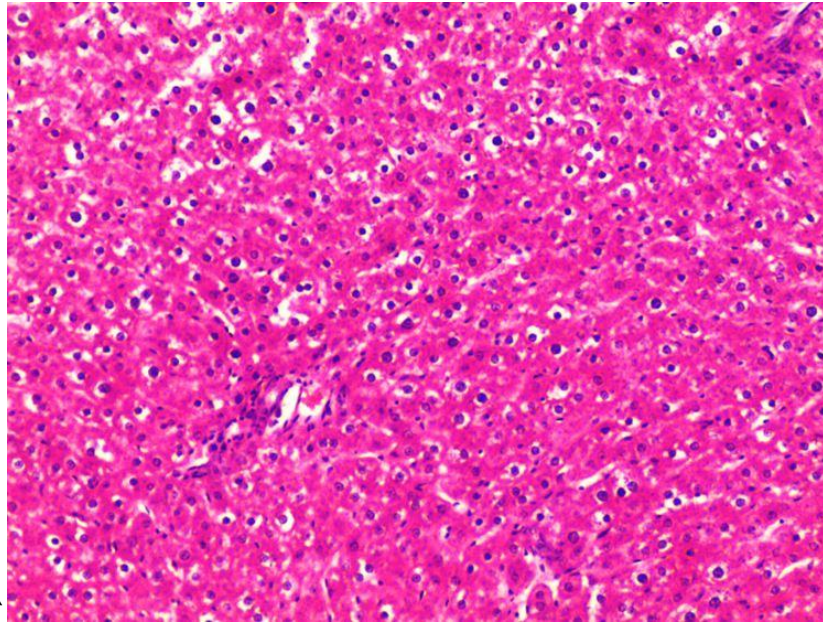


Figure 5. Acute oral toxicity - cinnamaldehyde treated rat liver - showing congestion and mild degenerative changes (H&E x10)

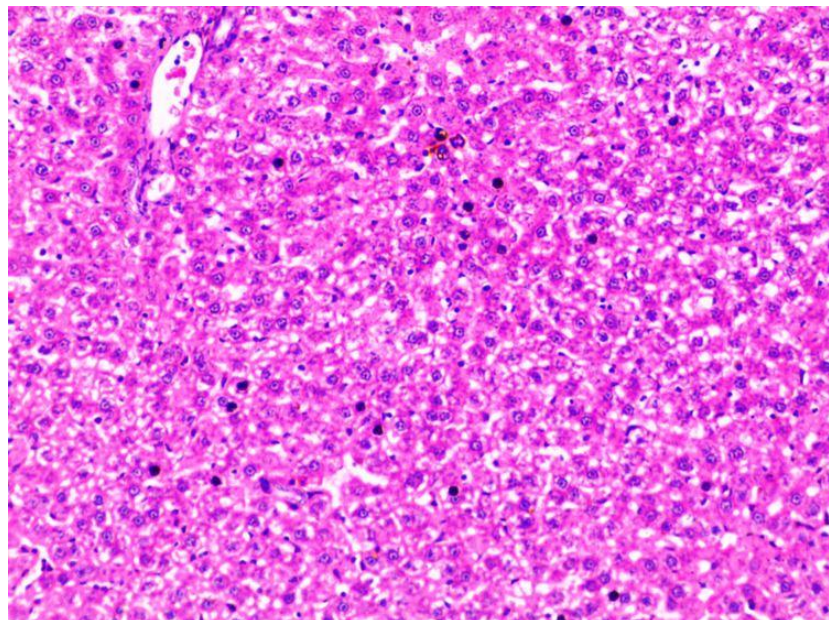


Figure 6. Acute oral toxicity - nano zinc cinnamaldehyde treated rat liver - showing degenerative changes (H&E x10)

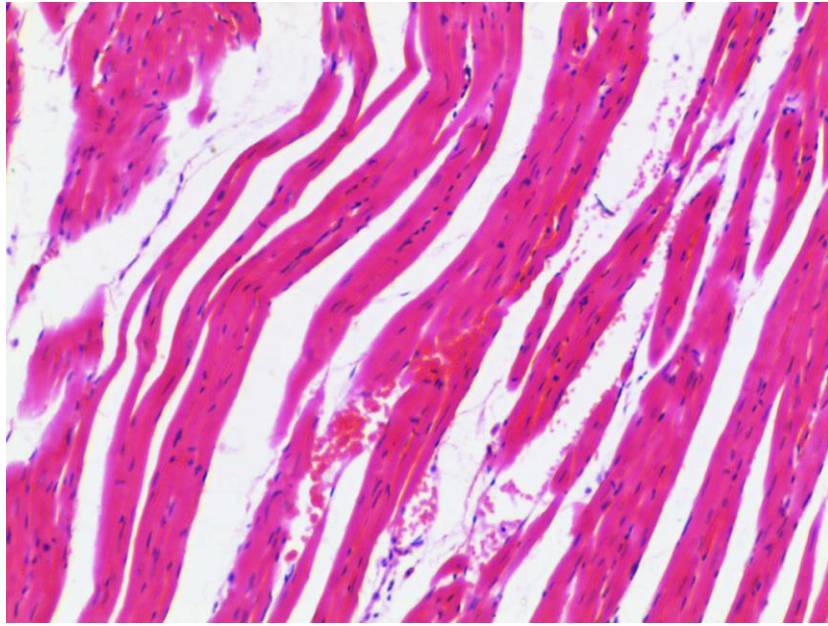


Figure 7. Acute oral toxicity - cinnamaldehyde treated rat myocardium - showing congestion and haemorrhage (H&E x10)

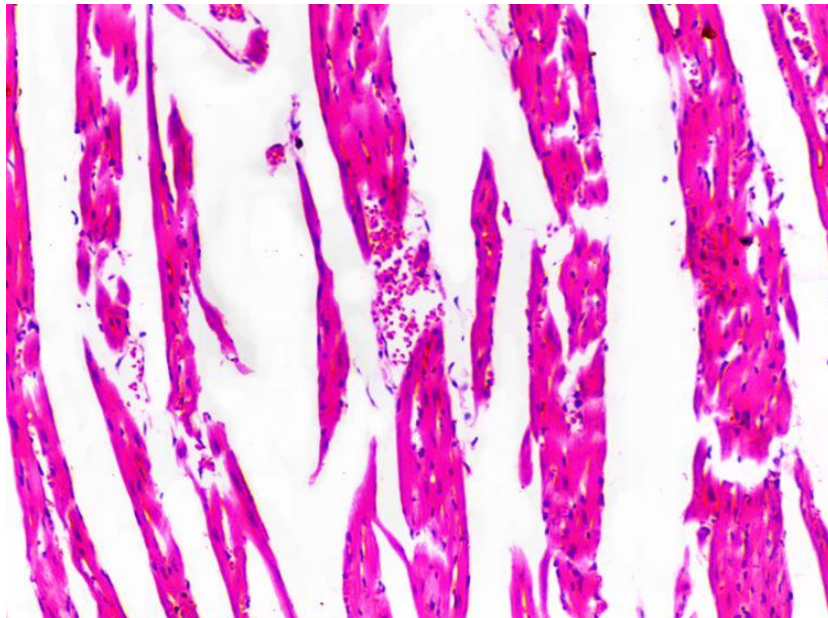


Figure 8. Acute oral toxicity - nano zinc cinnamaldehyde treated rat myocardium - showing congestion and haemorrhage (H&E x10)

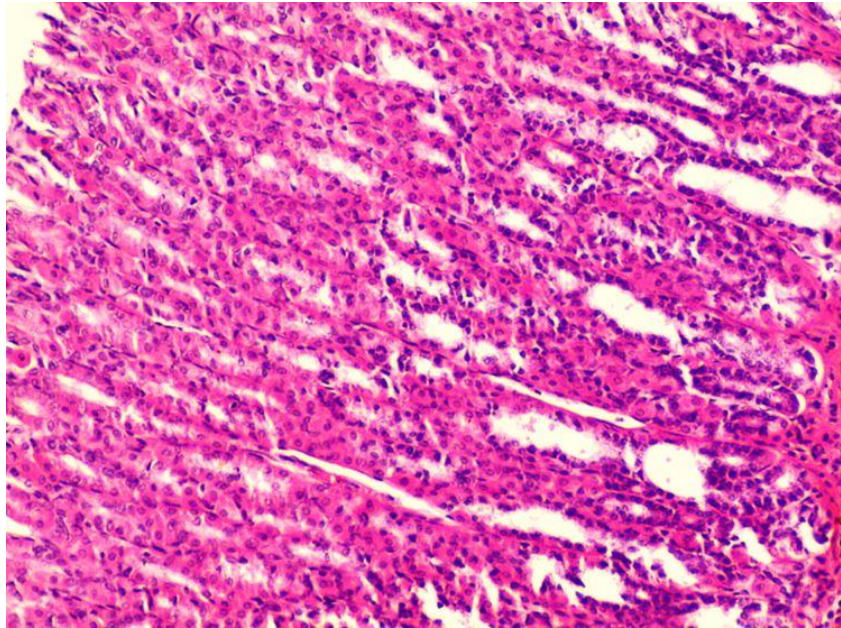


Figure 9. Acute oral toxicity - cinnamaldehyde treated rat stomach - showing mild degenerative changes (H&E x10)

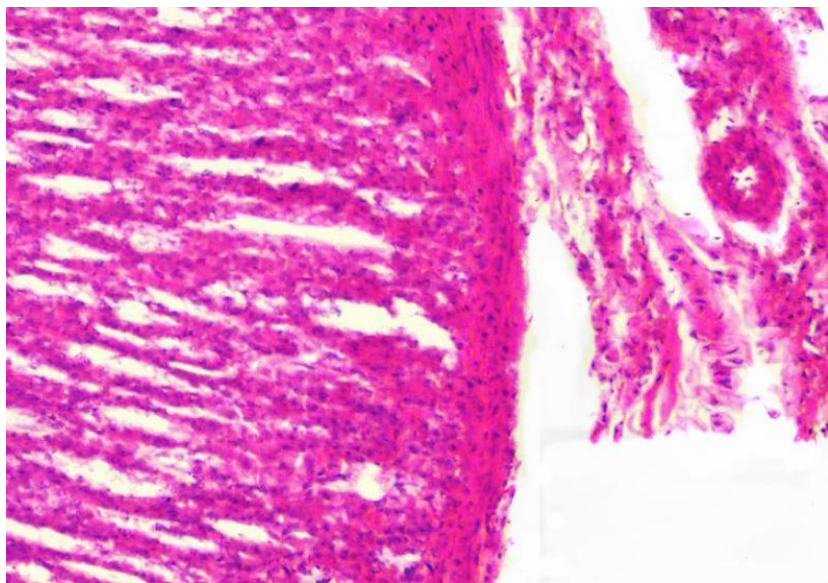


Figure 10. Acute oral toxicity - nano zinc cinnamaldehyde treated rat stomach - showing degenerative changes and infiltration in submucosa (H&E x10)

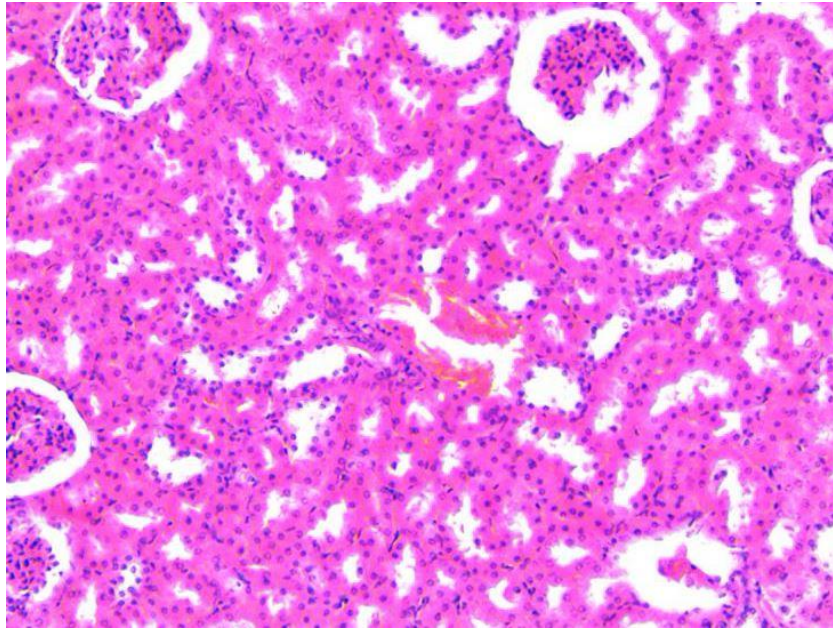


Figure 11. Acute oral toxicity - cinnamaldehyde treated rat kidney - showing congestion and mild degenerative changes (H&E x10)

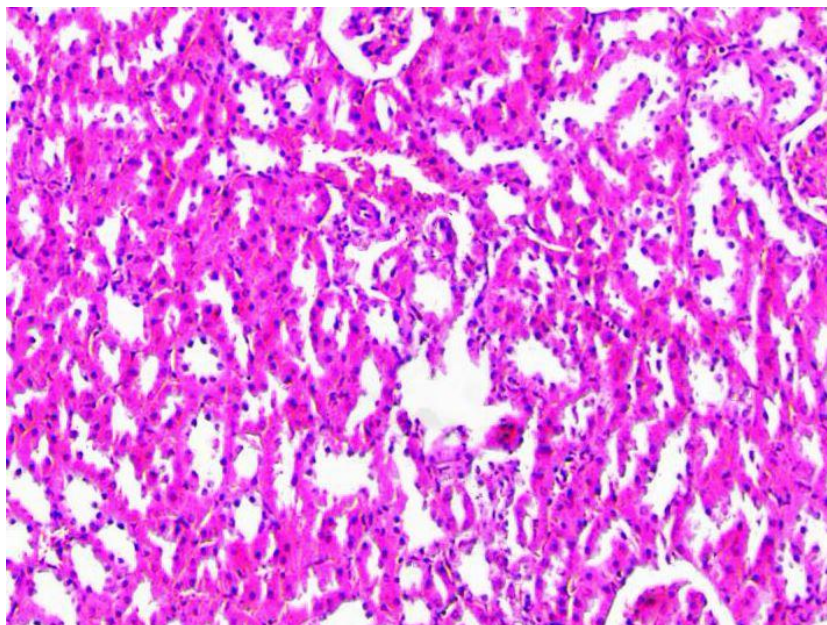


Figure 12. Acute oral toxicity - nano zinc cinnamaldehyde treated rat kidney - showing congestion and mild degenerative changes (H&E x10)

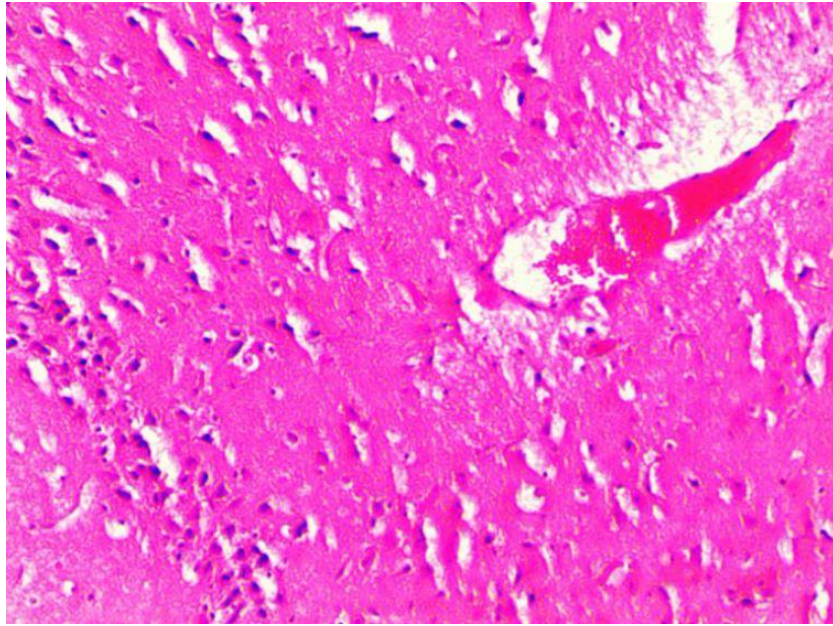


Figure 13. Acute oral toxicity - cinnamaldehyde treated rat brain - showing congestion (H&E x10)

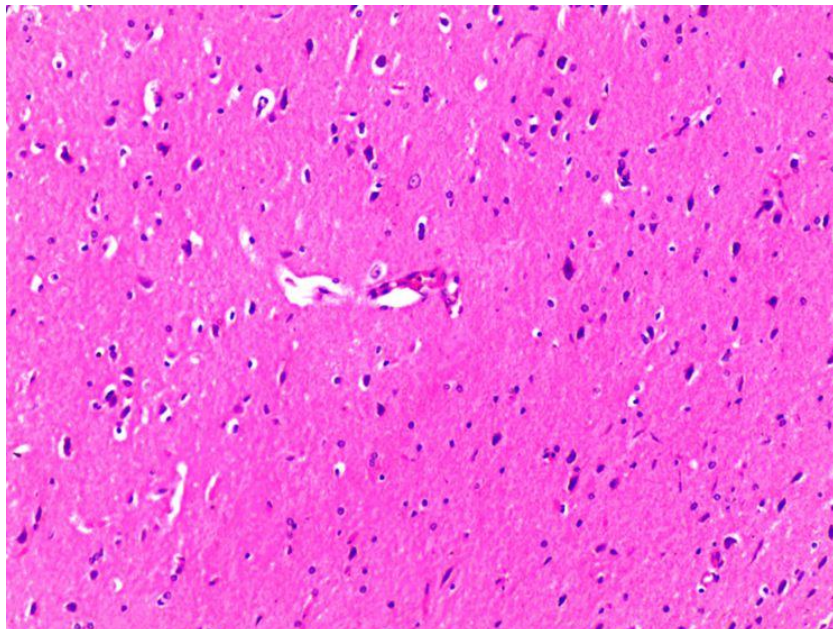


Figure 14. Acute oral toxicity -nano zinc cinnamaldehyde treated rat brain - showing congestion (H&E x10)

4.3 PHARMACOKINETIC ANALYSIS OF CINNAMALDEHYDE

4.3.1 Detection of Cinnamaldehyde (CNMA) and Cinnamic Acid (CA)

A chromatogram without showing any disturbance was obtained with the mobile phase acetonitrile (50%), 0.1% glacial acetic acid (25%) and water (25%) at a detection wavelength of 285nm. Chromatograms of CNMA and its metabolite CA from 0.01 µg/ml to 1.0 µg/ml concentrations are presented in figure 15.

4.3.2 Standard Concentration versus Area

The standard concentration versus area under curves for cinnamaldehyde (0.005, 0.01, 0.05, 0.1, 0.25, 0.5 and 1.0 µg/ml) and cinnamic acid (0.05, 0.1, 0.25, 0.5 and 1.0 µg/ml) are given in table 6 and 7 respectively. The experiment was repeated five times with freshly prepared standard concentrations each time, for both the compounds in acetonitrile.

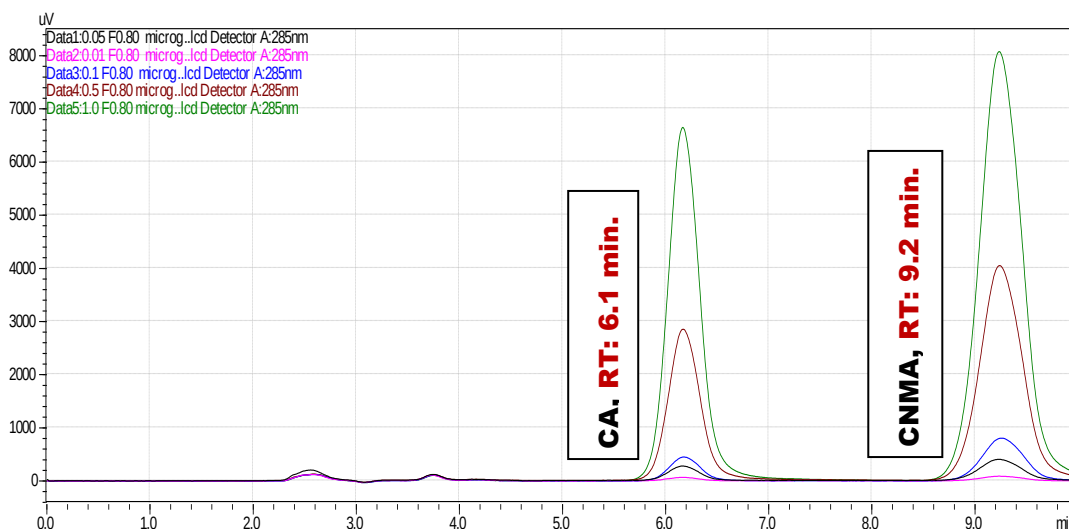


Figure 15. Chromatogram of cinnamaldehyde and cinnamic acid (0.01 to 1.0 µg/ml) in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)

4.3.3 Response Linearity

Individual graphs plotted, taking concentration of CNMA and CA on the x- axis and area on the y- axis, gave a linear relation with R^2 value of 0.999 and a regression equation as $\text{area} = 26319 (\text{Conc.}) + 157.9$ for CNMA (Figure 16) and for CA a

Table 6. Cinnamaldehyde standard concentrations versus area under curve in acetonitrile: 0.1% glacial acetic acid: water (50:25:25)

Concentration ($\mu\text{g/ml}$)	Trail 1	Trail 2	Trail 3	Trail 4	Trail 5	Mean $\pm$ SE	SD	CV
0.005	2604	1828	1792	1879	2225	2065.6 $\pm$ 155.1472	310.2944	16.79511
0.01	3718	2664	3176	2954	3320	3166.4 $\pm$ 176.8428	353.6855	12.48839
0.05	14077	14527	12071	12198	12470	13068.6 $\pm$ 512.6048	1025.21	8.770787
0.1	27755	27527	25531	25534	24888	26247.0 $\pm$ 582.253	1164.506	4.960404
0.25	67877	63165	63697	65565	60848	64230.4 $\pm$ 1181.928	2363.856	4.114673
0.5	135199	133271	133782	132810	131500	133312.4 $\pm$ 604.9255	1209.851	1.01465
1.0	264280	261682	261378	263623	264220	263036.6 $\pm$ 627.5444	1255.089	0.533474

Table 7. Cinnamic acid standard concentration versus area under curve in acetonitrile: 0.1% glacial acetic acid: water (50:25:25)

Concentration ($\mu\text{g/ml}$)	Trail 1	Trail 2	Trail 3	Trail 4	Trail 5	Mean $\pm$ SE	SD	CV
0.05	1565	1395	1668	1813	1192	1526.6 $\pm$ 107.8817	241.2308	15.80183
0.1	3256	3066	3939	3567	2646	3294.8 $\pm$ 219.5316	490.8877	14.89886
0.25	10098	9608	11452	9078	11997	10446.6 $\pm$ 552.8294	1236.164	11.83317
0.5	27865	26902	25214	29557	24883	26884.2 $\pm$ 863.1487	1930.059	7.179158
1.0	61346	59565	59287	57801	69936	61587.0 $\pm$ 2161.899	4834.154	7.849308

regression line was obtained with a R^2 value of 0.985 and a regression equation as area = 58903 (Conc.) (Figure17)

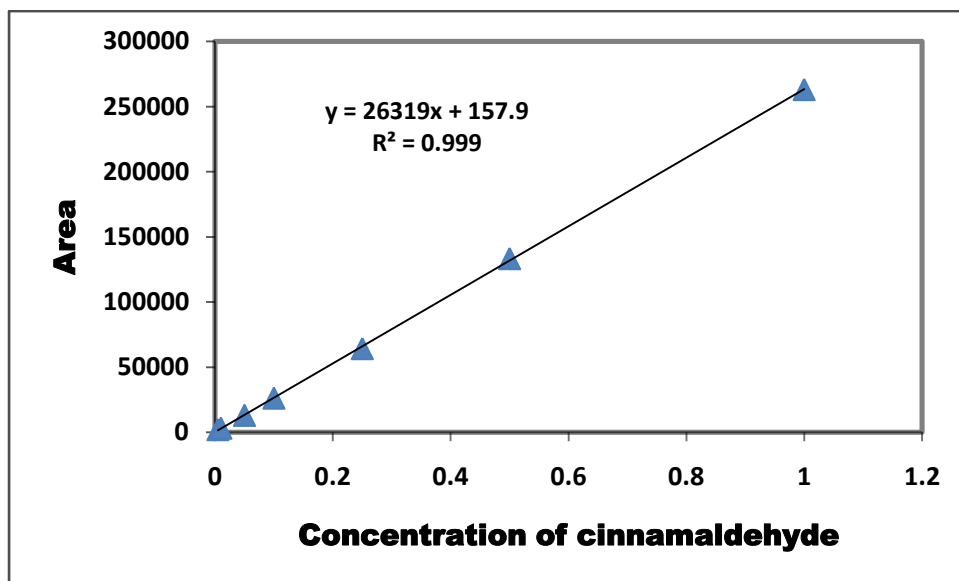


Figure 16. Response linearity of cinnamaldehyde in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)

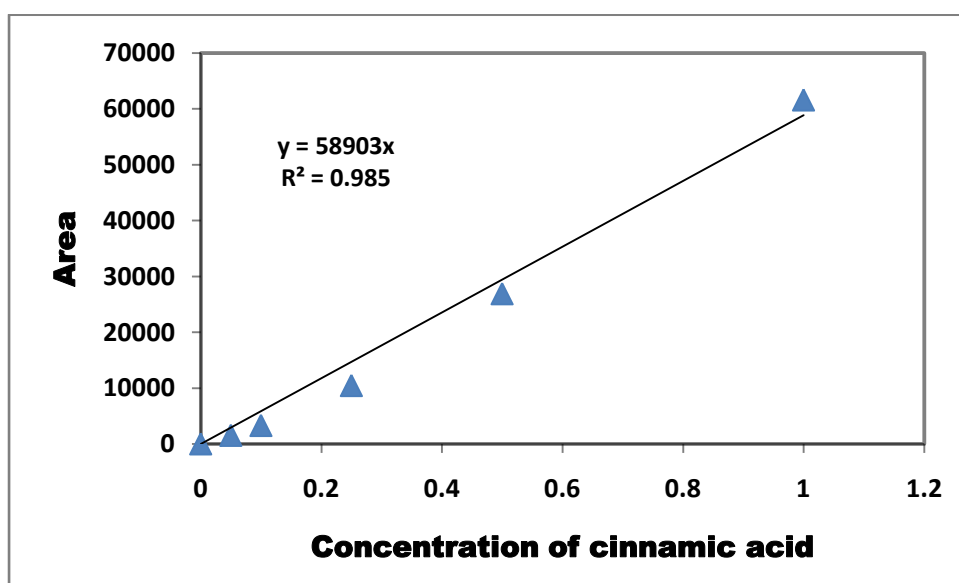


Figure 17. Response linearity of cinnamic acid in acetonitrile : 0.1% glacial acetic acid : water (50:25:25)

4.3.4. Standardization of Recovery of CNMA and CA

Acetonitrile, methanol, glacial acetic acid and water were used in different combination and proportions for extraction of CNMA and CA simultaneously from plasma spiked samples at concentrations of 0.005, 0.01, 0.05, 0.1, 0.25, 0.5 and 1.0 µg/ml. It has been found that 9 parts acetonitrile and 1 part 0.1% glacial acetic acid

combination showed good extraction capacity from plasma with a mean recovery percentage of 83.87 to 94.22 for CNMA and 87.27 to 89.83 for CA (Figure 18; Table 8 and 9).

HPLC conditions for detection of cinnamaldehyde and cinnamic acid:

Mobile phase: acetonitrile: 0.1% glacial acetic acid: water (50:25:25)

Detector wavelength (λ) 285nm; column oven 40°C, flow rate 0.8ml/min; run time 11 min.

Extraction solvent: acetonitrile: 1% glacial acetic acid (90:10).

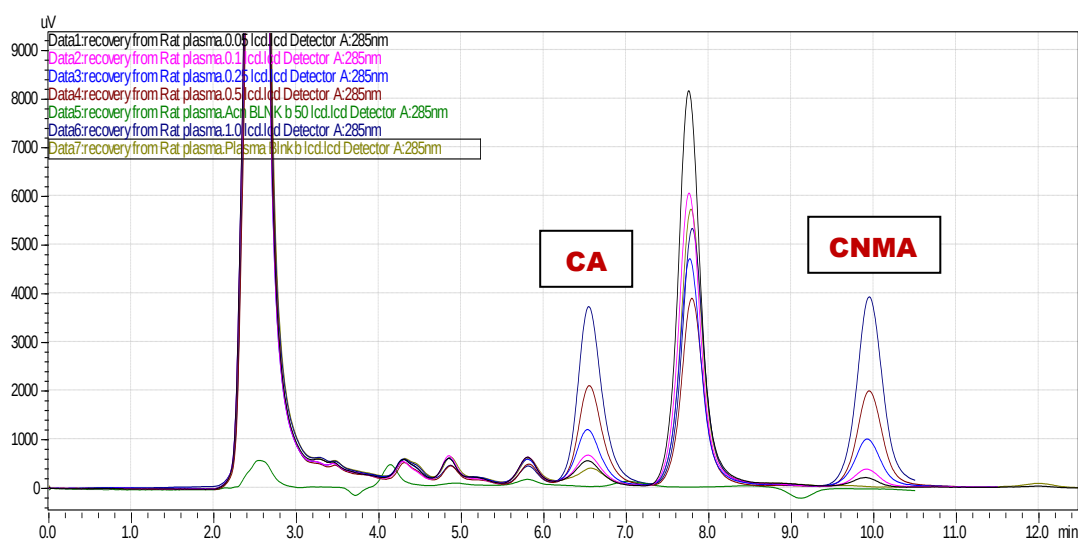


Figure 18. Representative chromatogram of cinnamaldehyde and cinnamic acid showing recovery from spiked plasma samples in acetonitrile: 0.1% glacial acetic acid : water (50:25:25)

4.3.5 Pharmacokinetics in Normal Rats

Eight adult healthy male animals were given CNMA at a dose rate of 500 mg/kg b.wt in 0.1% tween 20 by oral gavage needle. Individual animal plasma concentrations of CNMA and its metabolite CA were shown in table 10 and 11; figure 19 and 20 respectively. Plasma concentration of cinnamaldehyde and its metabolite cinnamic acid at different time intervals were shown in figure 21.

Table 8. Per cent recovery of cinnamaldehyde from spiked plasma samples with extraction solvent containing acetonitrile and 0.1% glacial acetic acid (90:10)

Concentration ($\mu\text{g/ml}$)	Trail 1	Trail 2	Trail 3	Trail 4	Trail 5	Mean $\pm$ SE	SD	CV
0.005	85.38	74.48	73.03	87.89	98.55	83.87 $\pm$ 4.69	10.48	12.50
0.01	97.43	85.90	92.63	70.21	96.51	88.54 $\pm$ 5.01	11.21	12.66
0.05	87.41	97.66	76.98	91.62	93.11	89.36 $\pm$ 3.50	7.83	8.76
0.1	84.05	89.01	100.46	86.24	104.99	92.95 $\pm$ 4.13	9.23	9.93
0.25	79.97	104.08	99.80	78.56	102.48	92.98 $\pm$ 5.64	12.62	13.57
0.5	84.52	94.17	90.62	101.51	100.29	94.22 $\pm$ 3.14	7.02	7.45
1.00	79.29	95.58	74.25	118.41	89.98	91.50 $\pm$ 7.71	17.25	18.85

Table 9. Per cent recovery of cinnamic acid from spiked plasma samples with extraction solvent containing acetonitrile and 0.1% glacial acetic acid (90:10)

Concentration ($\mu\text{g/ml}$)	Trail 1	Trail 2	Trail 3	Trail 4	Trail 5	Mean $\pm$ SE	SD	CV
0.05	85.42	76.18	91.05	98.98	84.70	87.27 $\pm$ 3.77	8.43	9.66
0.1	82.34	77.55	99.64	90.23	94.24	88.80 $\pm$ 3.98	8.91	10.03
0.25	83.76	79.70	94.99	90.23	99.52	89.64 $\pm$ 3.61	8.06	8.99
0.5	86.37	83.39	78.16	91.62	106.89	89.29 $\pm$ 4.91	10.98	12.30
1.0	83.01	80.60	88.34	102.57	94.63	89.83 $\pm$ 4.00	8.93	9.94

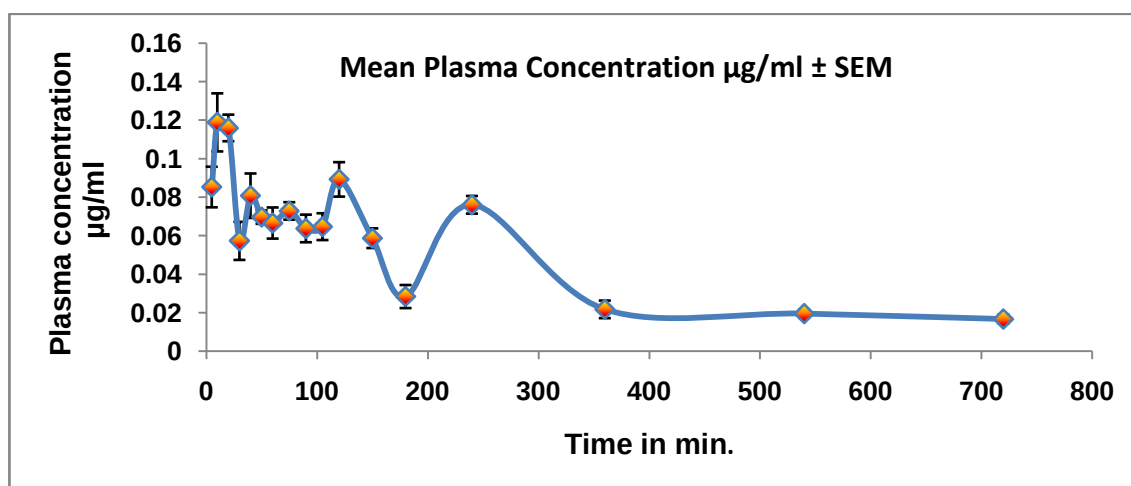


Figure 19. Cinnamaldehyde mean plasma concentration versus time curve in rats (n=8) dosed with cinnamaldehyde at the rate of 500 mg/kg b.wt., orally

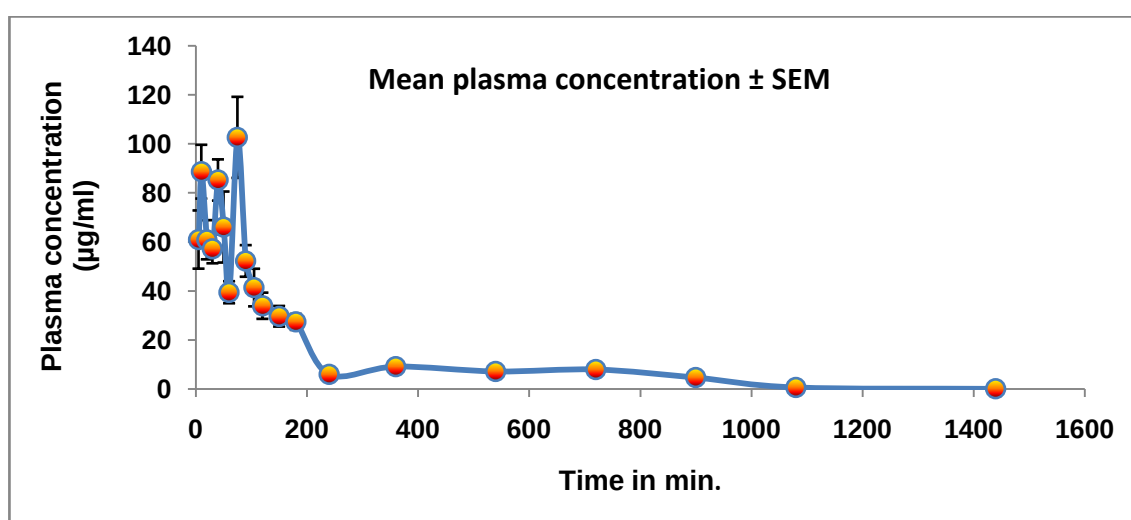


Figure 20. Cinnamic acid mean plasma concentration versus time curve in rats (n=8) dosed with cinnamaldehyde at the rate of 500 mg/kg b.wt., orally

4.3.6 Noncompartmental Modelling

The calculated non compartmental model kinetic parameters of CNMA and CA were given in tables 10 and 11. In this model the mean elimination rate constant (K_e) of CNMA and CA were 0.00818 ± 0.00039 and 0.01322 ± 0.00025 per minute respectively. Mean $t_{1/2}$ of CNMA was 85.4975 ± 3.8862 min. while that of CA was 52.4795 ± 0.95321 minutes. Mean values of AUC_{0-t} and $AUMC_{0-t}$ of CNMA and CA were 28.1315 ± 1.7439 , 15588.9 ± 869.325 and 3500.25 ± 345.331 , 1181696 ± 74087 respectively. Mean residence time (MRT) of CNMA and CA were 123.373 ± 5.6078 min. and 75.728 ± 1.37548 min. respectively.

Table 10. Calculated noncompartmental kinetic model parameters of cinnamaldehyde (dosed with CNMA @ 500 mg/kg b.wt, orally) in rats

Parameters	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean $\pm$ SE	CV
AUC_{0-t}	27.3558	30.542	24.1664	25.0552	33.5379	28.1315 $\pm$ 1.7439	13.862
AUMC_{0-t}	3708.65	3925.83	2580.9	2844.62	4441.24	3500.25 $\pm$ 345.331	22.0608
MRT (min.)	135.571	128.539	106.797	113.534	132.424	123.373 $\pm$ 5.6078	10.164
t_{1/2} (min.)	93.9505	89.0773	74.0104	78.679	91.7702	85.4975 $\pm$ 3.8862	10.164
Ke (1/min)	0.00738	0.00778	0.00936	0.00881	0.00755	0.00818 $\pm$ 0.00039	10.586
Cl.(μg/ml/min.)	18.2776	16.3709	20.6899	19.9559	14.9085	18.0406 $\pm$ 1.0803	13.3901
Vss (μg/ml)	2477.91	2104.29	2209.62	2265.68	1974.25	2206.35 $\pm$ 84.1316	8.52647

Table 11. Calculated noncompartmental kinetic model parameters of cinnamic acid (dosed with CNMA @ 500 mg/kg b.wt, orally) in rats

Parameters	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean $\pm$ SE	CV
AUC_{0-t}	17642.4	15612.1	13372.7	13930.1	17387.2	15588.9 $\pm$ 869.325	12.4696
AUMC_{0-t}	1383774	1199249	1043236	989175	1293044	1181696 $\pm$ 74087	14.0191
MRT (min.)	78.4345	76.8153	78.0126	71.0097	74.3677	75.728 $\pm$ 1.37548	4.06148
t_{1/2} (min.)	54.3551	53.233	54.0627	49.2097	51.5368	52.4795 $\pm$ 0.95321	4.06148
Ke (1/min)	0.01275	0.01302	0.01282	0.01408	0.01345	0.01322 $\pm$ 0.00025	4.17434
Cl.(μg/ml/min.)	0.00036	0.00042	0.00048	0.00051	0.00039	0.00043 $\pm$ 2.7E-05	14.1894
Vss (μg/ml)	0.02834	0.03203	0.03739	0.03589	0.02876	0.03248 $\pm$ 0.00183	12.5961

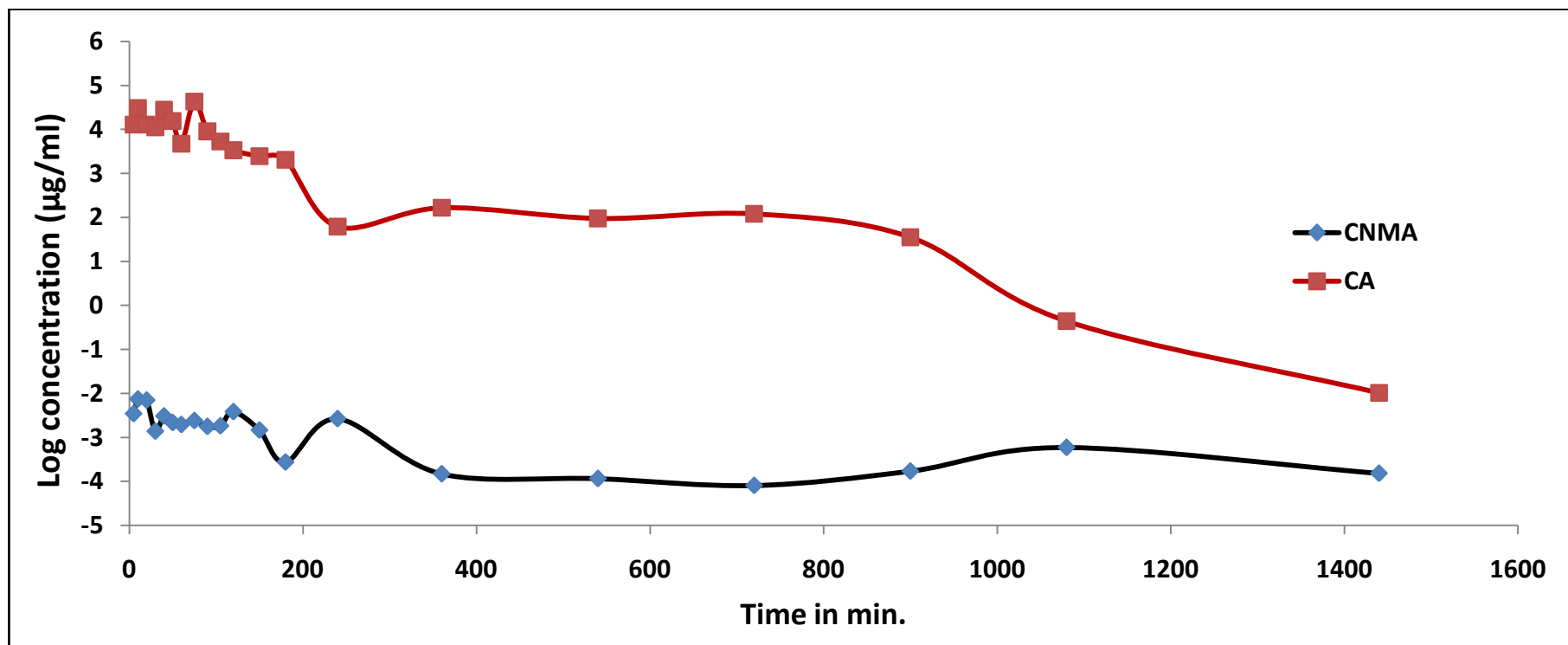


Figure 21. Cinnamaldehyde (CNMA) and Cinnamic acid (CA) mean plasma concentration versus time curve in rats (n=8) dosed with cinnamaldehyde at the rate of 500 mg/kg b.wt., orally

4.4 ANTI CARCINOGENIC STUDIES

The anti-breast cancer effects of cinnamaldehyde and its nano preparations were evaluated in DMBA induced mammary cancer model. Various parameters assessed in the study were, tumor morphometrics, tumorigenicity marker, antioxidant markers (SOD and GSH) peroxidation marker (TBARS), plasma biochemical profile, immunohistochemistry (IHC) studies and histopathology.

4.4.1 Tumor latency period

Pre-treatment with cinnamaldehyde increased the time of onset of tumors to 90 day when compared to DMBA alone administered group in which tumor latency period was recorded as 70 days after carcinogen administration. The difference in the pattern of occurrence with time was shown in (Table 12; Figure 22).

4.4.2 Tumor Morphometrics

4.4.2.1 Tumor Volume. The mean tumor volume (cm^3) in the rats at the end of the study in various treatment groups were 149.2 ± 28.80 (DMBA-C), 52.55 ± 15.58 (CNMA-P), 79.80 ± 20.85 (CNMA-T), 74.0 ± 9.99 (CZN-T) and 55.99 ± 7.37 (TAM). Statistical analysis revealed that the values in group II, group IV and group VI differed significantly ($P < 0.05$) when compared to CNMA prophylactic (III) (Table 13; Figure 25, 26 and 27).

4.4.2.3 Tumor Multiplicity. Average number of tumors in different treatment groups ranged from 0 to 5, with highest tumor multiplicity in DMBA control group (Table 13; Figure 23 and 24).

4.4.2.4 Tumor Burden. The mean tumor burden (cm^3) values are presented in the table 13 and figure 21, 24 and 28. Analysis of the results indicated that tumor burden in group DMBA-C (149.53 ± 11.89) was significantly ($P < 0.05$) higher compared CNMA-P, CNMA-T and CZN-T groups.

Table 12. Effect of cinnamaldehyde prophylactic treatment on tumor, number and latency period in rats administered with DMBA

Days	DMBA	CNMA-P
15	0	0
30	0	0
45	0	0
60	0	0
70	6	0
80	8	0
90	13	4
100	19	7
110	26	9
120	33	13

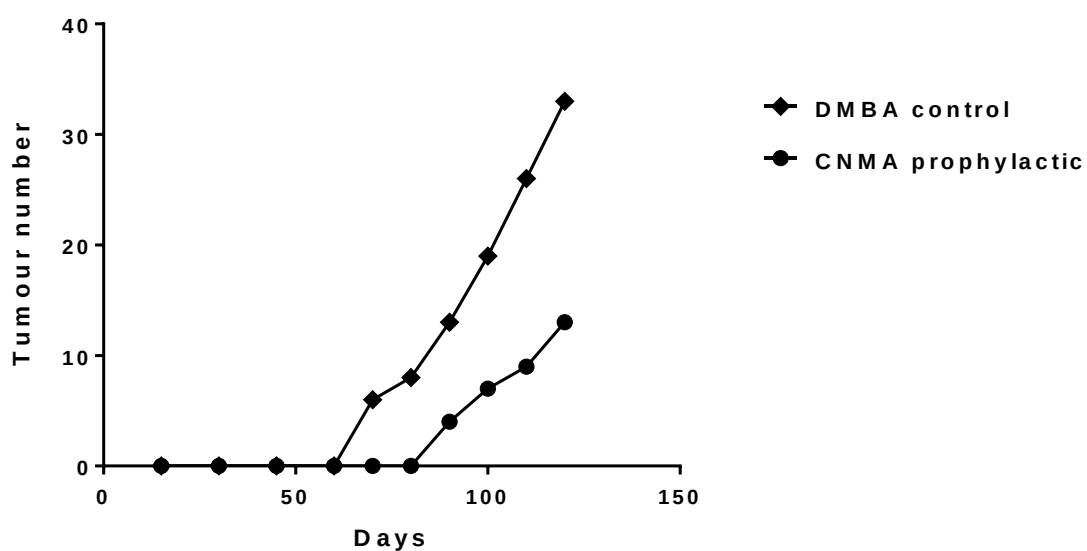


Figure 22. Effect of prophylactic cinnamaldehyde treatment on tumour latency period in rats administered with DMBA for mammary tumour production



Figure 23. Normal distribution pattern of mammary glands in rat



Figure 24. Mammary tumors in DMBA administered rats

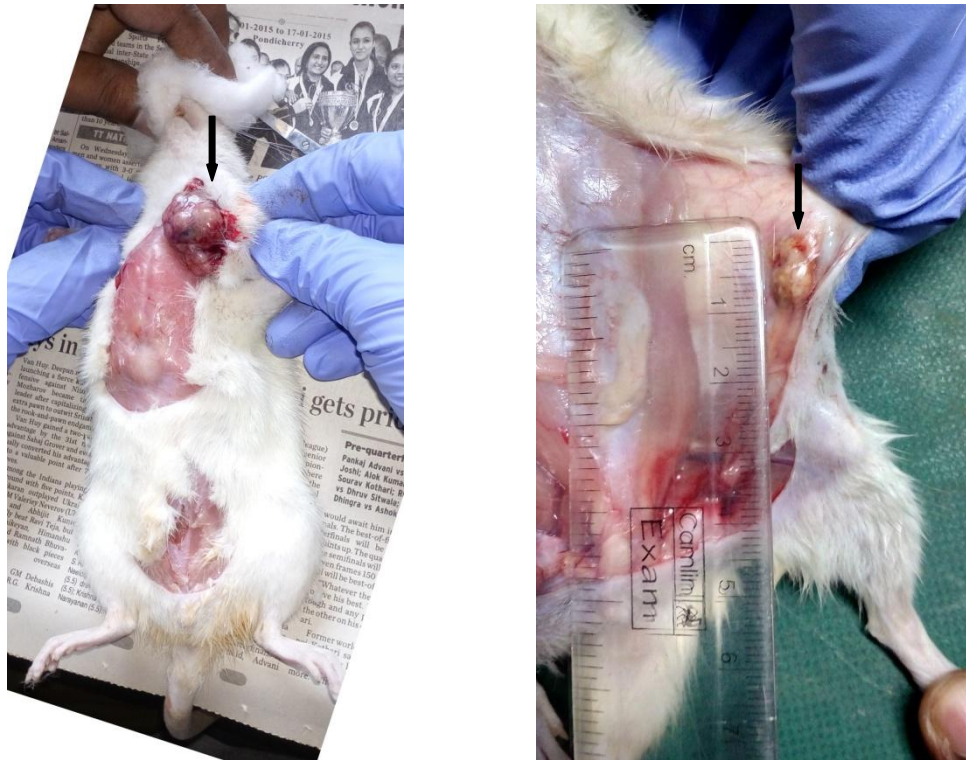


Figure 25. Mammary gland tumor in thoracic and inguinal region in DMBA administered rat



Figure 26. Excised mammary tumor mass from DMBA administered rat

Table 13. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on tumor morphometrics

Group	Tumor volume (cm ³)	Total no. of tumors	Tumor burden (cm ³)	Tumor multiplicity
I	0.00 ± 0.00 ^b	0	0.00 ± 0.00 ^b	0
II	149.2 ± 28.8 ^a	33	149.53 ± 11.89 ^a	4.71
III	52.55 ± 15.58 ^{bc}	13	39.21 ± 12.02 ^{bc}	1.86
IV	79.80 ± 20.85 ^c	21	78.75 ± 13.25 ^c	3.00
V	0.00 ± 0.00 ^b	0	0.00 ± 0.00 ^b	0
VI	74.0 ± 9.99 ^c	18	73.45 ± 5.08 ^c	2.57
VII	55.99 ± 7.37 ^{bc}	16	62.63 ± 10.71 ^c	2.29

Values are mean ± SEM (n=7); One way ANOVA, GraphPad Prism (7.02).

Means with different superscripts in the same column differ significantly (P < 0.05)

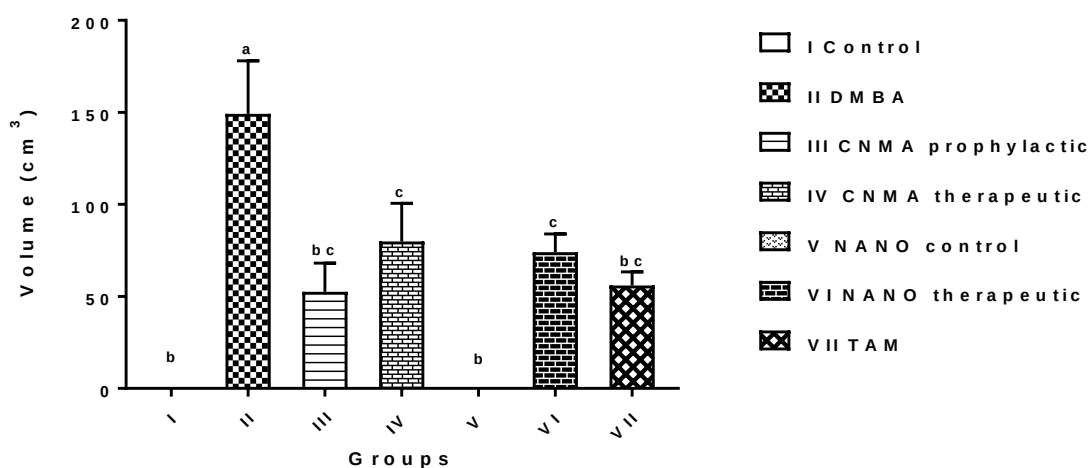


Figure 27. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on tumor volume (cm³) in rats administered with DMBA.

Values are mean ± SEM (n=7); One way ANOVA, GraphPad Prism (7.02).

Bars with different superscripts differ significantly (P < 0.05)

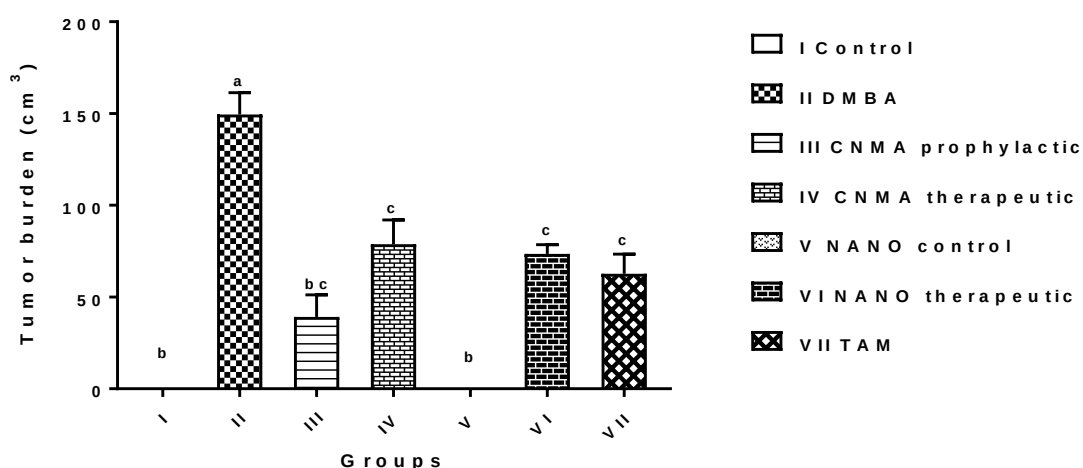


Figure 28. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on tumor burden (cm^3) in rats administered with DMBA.

Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02).

Bars with different superscripts differ significantly ($P < 0.05$)

4.4.3 Mean body weight

At the end of the study the recorded mean body weight of rats in different study groups i.e. control (C), DMBA control (DMBA-C), cinnamaldehyde prophylactic (CNMA-P), cinnamaldehyde treatment (CNMA-T), nano zinc cinnamaldehyde control (CZN-C), nano zinc cinnamaldehyde treatment (CZN-T) and tamoxifen treatment (TAM) were 282.0 ± 5.31 , 196.30 ± 6.94 , 229.90 ± 6.53 , 242.0 ± 11.54 , 273.60 ± 3.27 , 237.30 ± 9.57 and 237.70 ± 2.74 respectively. Significant reduction in body weight was recorded in all DMBA administered groups with a highest degree in DMBA-C when compared to control and nano cinnamaldehyde control (Table 14, Figure 29).

Table 14. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on body weight in rats

Group	Body wt. (g)
I	282.0 ± 5.31 ^b
II	196.3 ± 6.94 ^a
III	229.9 ± 6.53 ^c
IV	242.0 ± 11.54 ^c
V	273.6 ± 3.27 ^b
VI	237.3 ± 9.57 ^c
VII	237.7 ± 2.74 ^c

Values are mean ± SEM (n=7) one way ANOVA, GraphPad Prism (7.02). Means with different superscripts in the same column differ significantly (P < 0.05)

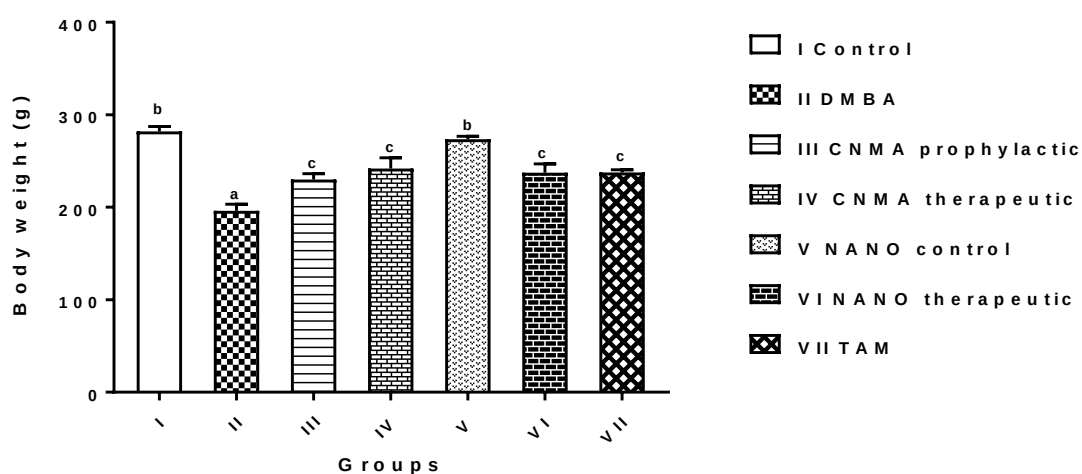


Figure 29. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on body weight (g) in rats.

Values are mean ± SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly (P < 0.05)

4.5 TUMORIGENICITY MARKER - TOTAL SIALIC ACID

The tumorigenicity marker total sialic acid levels ($\mu\text{g/ml}$) in various groups viz. control (C), DMBA-C, CNMA-P, CNMA-T, CAN-C, CZN-T and TAM were 23.99 ± 1.35 , 161.9 ± 5.37 , 61.63 ± 4.83 , 103.2 ± 5.14 , 27.49 ± 1.04 , 90.52 ± 1.16 and 74.86 ± 3.84 , respectively. In all these groups the TSA levels were significantly ($P < 0.05$) increased compared to both the control groups i.e. control and nano control groups. However, among various DMBA administered groups it was found that in CNMA-P, CNMA-T, CZN-T and TAM groups the TSA levels were significantly ($P < 0.05$) lowered compared to DMBA-C group (Table 15; Figure 30).

Table 15. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma total sialic acid ($\mu\text{g/ml}$) levels in rats

Group	Total sialic acid ($\mu\text{g/ml}$)
I	23.99 ± 1.35^b
II	161.9 ± 5.37^a
III	61.63 ± 4.83^d
IV	103.2 ± 5.14^c
V	27.49 ± 1.04^b
VI	90.52 ± 1.16^{ce}
VII	74.86 ± 3.84^{de}

Values are mean $\pm$ SEM (n=7) one way ANOVA, GraphPad Prism 7.02
Means with different superscripts in the same column differ significantly ($P < 0.05$)

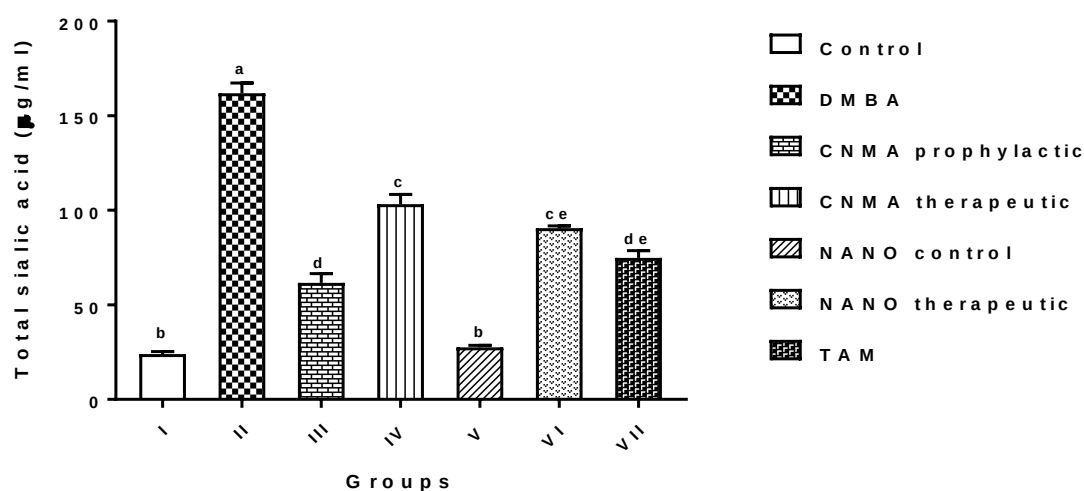


Figure 30. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma total sialic acid ($\mu\text{g/ml}$) levels in rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

4.6 ANTIOXIDANT MARKERS

To know the antioxidant status in different vital organs superoxide dismutase (SOD) and reduced glutathione (GSH) levels were studied.

4.6.1 Superoxide Dismutase (SOD)

4.6.1.1 SOD in Liver. The superoxide dismutase concentration (U/mg protein) in liver of CNMA-P, CNMA-T and CZN-C showed a significant ($P < 0.05$) increase when compared to DMBA-C (II) group. However the normal control (C) group exhibited the maximum concentration (Table 16, Figure 31).

4.6.1.2 SOD in Lung. In lung tissue the values of SOD (U/mg protein) ranged from 3.61 ± 0.25 to 5.04 ± 0.29 in different groups (I to VII) of rats, with no significant ($P > 0.05$) difference among different groups (Table 16; Figure 32).

4.6.1.3 SOD in Heart. The levels of SOD (U/mg of protein) in heart in groups I to VII were 6.81 ± 0.22 , 4.68 ± 0.16 , 6.43 ± 0.46 , 4.86 ± 0.41 , 6.38 ± 0.35 , 3.62 ± 0.33 and 3.93 ± 0.37 , respectively. Statistical analysis revealed a significant ($P < 0.05$) decrease

in DMBA control group compared to control group. Whereas the levels in group III and V were comparable to control (Table 16; Figure 33).

4.6.1.4 SOD in Kidney. Significant ($P < 0.05$) decrease in SOD levels (U/mg protein) was observed in kidney of rats belonging to groups DMBA-C (2.67 ± 0.14) and TAM (3.11 ± 0.29) compared to control (C). The concentration of SOD in CNMA-P, CNMA-T, CZN-C and CZN-T groups were comparable to control group (Table 16; Figure 34).

4.6.1.5 SOD in Brain. The mean SOD (U/mg of protein) levels in brain tissue homogenates of different groups ranged from 4.91 ± 0.91 to 7.00 ± 0.63 and they were statistically ($P < 0.05$) comparable (Table 16; Figure 35).

4.6.1.6 SOD in Mammary Tissue. The SOD levels (U/mg of protein) in homogenates of mammary gland tissue of different experimental groups are given in table 16 and figure 36. In DMBA control group rats (14.97 ± 1.12) SOD concentration was significantly ($P < 0.05$) elevated compared to other study groups.

4.6.1.7 SOD in Plasma. The antioxidant enzyme, SOD (U/mg of protein), content was significantly ($P < 0.05$) decreased in DMBA-C and TAM groups compared to control (C). However the levels in CNMA-P (0.496 ± 0.074), CNMA-T (0.47 ± 0.02), CZN-C (0.48 ± 0.02) and CZN-T (0.47 ± 0.02) groups were statistically comparable to control (C) group (0.641 ± 0.05) (Table 16; Figure 37).

Table 16. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (SOD, U/mg protein) in various tissues and plasma of rats

Group	Liver	Lung	Heart	Kidney	Brain	Mammary gland	Plasma
I	3.17 ± 0.19 ^b	5.04 ± 0.29 ^a	6.81 ± 0.22 ^a	5.17 ± 0.14 ^a	6.38 ± 0.23 ^a	5.99 ± 0.34 ^{bc}	0.64 ± 0.05 ^a
II	1.51 ± 0.18 ^a	4.07 ± 0.09 ^a	4.68 ± 0.16 ^c	2.67 ± 0.14 ^b	4.91 ± 0.91 ^a	14.97 ± 1.12 ^a	0.35 ± 0.02 ^b
III	2.83 ± 0.16 ^b	4.46 ± 0.51 ^a	6.43 ± 0.46 ^a	5.16 ± 0.29 ^a	6.33 ± 0.68 ^a	6.12 ± 0.83 ^{bc}	0.49 ± 0.07 ^{ab}
IV	2.69 ± 0.29 ^b	4.07 ± 0.12 ^a	4.86 ± 0.41 ^{bc}	5.21 ± 0.26 ^a	6.95 ± 0.28 ^a	7.63 ± 0.45 ^b	0.47 ± 0.02 ^{ab}
V	2.87 ± 0.21 ^b	3.71 ± 0.18 ^a	6.38 ± 0.35 ^{ab}	4.48 ± 0.63 ^{ac}	7.00 ± 0.63 ^a	6.01 ± 0.33 ^{bc}	0.48 ± 0.01 ^{ab}
VI	2.51 ± 0.38 ^{ab}	3.61 ± 0.25 ^a	3.62 ± 0.33 ^c	3.84 ± 0.18 ^{abc}	6.90 ± 0.96 ^a	6.85 ± 0.58 ^{bc}	0.47 ± 0.02 ^{ab}
VII	2.62 ± 0.33 ^{ab}	3.98 ± 0.58 ^a	3.93 ± 0.37 ^c	3.11 ± 0.29 ^{bc}	6.91 ± 0.65 ^a	4.44 ± 0.44 ^c	0.36 ± 0.01 ^b

Values are mean ± SEM (n=7) one way ANOVA, GraphPad Prism 7.02.

Means with different superscripts in the same column differ significantly (P < 0.05)

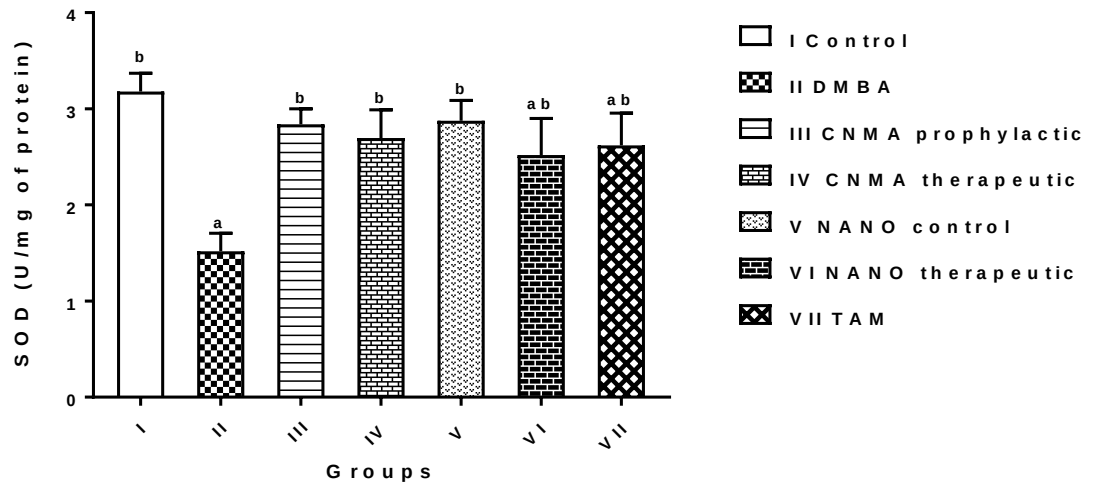


Figure 31. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the liver of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

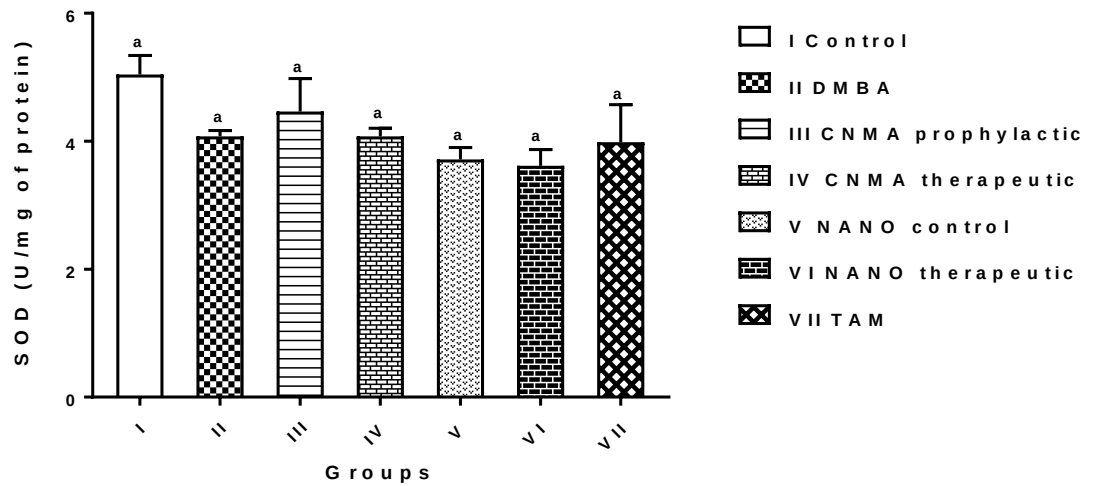


Figure 32. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the lung tissue of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

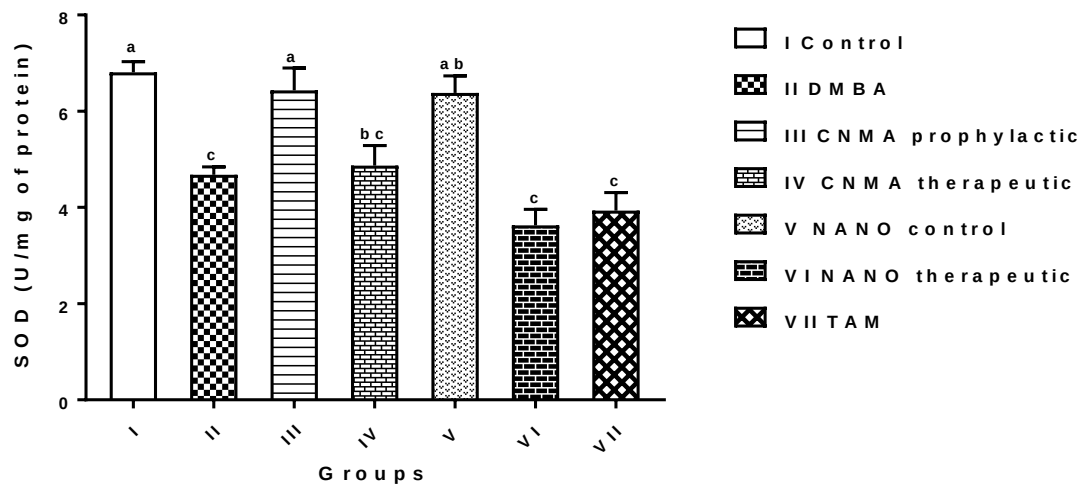


Figure 33. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the heart tissue of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

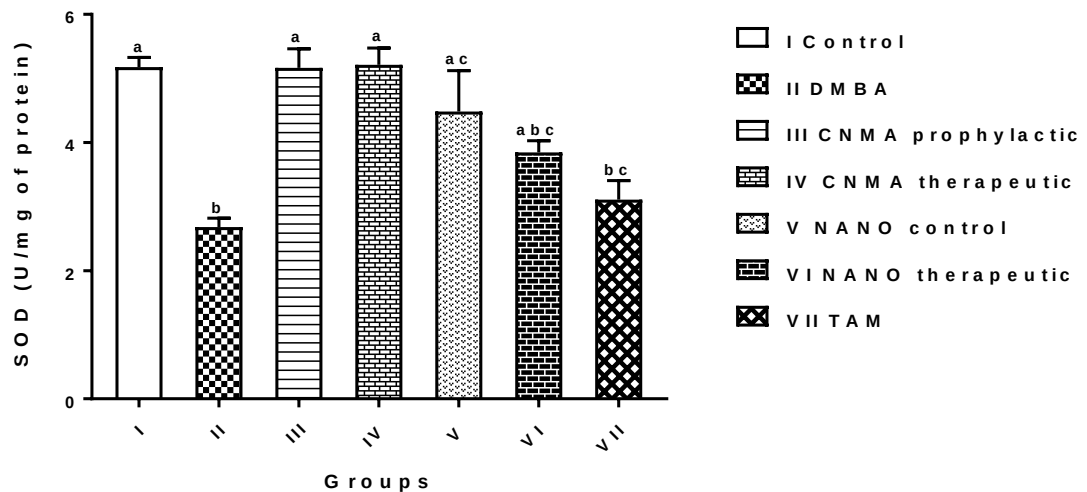


Figure 34. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the kidney of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

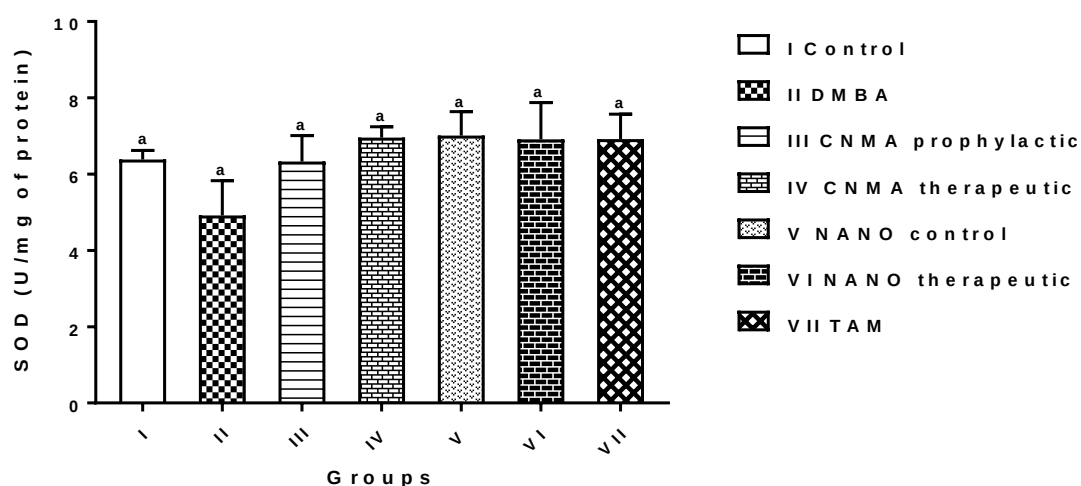


Figure 35. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the brain tissue of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

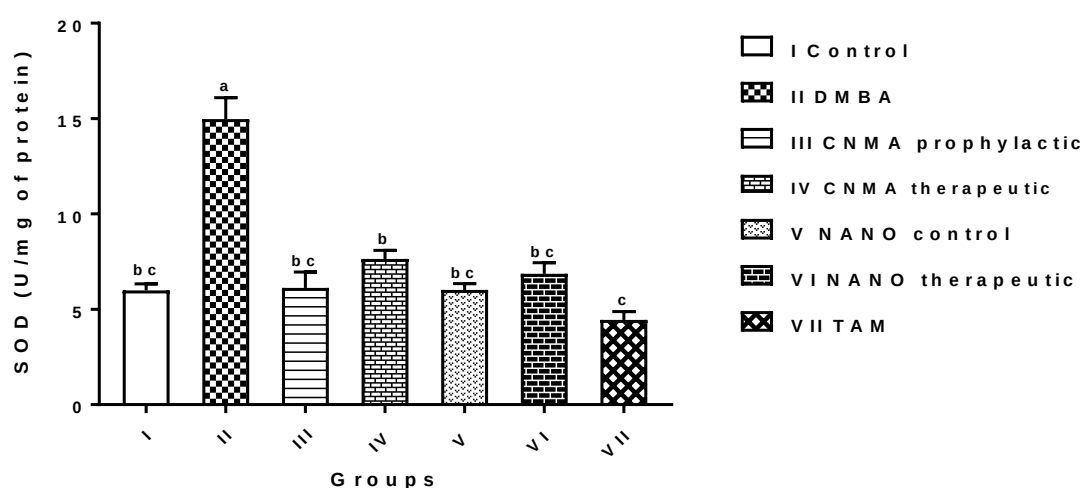


Figure 36. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the mammary gland tissue of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

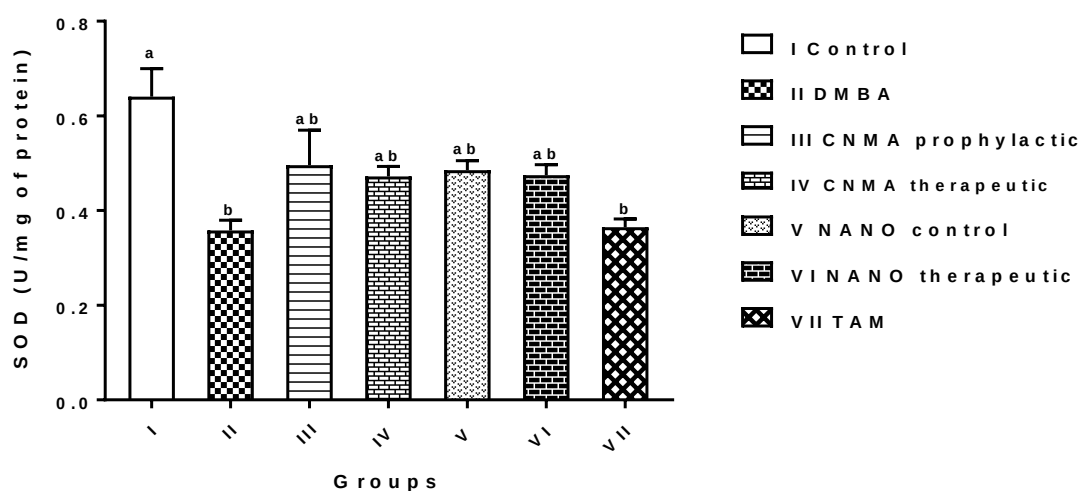


Figure 37. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on super oxide dismutase activity (U/mg of protein) in the plasma of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

4.6.2 Reduced Glutathione (GSH)

4.6.2.1 GSH in Liver. The non enzymatic antioxidant, GSH ($\mu\text{g}/\text{mg}$ of protein) concentrations in liver homogenates of different treatment groups were significantly ($P < 0.05$) lowered compared to group I. The values ranged from 21.44 ± 1.56 in DMBA-C group to 60.68 ± 4.26 in control (C) group (Table 17; Figure 38).

4.6.2.2 GSH in Lung. GSH levels in ($\mu\text{g}/\text{mg}$ of protein) lung tissue of different study groups are given in table 17 and figure 39. Compared to control (C) there was a significant ($P < 0.05$) fall was observed in DMBA-C, CNMA-P, CNMA-T, CZN-T and TAM groups, while the levels in CZN-C group (49.97 ± 1.17) was comparable with control group (55.41 ± 1.16).

4.6.2.3 GSH in Heart. Significant ($P < 0.05$) decrease in GSH ($\mu\text{g}/\text{mg}$ of protein) content was observed in heart tissue homogenates of DMBA-C (22.30 ± 1.81), CNMA-T (41.75 ± 1.57), CZN-T (34.48 ± 1.92) and TAM (32.22 ± 0.99) groups when compared to normal control (C) (52.83 ± 2.33). The mean GSH level in

cinnamaldehyde prophylactic group (46.54 ± 1.74) and CZN-C (47.15 ± 1.96) did not significantly differ from control (Table 17; Figure 40).

4.6.2.4 GSH in Kidney. The mean GSH content ($\mu\text{g}/\text{mg}$ of protein) in different experimental groups ranged from 31.19 ± 2.75 to 70.54 ± 3.23 . When compared to control (C) and CZN-C groups the concentration of GSH was significantly ($P < 0.05$) differed in DMBA-C, CNMA-P, CNMA-T, CZN-T and TAM groups (Table 17; Figure 41).

4.6.2.5 GSH in Brain. In brain homogenates of different groups (I to VII) the GSH levels ($\mu\text{g}/\text{mg}$ of protein) were 48.59 ± 1.73 , 27.27 ± 1.22 , 45.52 ± 1.47 , 41.92 ± 1.93 , 43.18 ± 1.73 , 37.49 ± 1.86 and 35.52 ± 2.75 respectively. When compared to control, the levels in group II, VI and VII were significantly ($P < 0.05$) lowered while in remaining groups the levels were comparable to control (Table 17; Figure 42).

4.6.2.6 GSH in Mammary Tissue. The levels of GSH ($\mu\text{g}/\text{mg}$ of protein) in different study groups are given in table 17 and figure 43. There was a significant ($P < 0.05$) decrease in all the DMBA treated groups when compared to control (33.91 ± 1.12).

4.6.2.7 GSH in Plasma. The concentration of GSH ($\mu\text{g}/\text{mg}$ of protein) in plasma recorded at the end of the study in the control rats (group C) was 26.03 ± 1.13 , which was significantly ($P < 0.05$) decreased in all the groups other than CZN-C group (23.16 ± 1.35). The values were 10.87 ± 0.69 , 19.35 ± 0.63 , 16.12 ± 0.81 , 15.79 ± 0.81 and 15.15 ± 0.91 in DMBA-C (II) through TAM (VII) respectively (Table 17; Figure 44).

Table 17. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione (GSH) activity ($\mu\text{g}/\text{mg}$ of protein) in various tissues and plasma of rats

Group	Liver	Lung	Heart	Kidney	Brain	Mammary gland	Plasma
I	60.68 ± 4.26^b	55.41 ± 1.16^b	52.83 ± 2.33^b	70.54 ± 3.23^b	48.59 ± 1.73^b	33.91 ± 1.12^b	26.03 ± 1.13^b
II	21.44 ± 1.56^a	20.77 ± 0.66^a	22.30 ± 1.81^a	31.19 ± 2.75^a	27.27 ± 1.22^a	17.87 ± 1.05^a	10.87 ± 0.68^a
III	48.63 ± 2.09^c	47.88 ± 2.43^c	46.54 ± 1.74^{bc}	51.32 ± 1.91^c	45.52 ± 1.47^{bc}	27.49 ± 1.02^{cd}	19.35 ± 0.63^{cd}
IV	37.65 ± 1.11^d	41.34 ± 0.86^d	41.75 ± 1.57^{cd}	43.98 ± 2.75^c	41.92 ± 1.93^{bcd}	25.02 ± 0.94^d	16.12 ± 0.81^{de}
V	53.00 ± 1.36^{bc}	49.97 ± 1.17^{bc}	47.15 ± 1.96^{bc}	64.61 ± 1.66^b	43.18 ± 1.73^{bcd}	30.11 ± 0.81^{bc}	23.16 ± 1.35^{bc}
VI	32.34 ± 1.43^d	39.89 ± 1.26^d	34.48 ± 1.92^{de}	43.05 ± 1.50^c	37.49 ± 1.86^{cd}	24.17 ± 1.44^d	15.79 ± 0.81^{de}
VII	31.27 ± 1.8^d	37.88 ± 1.32^d	32.22 ± 0.99^e	41.97 ± 1.78^c	35.52 ± 2.75^d	22.82 ± 0.99^d	15.15 ± 0.91^e

Values are mean $\pm$ SEM (n=7) one way ANOVA, GraphPad Prism 7.02.

Means with different superscripts in the same column differ significantly ($P < 0.05$)

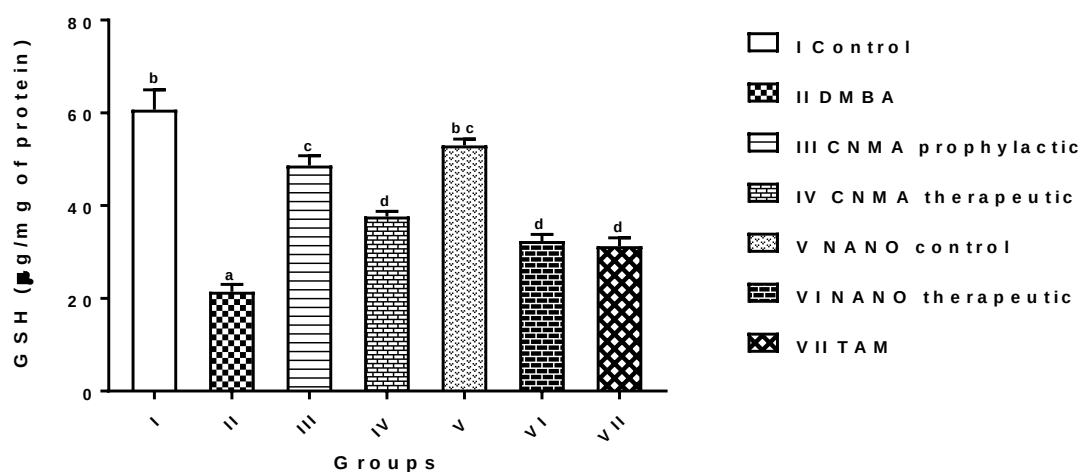


Figure 38. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ of protein) in the liver of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

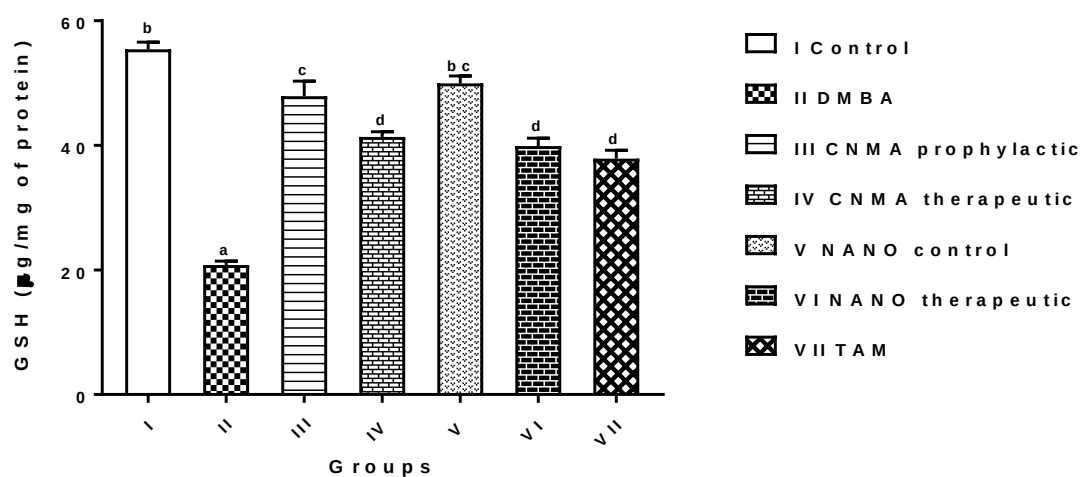


Figure 39. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ protein) in the lung tissue of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

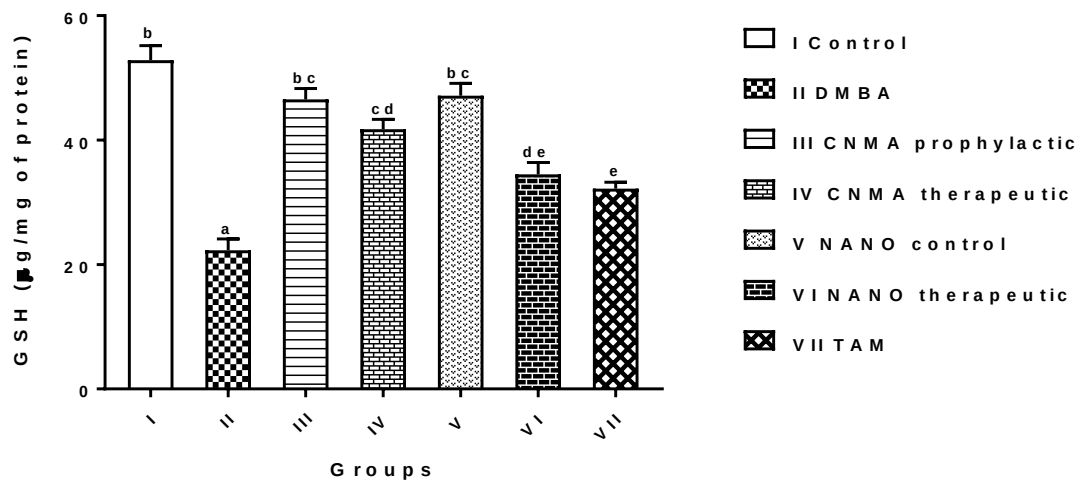


Figure 40. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ protein) in the heart tissue of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

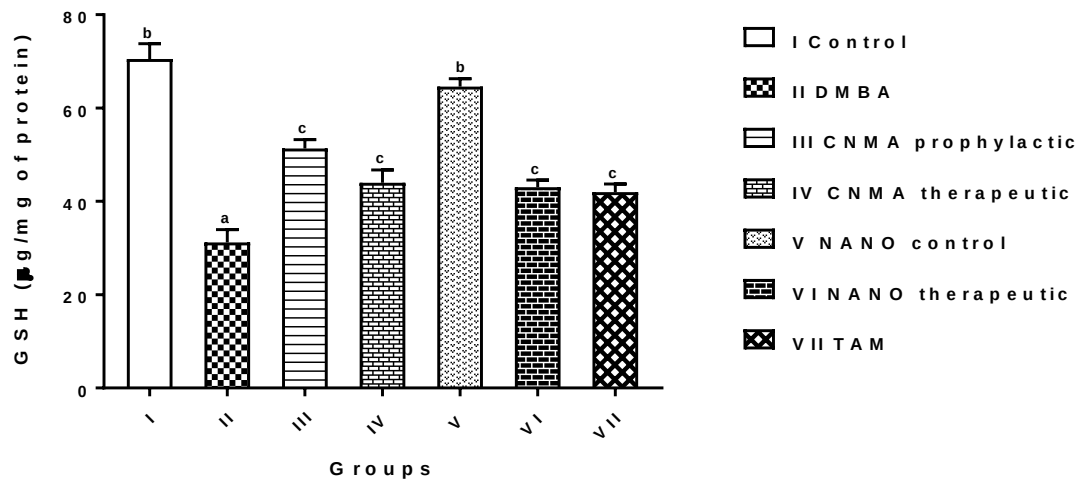


Figure 41. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ protein) in the kidney of rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

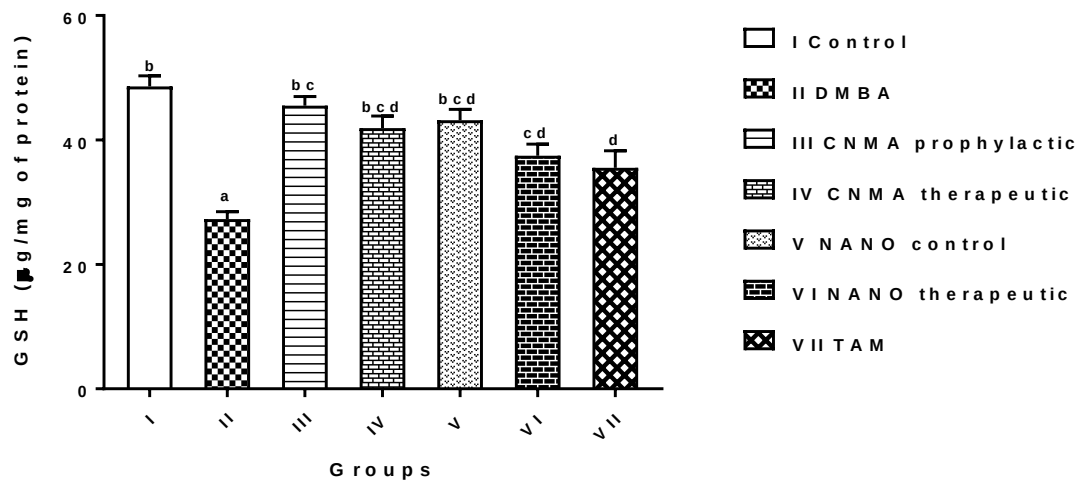


Figure 42. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ protein) in the brain tissue of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

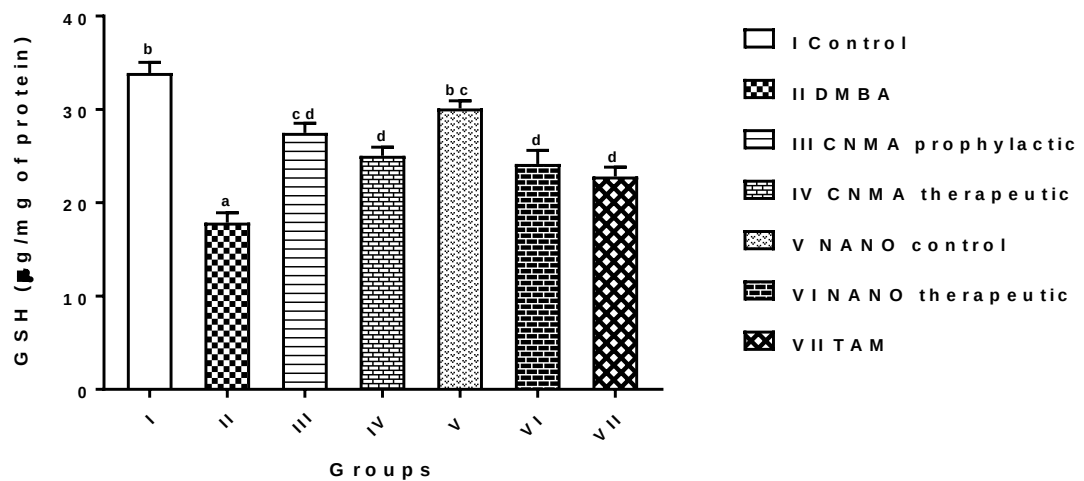


Figure 43. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ protein) in the mammary gland tissue of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

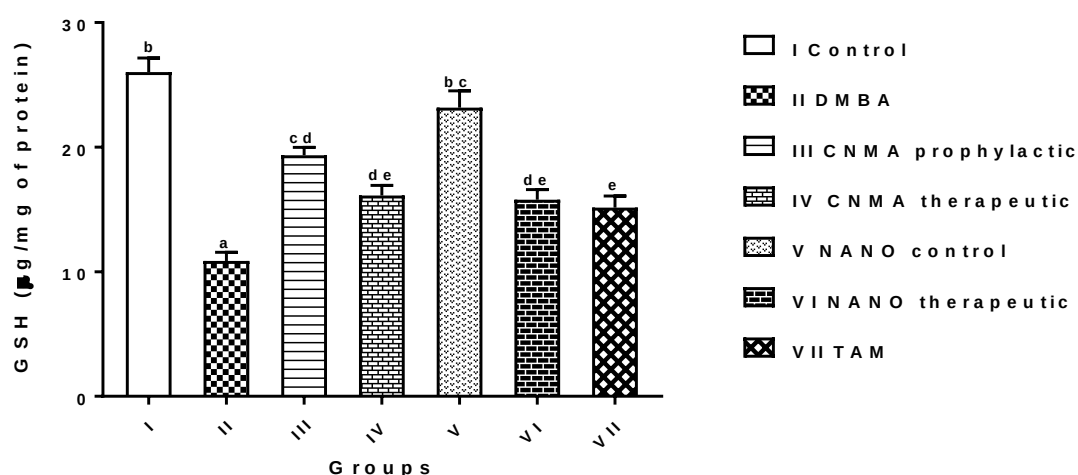


Figure 44. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on reduced glutathione activity ($\mu\text{g}/\text{mg}$ protein) in the plasma of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

4.7 PEROXIDATION MARKER - THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)

4.7.1 TBARS in Liver

The levels of TBARS ($\mu\text{mol}/\text{mg}$ of protein) in liver tissue of different study groups are depicted in table 18 and figure 45. When compared to control (C) (1.92 ± 0.14) there was a significant ($P < 0.05$) increase in the concentration of TBARS in all other groups except CZN-C. However there was a significant ($P < 0.05$) decrease was observed in CNMA-P, CNMA-T, CZN-T and TAM compared to DMBA-C.

4.7.2 TBARS in Lung

In lung homogenates the values of TBARS ($\mu\text{mol}/\text{mg}$ of protein) ranged from 4.06 ± 0.21 to 12.49 ± 1.00 in different groups. The values in DMBA-C, CNMA-T, CZN-T and TAM groups were significantly ($P < 0.05$) elevated compared to control though the values in group III and V were comparable to control (C) group (Table 18; Figure 46).

4.7.3 TBARS in Heart

Concentration of TBARS ($\mu\text{mol/mg}$ of protein) in heart of different study groups (I to VII) were 1.56 ± 0.17 , 6.36 ± 0.46 , 3.117 ± 0.21 , 4.10 ± 0.31 , 2.53 ± 0.21 , 4.83 ± 0.27 and 5.16 ± 0.26 , respectively. Statistical analysis revealed significant ($P < 0.05$) increase in TBARS level was observed in DMBA-C, CNMA-C, CNMA-T, CZN-T and TAM groups when compared to control (C) (Table 18; Figure 47).

4.7.4 TBARS in Kidney

Statistical analysis revealed significant ($P < 0.05$) increase in TBARS ($\mu\text{mol/mg}$ of protein) of kidney was observed in group II, IV, VI and VII when compared to group I (3.61 ± 0.26), III (4.27 ± 0.24), and V (5.14 ± 0.34) (Table 18; Figure 48).

4.7.5 TBARS in Brain

The mean TBARS ($\mu\text{mol/mg}$ of protein) levels in different experimental groups are given in table 18 and figure 49. The values of TBARS in groups II to VII were 5.47 ± 0.40 , 3.17 ± 0.15 , 3.49 ± 0.23 , 2.9 ± 0.11 , 4.54 ± 0.29 and 4.67 ± 0.26 , respectively, which were significantly ($P < 0.05$) higher, except group V, than that observed in the control (1.79 ± 0.18).

4.7.6 TBARS in Mammary tissue

From table 18 it was clear that the concentration of TBARS ($\mu\text{mol/mg}$ of protein) in group II (4.31 ± 0.32) and group VII (3.67 ± 0.26) were significantly ($P < 0.05$) increased from control group I (2.14 ± 0.29). While the levels in group III, IV, V and VI were comparable to control group I (Figure 50).

4.7.7 TBARS in Plasma

The plasma TBARS ($\mu\text{mol/mg}$ of protein) values in group DMBA-C (II) through TAM (VII) were significantly ($P < 0.05$) higher than the control (0.07 ± 0.004). Whereas TBARS levels in CZN-C (0.10 ± 0.007) were comparable to control (Table 18; Figure 51).

Table 18. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS activity ($\mu\text{mol}/\text{mg}$ protein) in various tissues and plasma of rats

Group	Liver	Lung	Heart	Kidney	Brain	Mammary gland	Plasma
I	1.92 ± 0.14^b	4.06 ± 0.2^b	1.56 ± 0.17^b	3.61 ± 0.26^b	1.79 ± 0.18^a	2.14 ± 0.29^a	0.07 ± 0.004^b
II	9.65 ± 0.45^a	12.49 ± 1.00^a	6.36 ± 0.46^d	12.97 ± 0.37^a	5.47 ± 0.40^d	4.31 ± 0.32^b	0.23 ± 0.014^a
III	4.23 ± 0.49^{cd}	5.09 ± 0.36^{bc}	3.11 ± 0.21^{ac}	4.27 ± 0.24^b	3.17 ± 0.15^b	2.79 ± 0.16^{ac}	0.11 ± 0.01^{cd}
IV	5.08 ± 0.23^{de}	6.55 ± 0.45^{cd}	4.10 ± 0.31^{ae}	6.49 ± 0.75^{cd}	3.49 ± 0.23^{bc}	2.97 ± 0.26^{ac}	0.11 ± 0.01^{cd}
V	3.08 ± 0.19^{bc}	5.41 ± 0.27^{bc}	2.53 ± 0.21^{bc}	5.14 ± 0.34^{bc}	2.90 ± 0.11^{ab}	2.93 ± 0.19^{ac}	0.10 ± 0.004^{bc}
VI	5.69 ± 0.34^{de}	6.90 ± 0.39^{cd}	4.83 ± 0.27^e	7.27 ± 0.48^d	4.54 ± 0.29^{cd}	3.10 ± 0.13^{ac}	0.12 ± 0.01^{cd}
VII	5.99 ± 0.48^e	7.64 ± 0.35^d	5.16 ± 0.26^{de}	7.51 ± 0.59^d	4.67 ± 0.26^d	3.67 ± 0.26^{bc}	0.13 ± 0.01^d

Values are mean $\pm$ SEM (n=7) one way ANOVA, GraphPad Prism 7.02

Means with different superscripts in the same column differ significantly ($P < 0.05$)

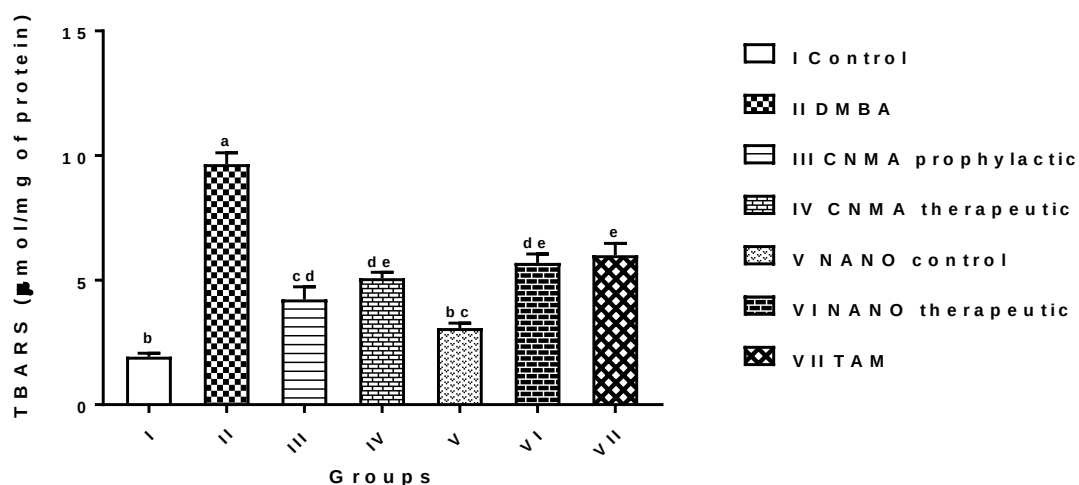


Figure 45. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the liver of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

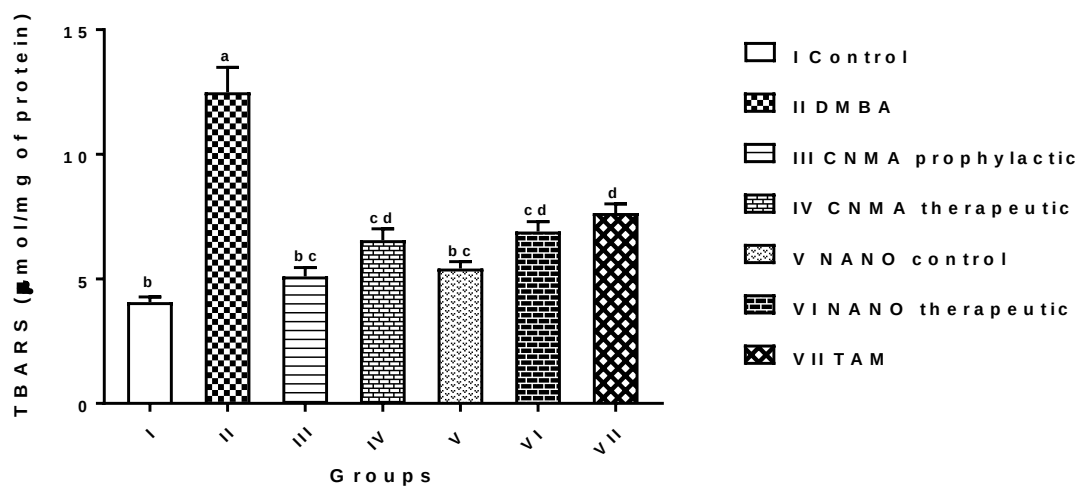


Figure 46. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the lung tissue of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

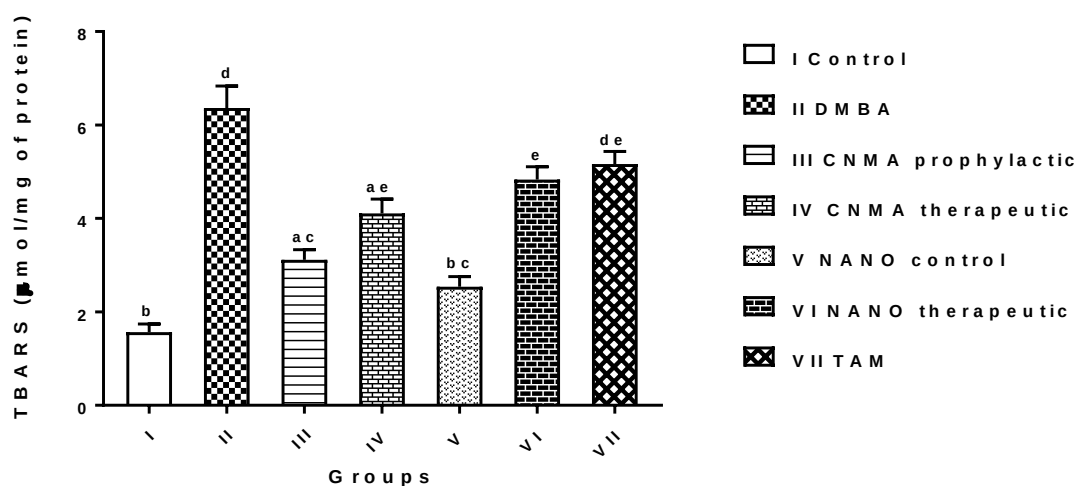


Figure 47. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the heart tissue of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

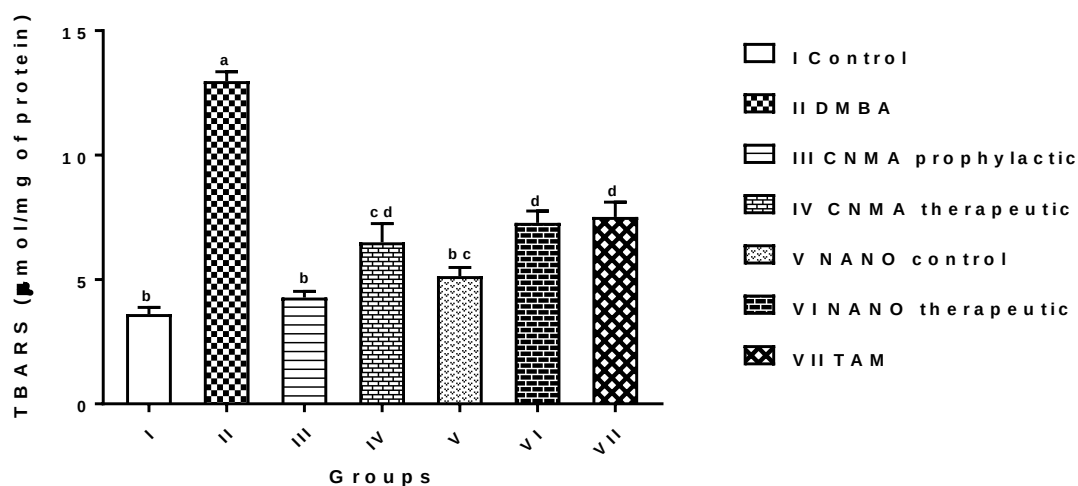


Figure 48. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the kidney of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

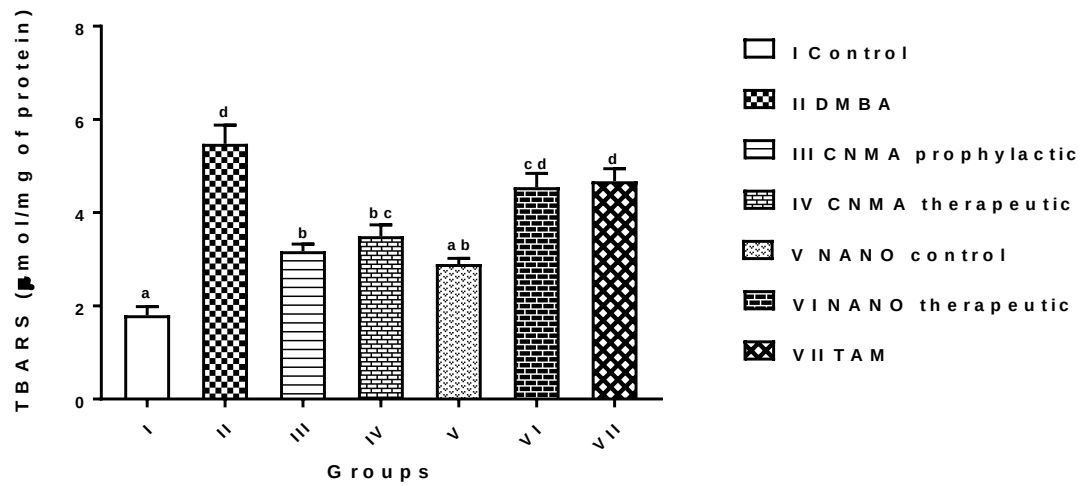


Figure 49. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the brain tissue of rats.

Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

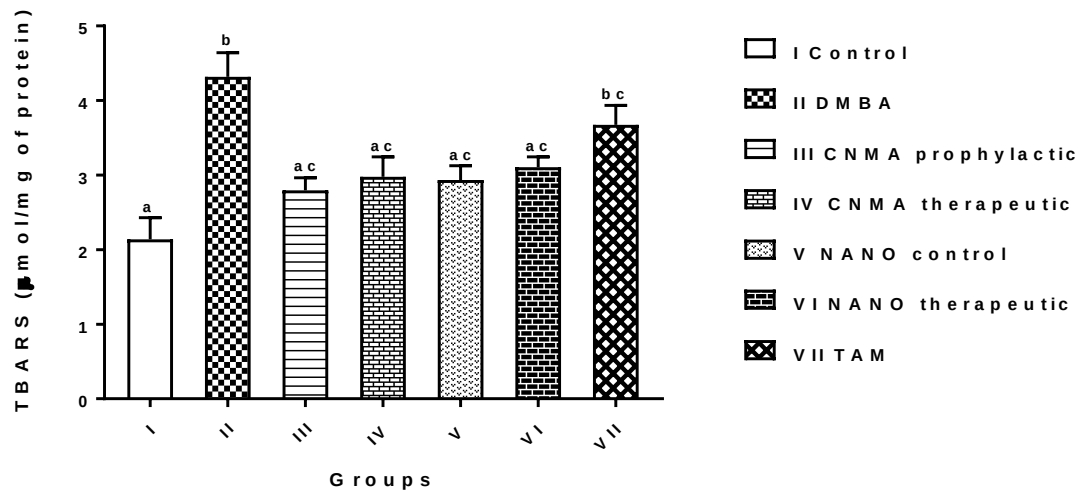


Figure 50. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the mammary gland tissue of rats.

Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

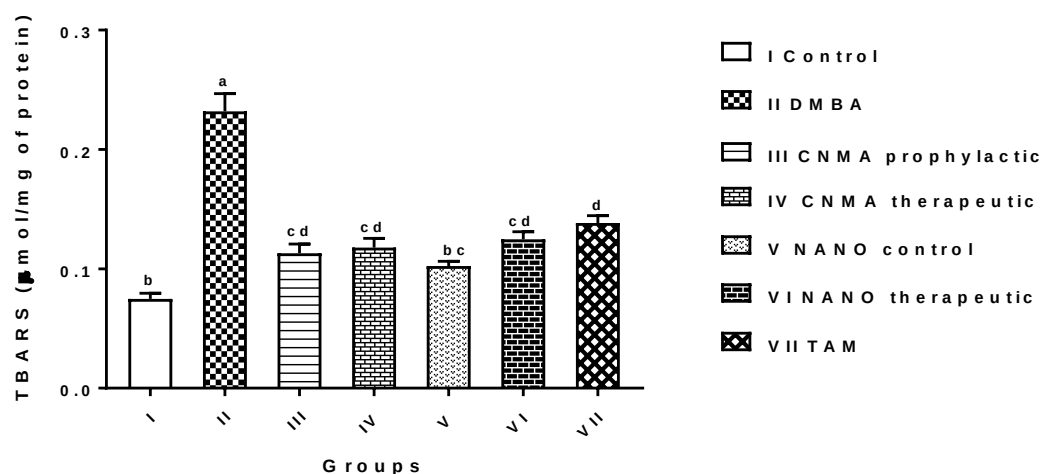


Figure 51. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on TBARS levels ($\mu\text{mol/mg}$ of protein) in the plasma of rats. Values are mean $\pm$ SEM ($n=7$); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

4.8 BIOCHEMICAL PROFILE

4.8.1 Total Proteins

The concentration of total protein (g/dl) in groups I to VII was 5.83 ± 0.34 , 4.32 ± 0.13 , 4.87 ± 0.27 , 4.59 ± 0.17 , 5.01 ± 0.08 , 4.79 ± 0.24 and 4.81 ± 0.40 , respectively. The results indicated significant ($P < 0.05$) decrease in DMBA control and CNMA-T group when compared to normal control (C). Whereas the levels in the remaining groups were comparable to control (Table 19, Figure 52).

4.8.2 Albumin

The albumin concentration (g/dl) in different DMBA administered groups showed a significant ($P < 0.05$) decrease compared to control and the values are ranged from 1.77 ± 0.29 to 3.14 ± 0.16 (Table 19; Figure 52).

4.8.3 Blood Urea Nitrogen (BUN)

The concentrations of BUN (mg/dl) in group II (72.32 ± 6.29), group VI (65.92 ± 2.33) and group VII (64.91 ± 2.72) were increased significantly ($P < 0.05$) compared to control. However, no difference was observed in group III (54.82 ± 2.23), group IV (60.96 ± 5.82) and group V (54.34 ± 1.21) compared to control (47.22 ± 2.23) (Table 19; Figure 53).

4.8.4 Creatinine

The plasma creatinine level (mg/dl) in the normal control was 0.58 ± 0.07 , which was comparable to that recorded in group III, IV, V and VI. While the concentrations in group II and group VII were significantly ($P < 0.05$) elevated when compared to control group rats (Table 19; Figure 54).

4.8.5 Total Bilirubin

Total bilirubin values (mg/dl) ranged from 0.73 ± 0.01 to 0.99 ± 0.09 in various groups. Except a significant ($P < 0.05$) increase in group II (DMBA-C) and group VII in all other groups the values were comparable to that of normal control (0.73 ± 0.01) (Table 19; Figure 54).

4.8.6 Total Cholesterol

The total cholesterol levels (mg/dl) in plasma of different treatment groups did not significantly ($P > 0.05$) differ from the control group (41.27 ± 0.64) (Table 19; Figure 53).

4.8.7 Glucose

Glucose levels in various treatment groups were ranged from 81.32 ± 5.99 to 97.95 ± 9.29 with no significant ($P > 0.05$) difference between groups group (Table 19; Figure 55).

4.8.9 Aspartate Amino Transferase (AST)

Barring the group II, the values of AST in all other groups were not significantly affected compared to normal control. In group II the AST levels was significantly ($P < 0.05$) elevated compared to control (Table 20; Figure 56).

4.8.10 Alanine Amino Transferase (ALT)

The activity of enzyme alanine amino transferase (IU/L) was significantly ($P < 0.05$) increased in the DMBA control group (101.96 ± 9.09). While the values of all other treatment groups were comparable to normal control (C) (Table 20; Figure 56).

4.8.11 Alkaline Phosphatase (ALP)

The alkaline phosphatase concentration (IU/L) levels in various treatment groups was given in table 20. The activity was significantly ($P < 0.05$) increased in group II, III, IV, VI and VII when compared to control rats (Figure 57).

Table 19. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on rat plasma biochemical profile – I

Group	Total protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Total bilirubin (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)
I	5.83 ± 0.34 ^a	3.14 ± 0.16 ^a	47.22 ± 2.23 ^a	0.58 ± 0.07 ^a	0.73 ± 0.01 ^a	41.27 ± 0.64 ^a	97.95 ± 9.29 ^a
II	4.32 ± 0.13 ^b	1.77 ± 0.29 ^b	72.32 ± 6.29 ^b	1.02 ± 0.08 ^b	0.99 ± 0.08 ^b	42.68 ± 3.99 ^a	87.40 ± 2.68 ^a
III	4.87 ± 0.27 ^{ab}	2.03 ± 0.06 ^{bc}	54.82 ± 2.23 ^{ac}	0.67 ± 0.03 ^{ac}	0.79 ± 0.02 ^{ac}	39.30 ± 2.25 ^a	87.32 ± 4.89 ^a
IV	4.59 ± 0.17 ^b	1.84 ± 0.15 ^b	60.96 ± 5.82 ^{abc}	0.78 ± 0.03 ^{abc}	0.84 ± 0.03 ^{abc}	40.30 ± 1.88 ^a	85.26 ± 3.20 ^a
V	5.01 ± 0.08 ^{ab}	2.50 ± 0.03 ^{ac}	54.34 ± 1.21 ^{ac}	0.72 ± 0.07 ^{ac}	0.78 ± 0.02 ^{ac}	38.47 ± 1.43 ^a	83.04 ± 4.12 ^a
VI	4.79 ± 0.24 ^{ab}	2.10 ± 0.07 ^{bc}	65.92 ± 2.33 ^{bc}	0.85 ± 0.08 ^{abc}	0.85 ± 0.01 ^{abc}	40.32 ± 3.27 ^a	81.32 ± 5.59 ^a
VII	4.81 ± 0.40 ^{ab}	1.97 ± 0.05 ^{bc}	64.91 ± 2.72 ^{bc}	0.91 ± 0.04 ^{bc}	0.92 ± 0.02 ^{bc}	38.83 ± 1.38 ^a	87.43 ± 4.60 ^a

Values are mean ± SEM (N=7) one way ANOVA, GraphPad Prism 7.02

Means with different superscripts in the same column differ significantly (P < 0.05)

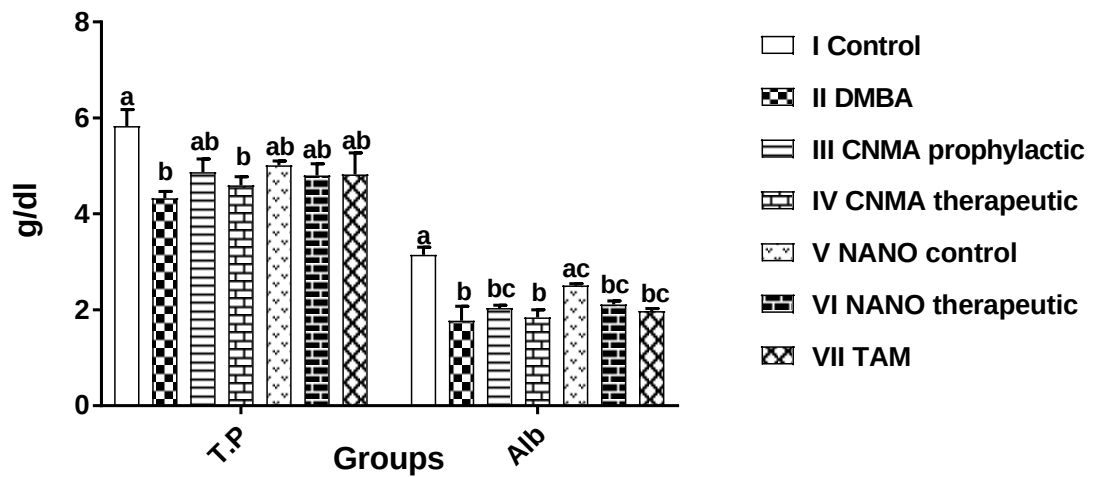


Figure 52. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma total protein (T.P) and albumin (Alb) levels (g/dl) in rats.

Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02).

Bars with different superscripts differ significantly ($P < 0.05$)

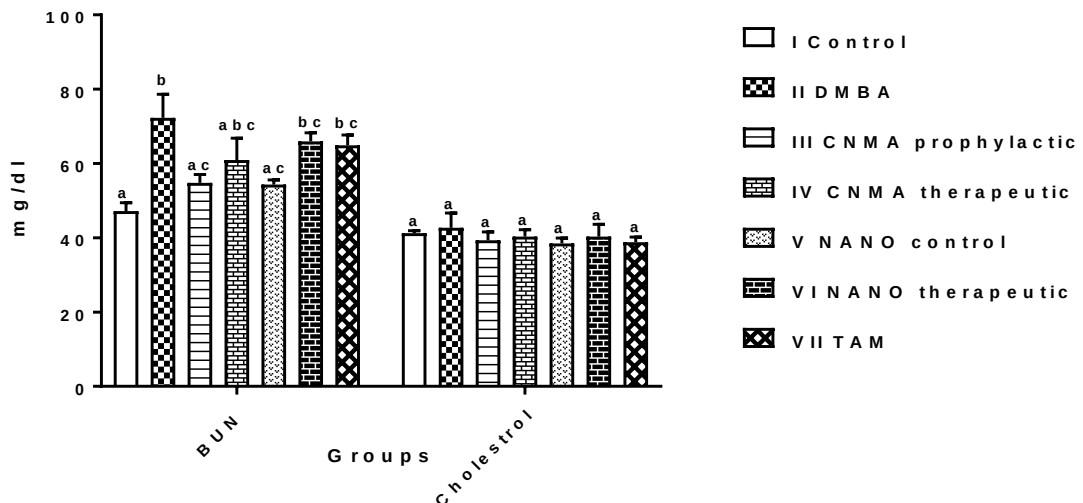


Figure 53. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma blood urea nitrogen (BUN) and cholesterol levels (mg/dl) in rats.

Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02).

Bars with different superscripts differ significantly ($P < 0.05$)

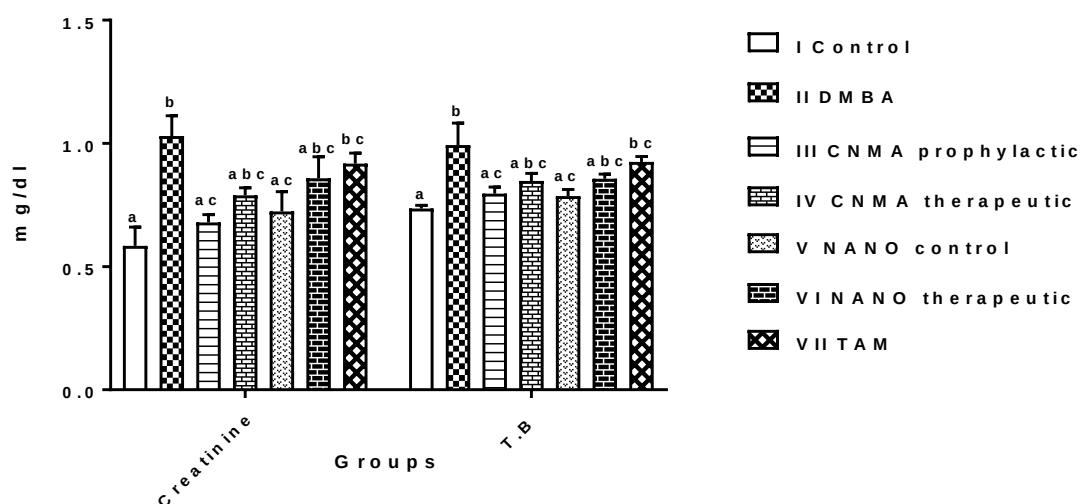


Figure 54. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma creatinine and total bilirubin (T.B) (mg/dl) in rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

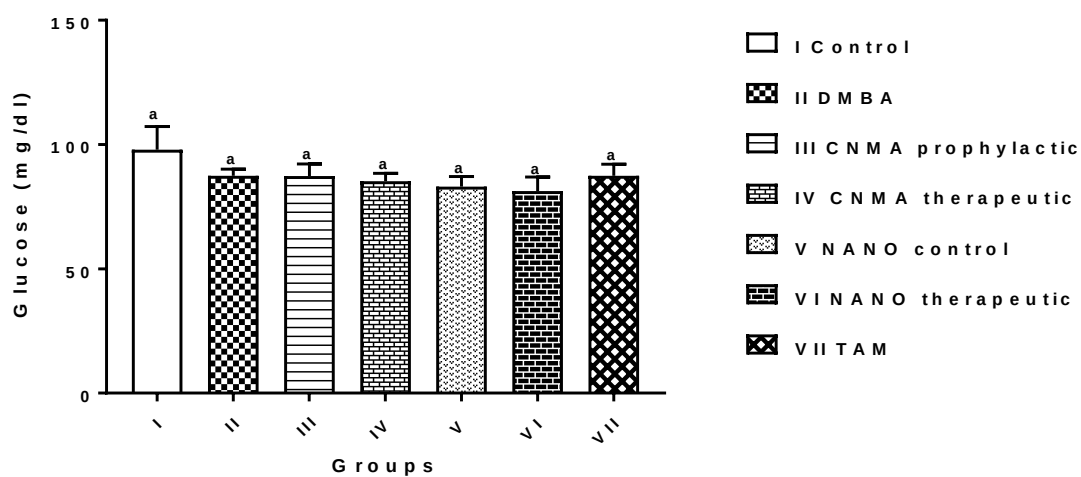


Figure 55. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma glucose (mg/dl) in rats. Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02). Bars with different superscripts differ significantly ($P < 0.05$)

Table 20. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on rat plasma biochemical profile – II

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I	93.68 ± 2.99 ^b	56.73 ± 6.59 ^b	31.17 ± 1.83 ^a
II	161.30 ± 8.76 ^a	101.95 ± 9.09 ^a	61.38 ± 3.28 ^c
III	106.39 ± 14.36 ^b	67.41 ± 6.36 ^b	47.84 ± 2.03 ^{bd}
IV	114.89 ± 7.12 ^b	82.95 ± 7.85 ^{ab}	53.28 ± 1.72 ^{cd}
V	102.40 ± 10.98 ^b	70.43 ± 1.99 ^b	40.20 ± 1.84 ^{ab}
VI	115.56 ± 6.45 ^b	84.44 ± 6.45 ^{ab}	54.39 ± 2.48 ^{cd}
VII	122.17 ± 10.32 ^{ab}	81.95 ± 4.57 ^{ab}	57.19 ± 2.04 ^{cd}

Values are mean ± SEM (N=7) one way ANOVA, GraphPad Prism 7.02
Means with different superscripts in the same column differ significantly (P < 0.05)

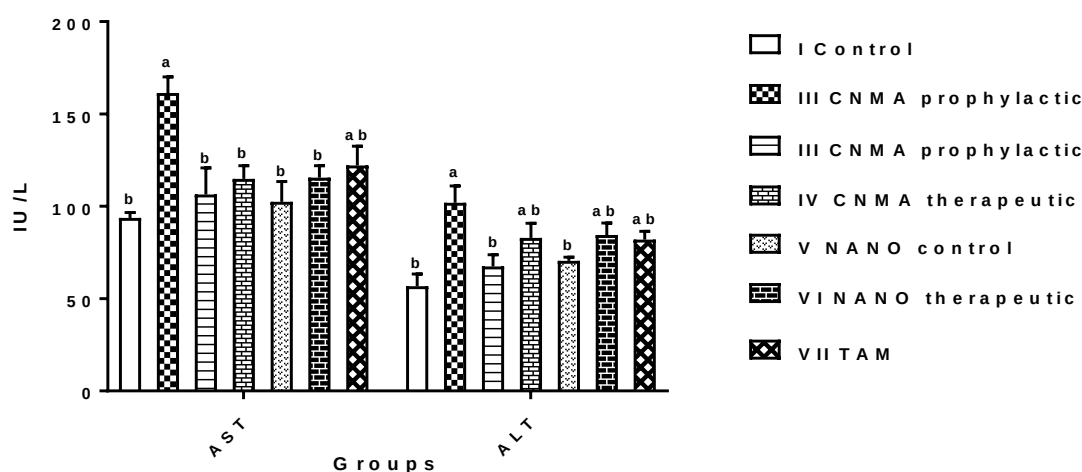


Figure 56. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma AST and ALT levels (IU/L) in rats.

Values are mean ± SEM (n=7); One way ANOVA, GraphPad Prism (7.02).
Bars with different superscripts differ significantly (P < 0.05)

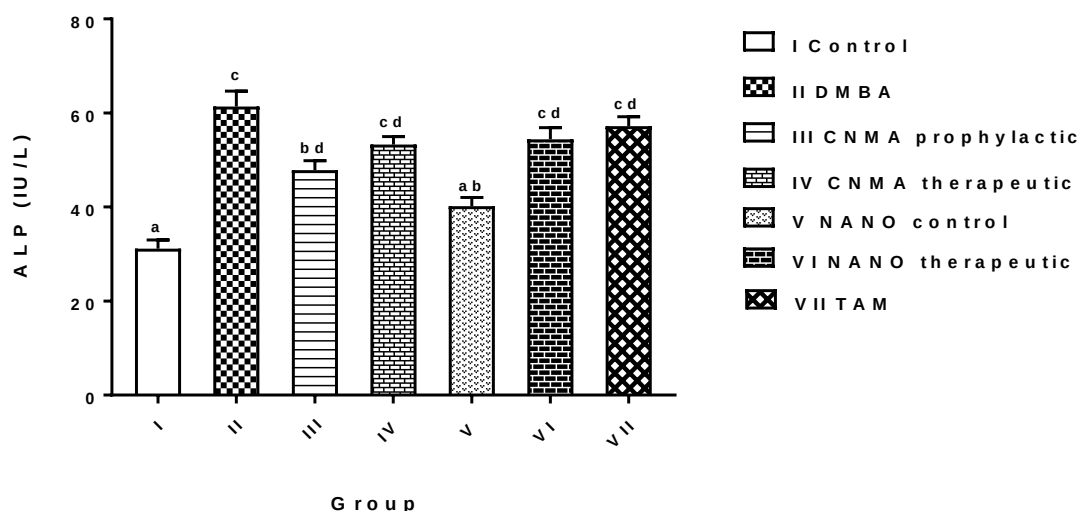


Figure 57. Effect of cinnamaldehyde and nano zinc cinnamaldehyde on plasma ALP levels (IU/L) in rats.
 Values are mean $\pm$ SEM (n=7); One way ANOVA, GraphPad Prism (7.02).
 Bars with different superscripts differ significantly ($P < 0.05$)

4.9 HISTOPATHOLOGY

The light microscopic examination of mammary tumor tissues from DMBA control group revealed different types of morphologies viz. tubular adenocarcinoma, papillary adenocarcinoma, cribriform carcinoma and comedo carcinoma when compared to the normal mammary tissue (Figure 58). Invasive tubular adenocarcinoma sections showed proliferation of ductal/tubular or alveolar structures separated by thin connective tissue (Figure 59 to 61). The tubular structures were lined by multiple layers of epithelial cells and with invasion of neoplastic cells into the stroma. The neoplastic cells were round to oval in shape and increased in size with prominent nucleoli. Mammary tumors of papillary carcinoma type showed proliferated neoplastic cells giving finger like projections into the alveolar lumen and supported by fibrous tissue core (Figure 62 and 63). Tumors with cribriform pattern showed the neoplastic cells within the lobule that were arranged in irregularly shaped alveoli forming secondary

lumina and giving sieve like appearance (Figure 65). Comedocarcinoma was characterized by presence of distended ductal structures with growth of intraductal epithelium and accumulation of necrotic cellular debris (Figure 64).

Mammary tumors of tamoxifen treated rats showed regressive changes like desquamation of epithelium, necrosis of neoplastic cells, presence of multinucleated cells and connective tissue proliferation with presence of abundant collagen and hyalinization in the stroma (Figure 67 to 72). Most of the alveoli were lined by single layer of epithelial cells. Due to these regressive changes the structure of tumors was completely altered. Histologically the breast tumor tissues of rats from CNMA-P and CZN-T groups showed improved cellular structures. The ductal and alveolar structures revealed near normal changes without proliferated neoplastic cells and most of the alveoli were lined by uniform single layer of epithelial cells (Figure 73 to 76). In addition to these changes increased number of alveoli and some dilated alveoli were found in nano zinc cinnamaldehyde treated group (Figure 76). Whereas tumor tissues of CNMA-T group rats are histologically almost similar to DMBA control group (Figure 77).

5.0 TRANSMISSION ELECTRON MICROSCOPIC STUDIES

Tumor control group rat tumor TEM studies revealed typical appearance of neoplastic cells with diversity in size and shape of nucleus (pleomorphic nuclei) and nuclear indentation (Figure 78 and 79). The ultra structural morphologies of tumor cells from different treatment groups consisted of presence of electron dense vesicles/vacuoles and nuclear pleomorphism (Figure 80 and 81) in CNMA-P group, connective tissue proliferation, presence of vesicles and pleomorphic cells containing different sizes and enlarged nuclei (Figure 82 to 84) in TAM-C group, perinuclear

vesicles, fibrous tissue proliferation and dividing/fragmented nuclei (Figure 85 to 88) in CNMA-T and CZN-T groups.

6.0 IMMUNOHISTOCHEMISTRY

Immunohistological studies showed that all the tumors induced by DMBA in this study were positive for both estrogen receptor (ER) and progesterone receptor (PR) (Figure 89 and 90).

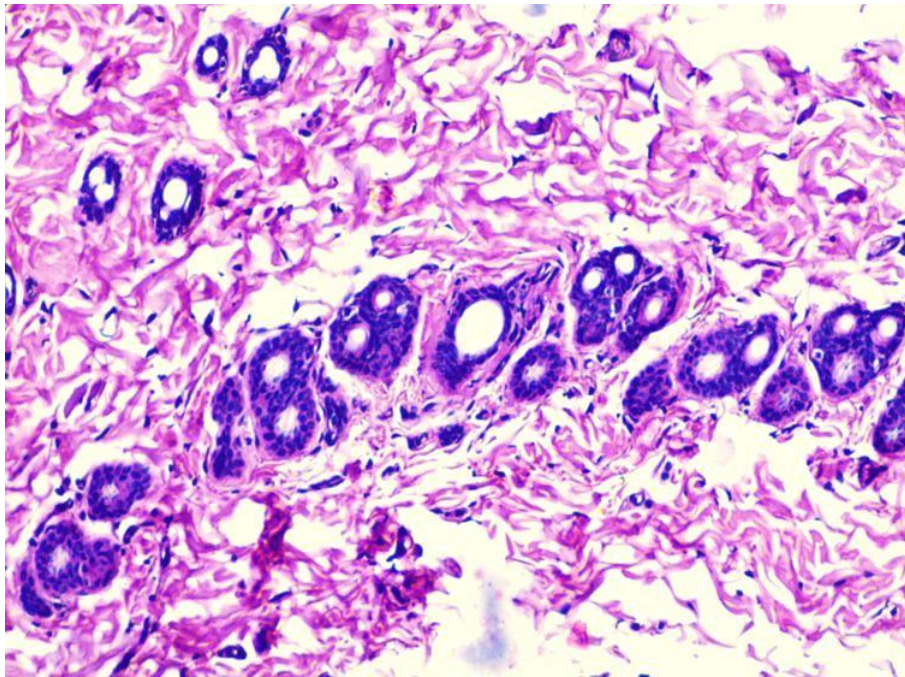


Figure 58. Normal control - rat mammary tissue - normal architecture of mammary gland (H&E x10)

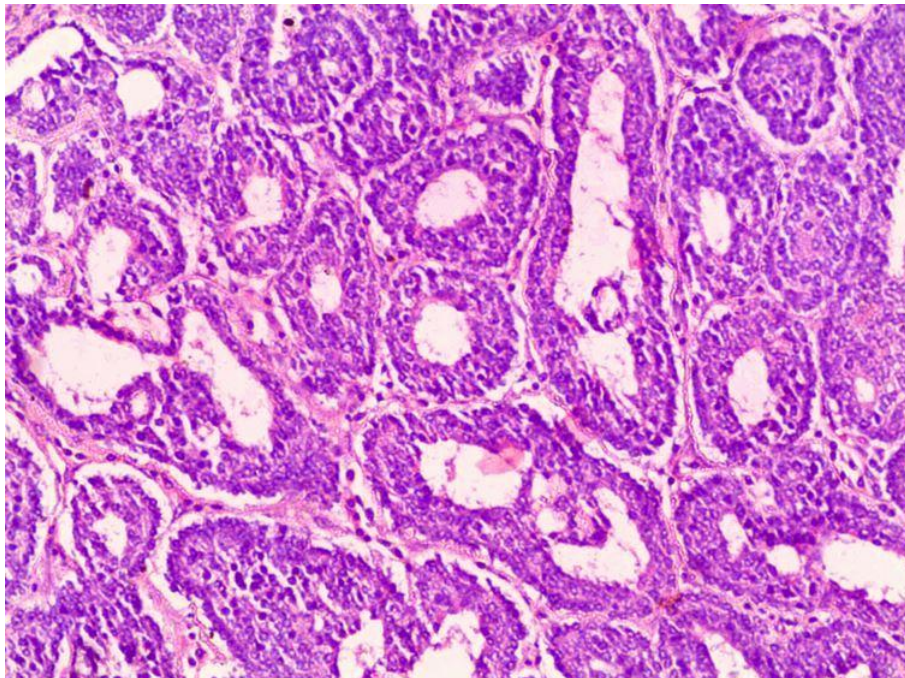


Figure 59. DMBA control - rat mammary tumor - showing tubular adenocarcinoma - proliferation of tubular structures separated by thin connective tissue (H&E x10)

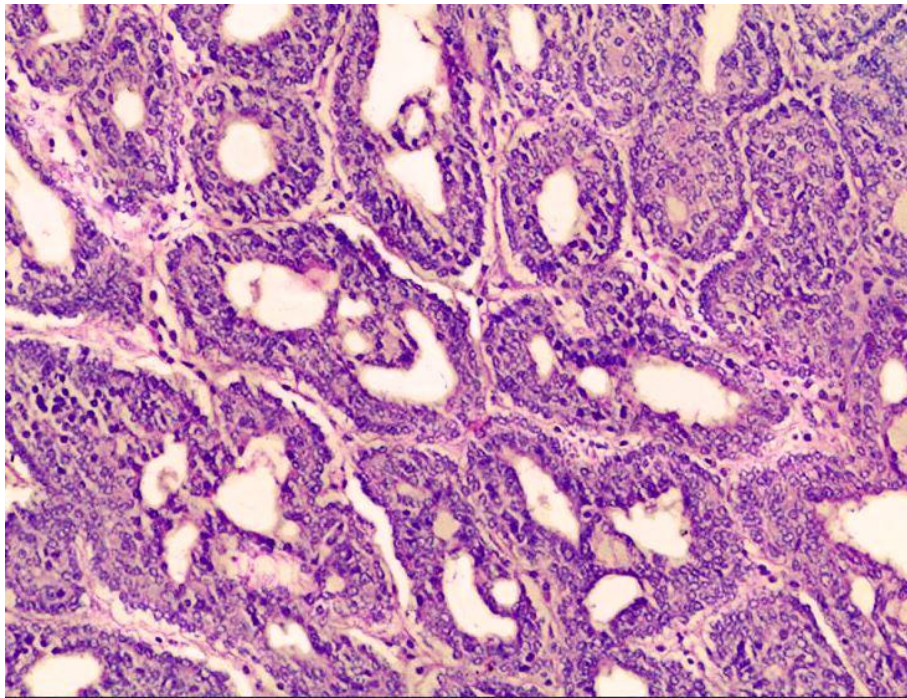


Figure 60. DMBA control - rat mammary tumor - showing invasive tubular adenocarcinoma (H&E x10)

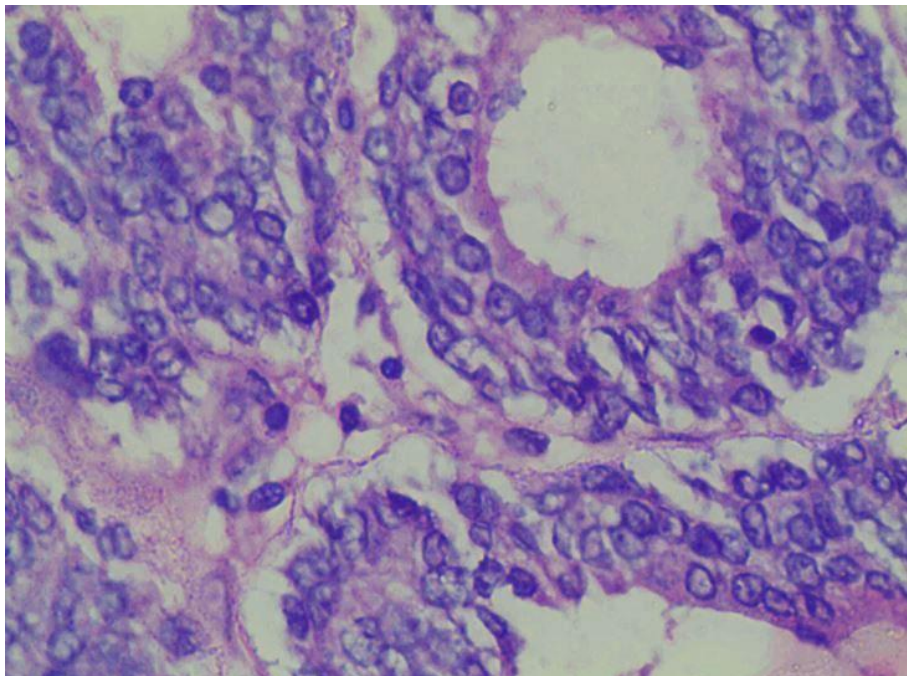


Figure 61. DMBA control - rat mammary tumor - showing tubular adenocarcinoma - alveoli lined by multiple layers of epithelial cells (H&E x40)

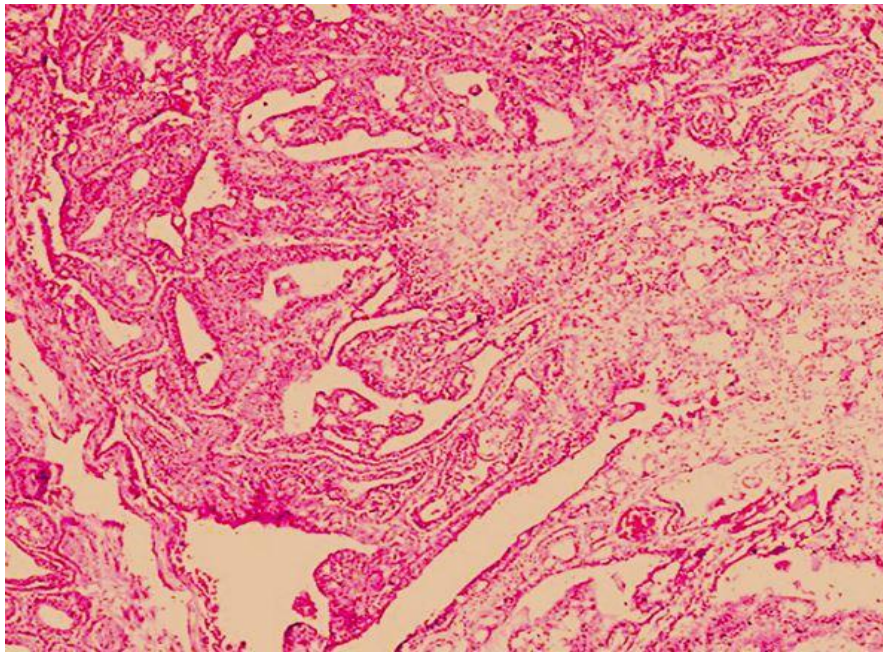


Figure 62. DMBA control - rat mammary tumor - showing papillary adenocarcinoma (H&E x4)

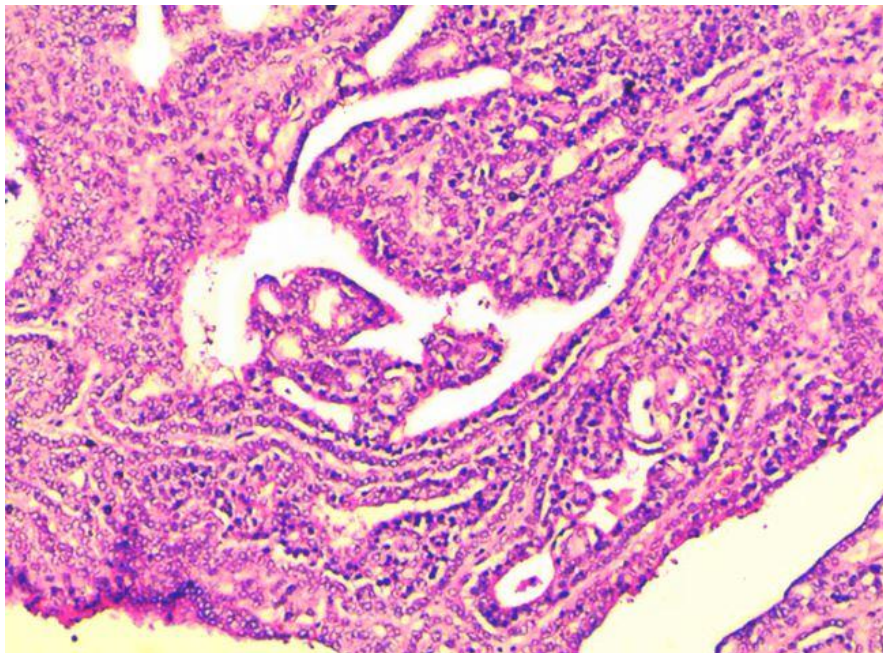


Figure 63. DMBA control - rat mammary tumor - showing papillary adenocarcinoma - proliferation of neoplastic cells giving finger like projections in to the lumen (H&E x10)

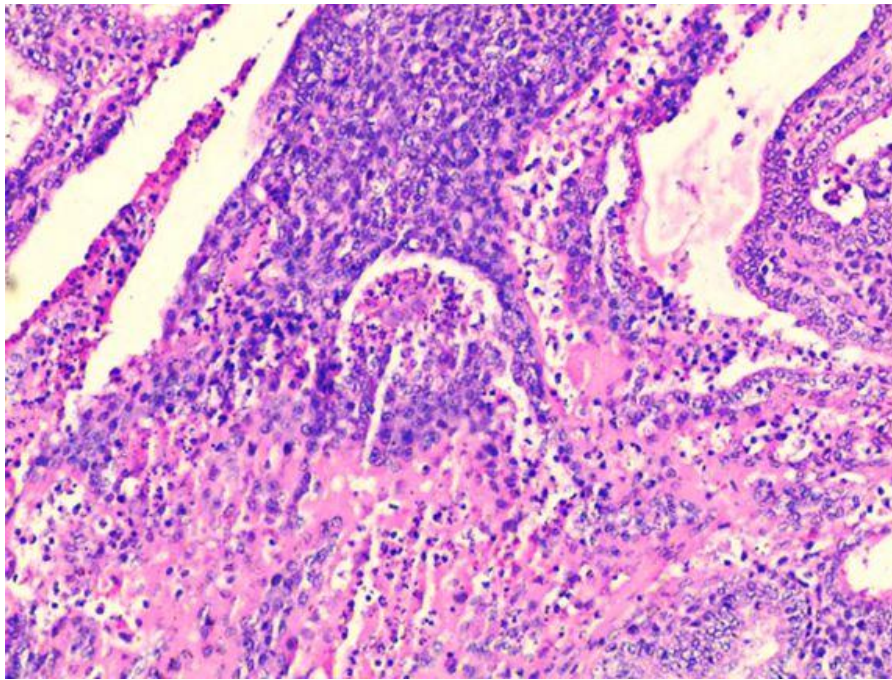


Figure 64. DMBA control - rat mammary tumor - showing comedo carcinoma with accumulation of necrotic cellular debris in the distended ducts (H&E x10)

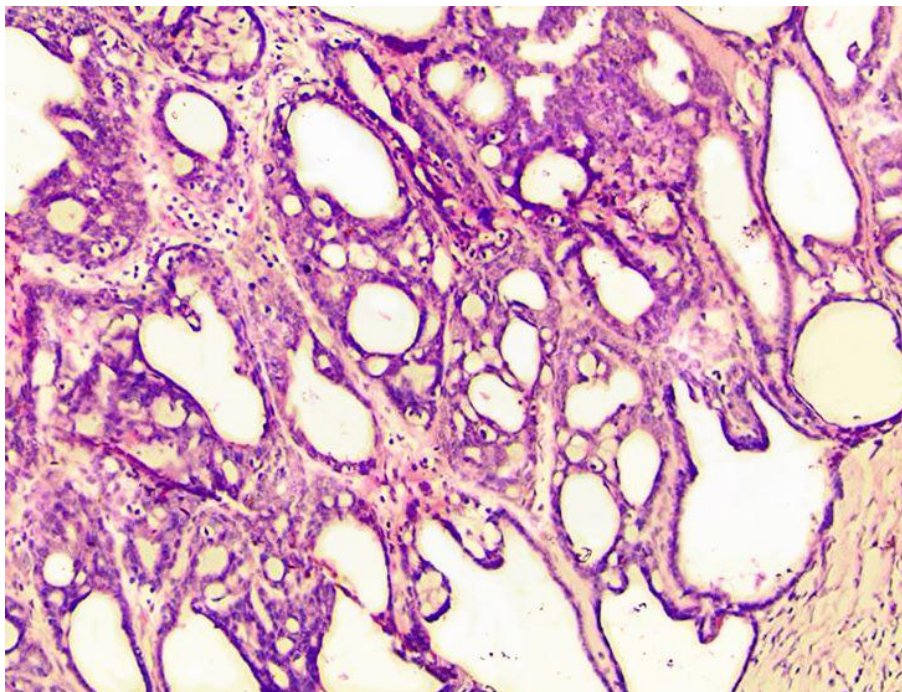


Figure 65. DMBA control - rat mammary tumor - showing cribriform carcinoma with irregular shaped secondary lumina within the lobule showing sieve like pattern (H&E x10)

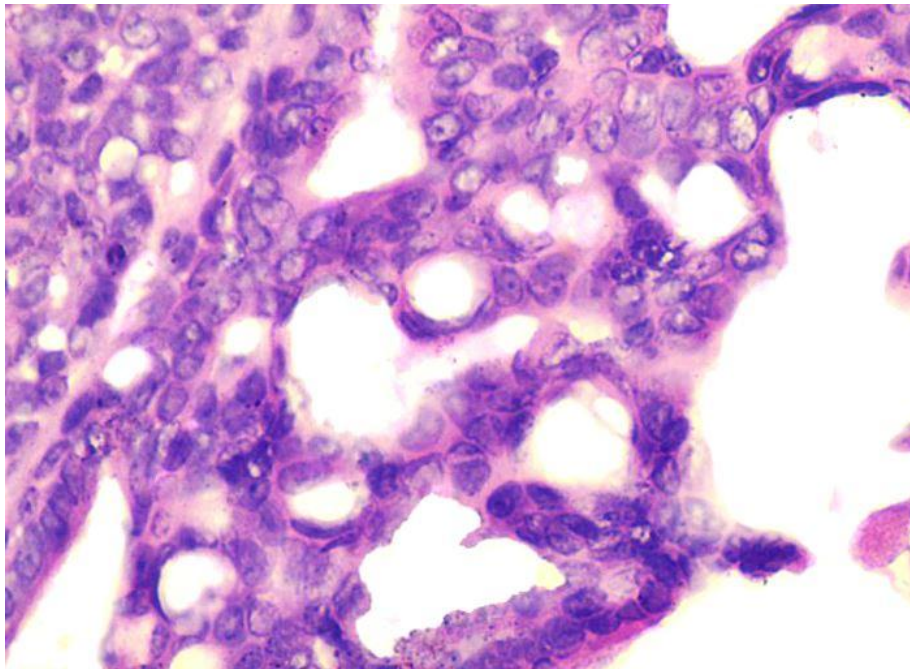


Figure 66. DMBA control - rat mammary tumor - showing cribriform carcinoma with neoplastic cells in the lobule forming secondary lumina (H&E x40)

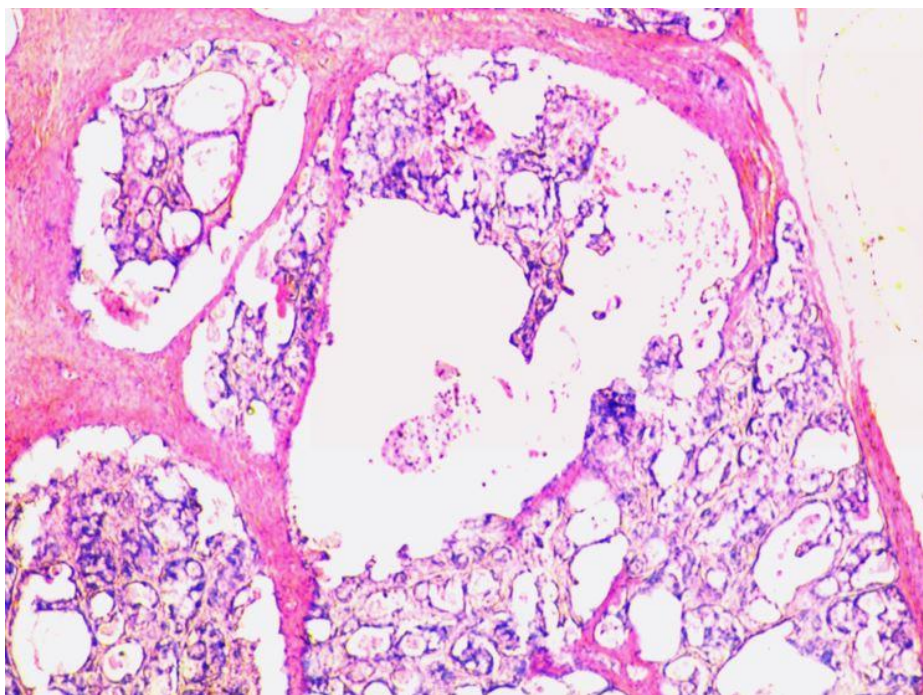


Figure 67. Tamoxifen control - rat mammary tumor - showing area with regressive changes (H&E x4)

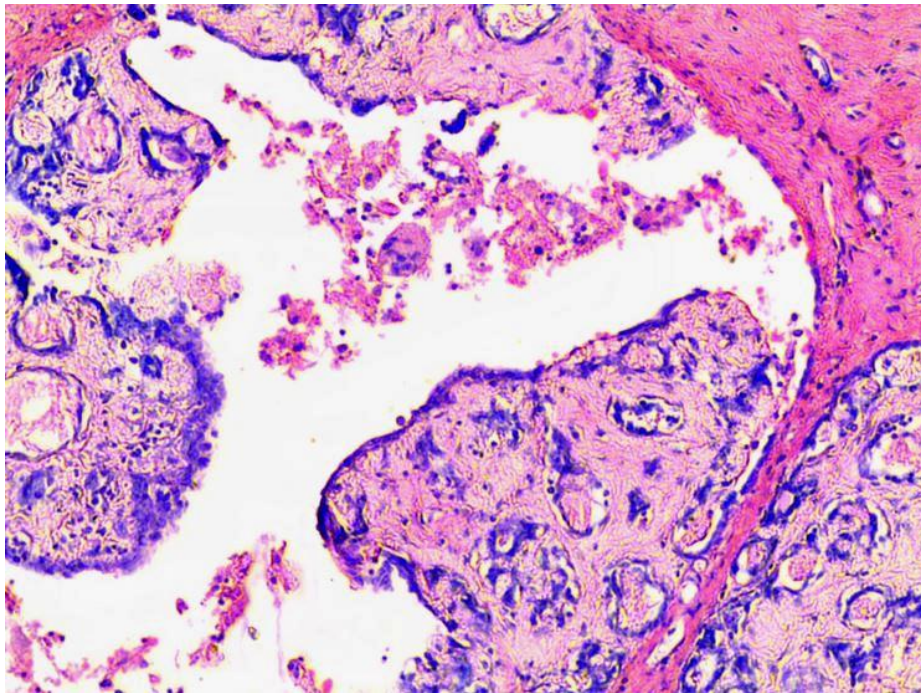


Figure 68. Tamoxifen control - rat mammary tumor - showing desquamation, less cellularity, connective tissue hyalinization and presence of multi nucleated cells (H&E x10)

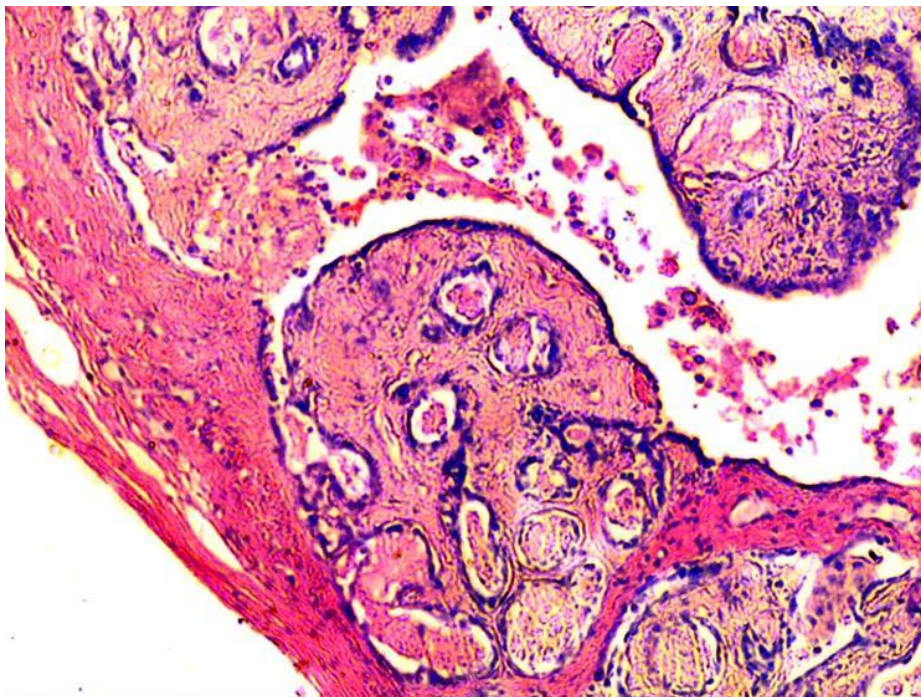


Figure 69. Tamoxifen control - rat mammary tumor - showing regressive changes and desquamation of epithelium (H&E x10)

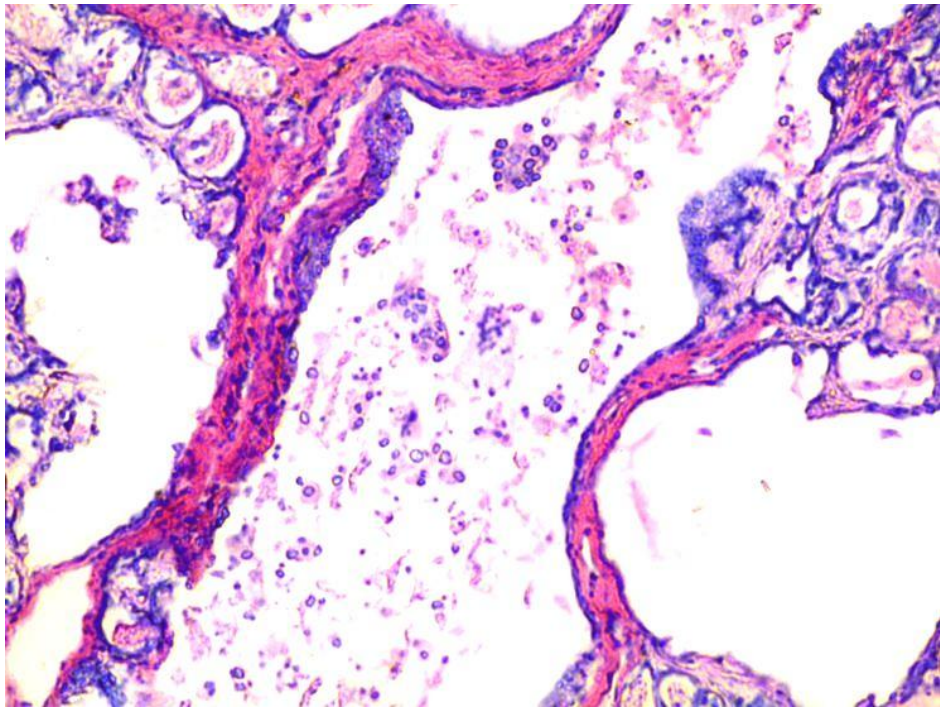


Figure 70. Tamoxifen control - rat mammary tumor - showing desquamation of epithelium (H&E x10)

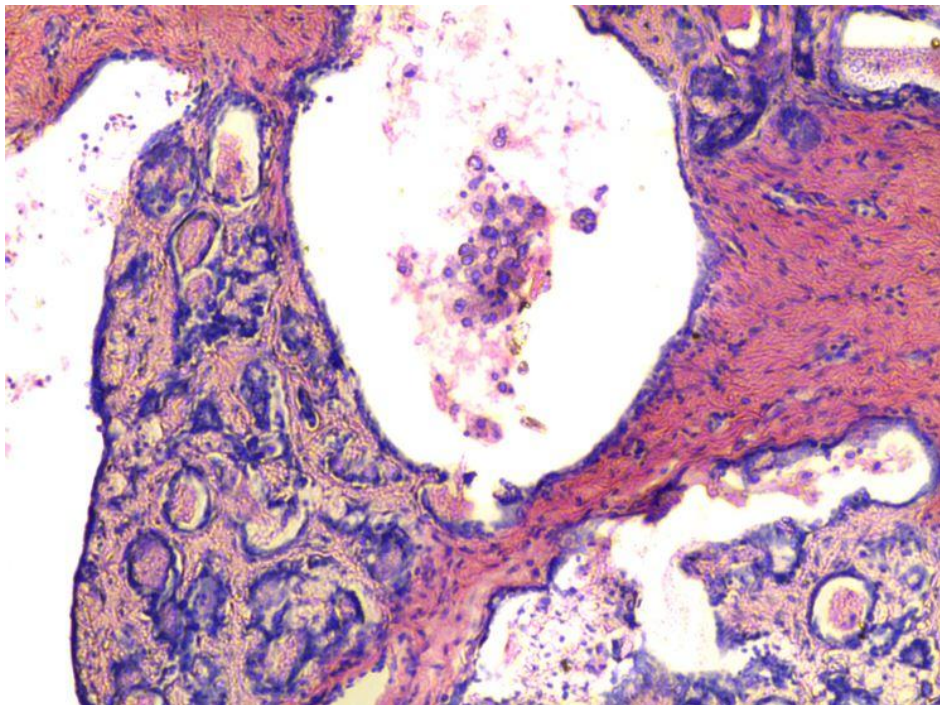


Figure 71. Tamoxifen control – rat mammary tumor - area showing regressive changes with desquamated epithelium (H&E x10)

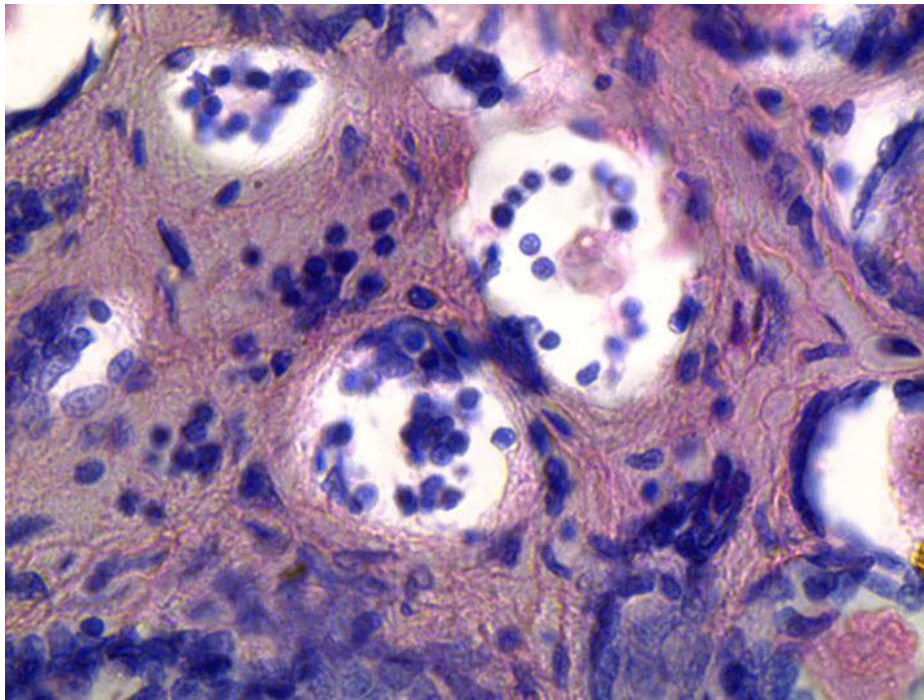


Figure 72. Tamoxifen control – rat mammary tumor - showing alveoli lined with single layer of epithelium, presence of cellular debris and connective tissue proliferation in the stroma (H&E x40)

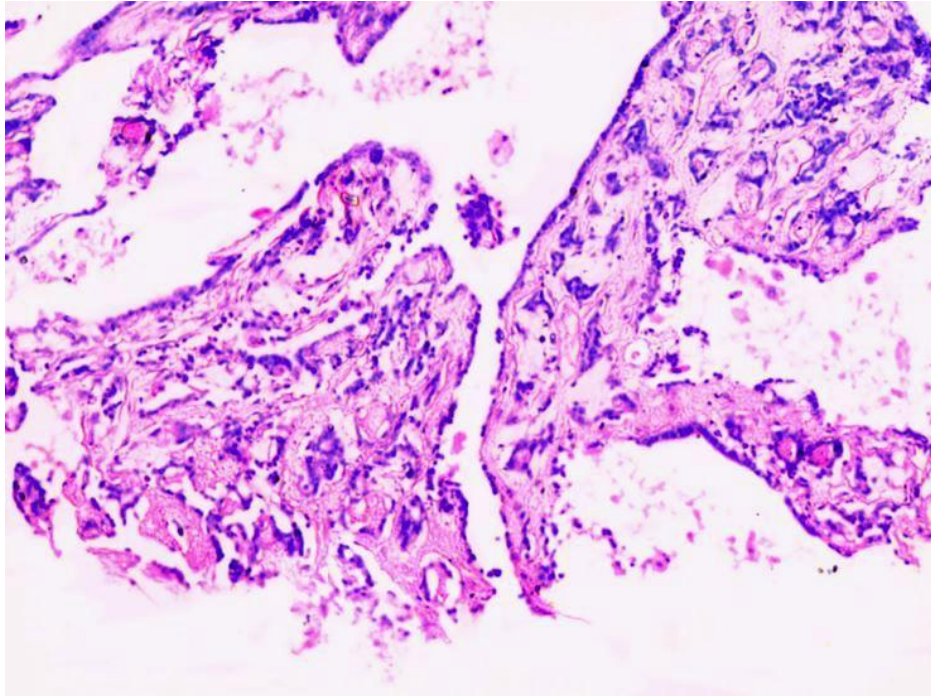


Figure 73. CNMA prophylactic – rat mammary tumor - showing most of the alveoli were contain single layer of epithelia cells and stroma filled with abundant connective tissue (H&E x4)

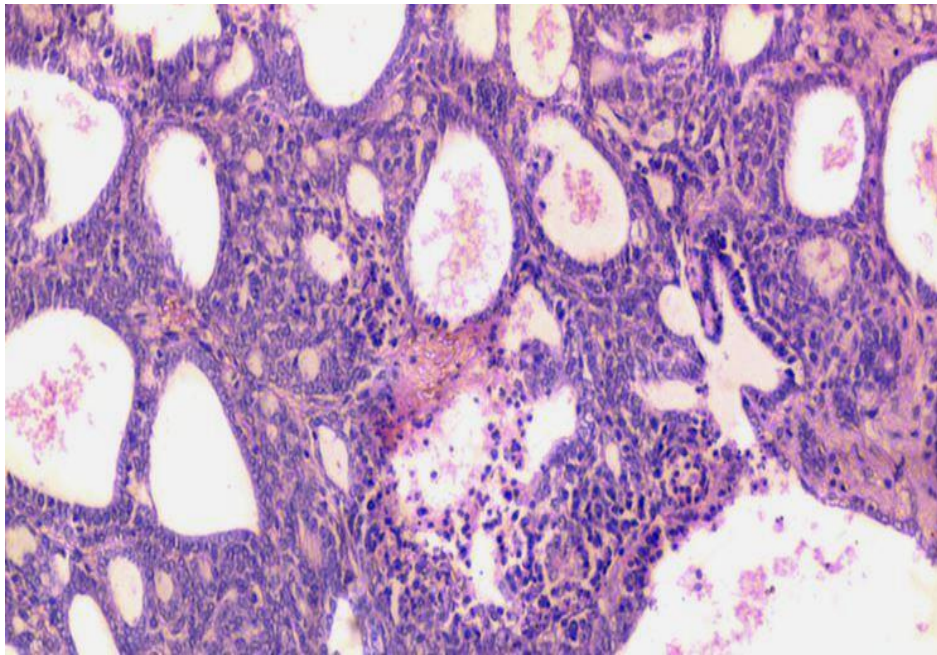


Figure 74. CNMA prophylactic - rat mammary tumor - showing few alveoli were uniformly lined by single layer of epithelial cells and areas of epithelial desquamation (H&E x10)

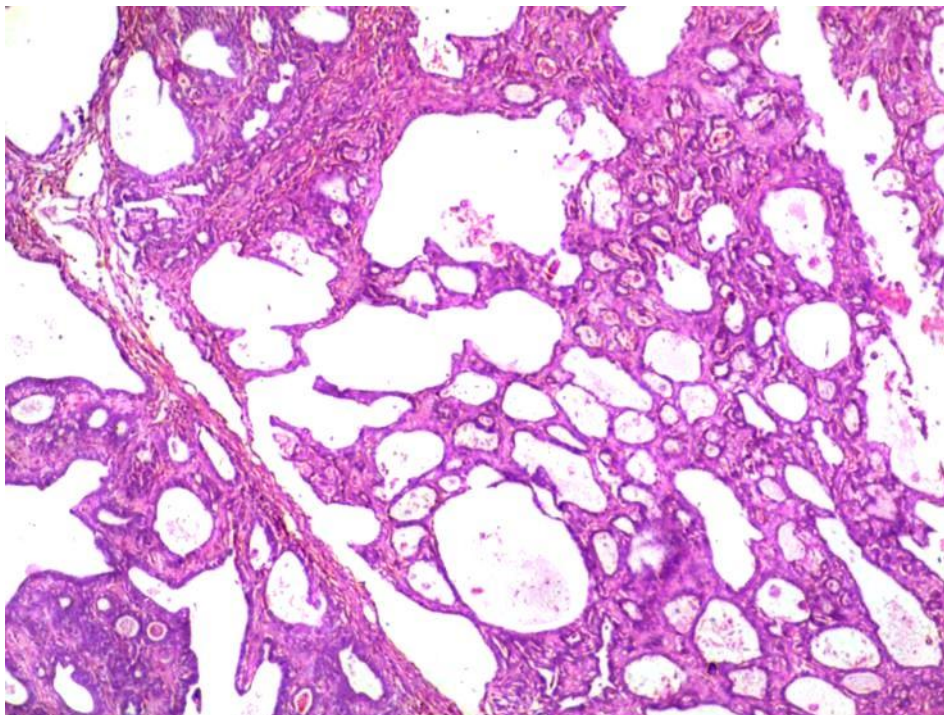


Figure 75. Nano zinc cinnamaldehyde treatment – rat mammary tumor - showing different sizes of alveoli lined by single layer of epithelial cells and also alveoli were increased in number (H&E x4)

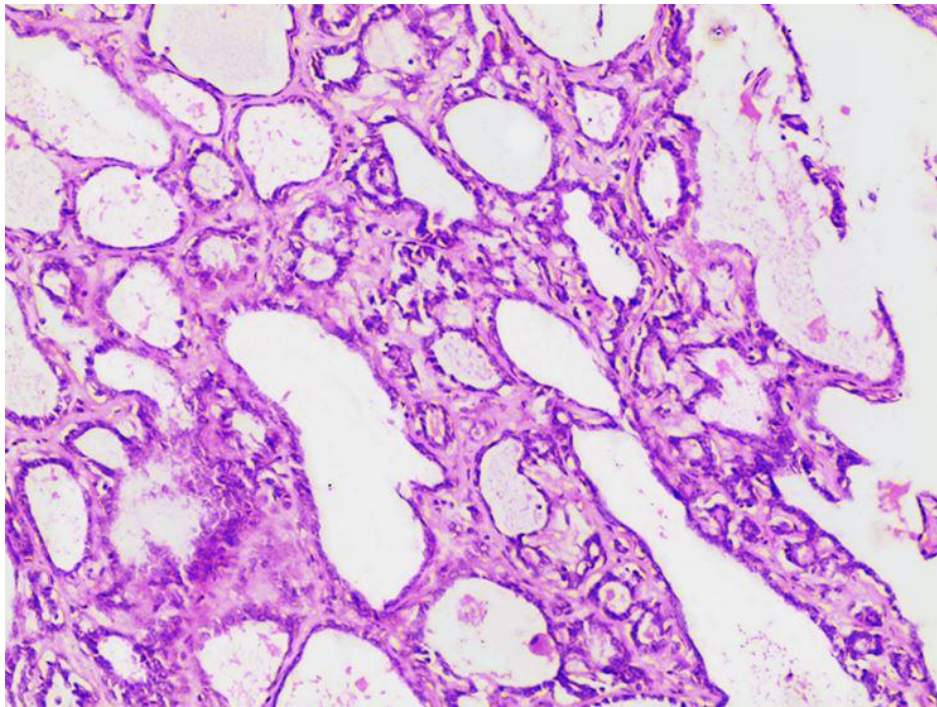


Figure 76. Nano zinc cinnamaldehyde treatment - rat mammary tumor - showing alveoli lined with single layer of epithelium and few alveoli were dilated (H&E x10)

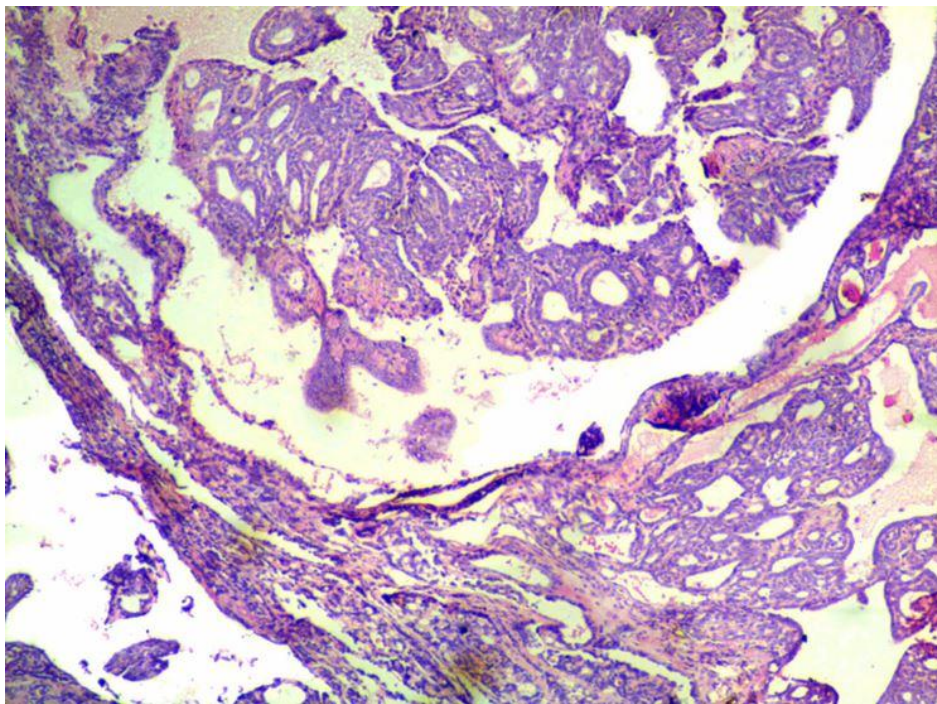


Figure 77. Cinnamaldehyde treatment - rat mammary tumor - adenocarcinoma (H&E x4)

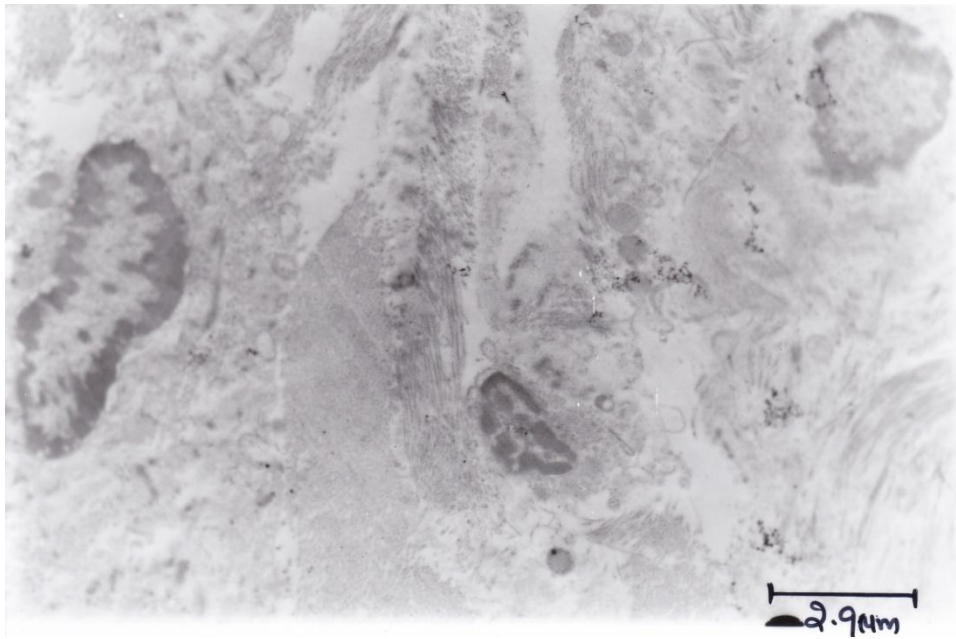


Figure 78. Mammary tumor, rat - DMBA control - TEM showing nuclear pleomorphism and nuclear folding (x3.5 K)

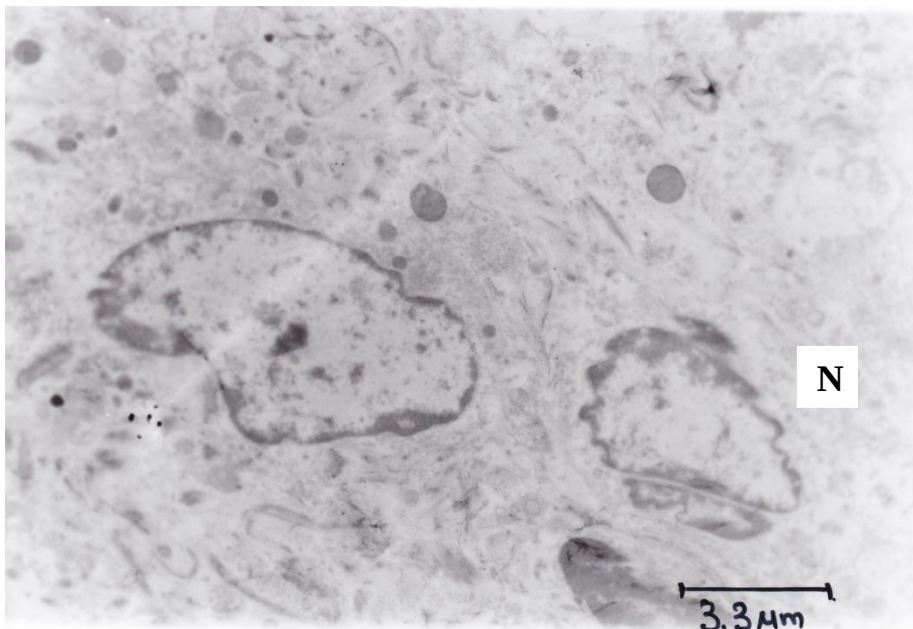


Figure 79. Mammary tumor, rat - DMBA control - TEM showing nuclear indentation and pleomorphism (x3 K)



Figure 80. Mammary tumor, rat - CNMA prophylactic - TEM showing a desquamated epithelial cell with an eccentrically placed indented nucleus and electron dense vesicles in cytoplasm (x3.5 K)

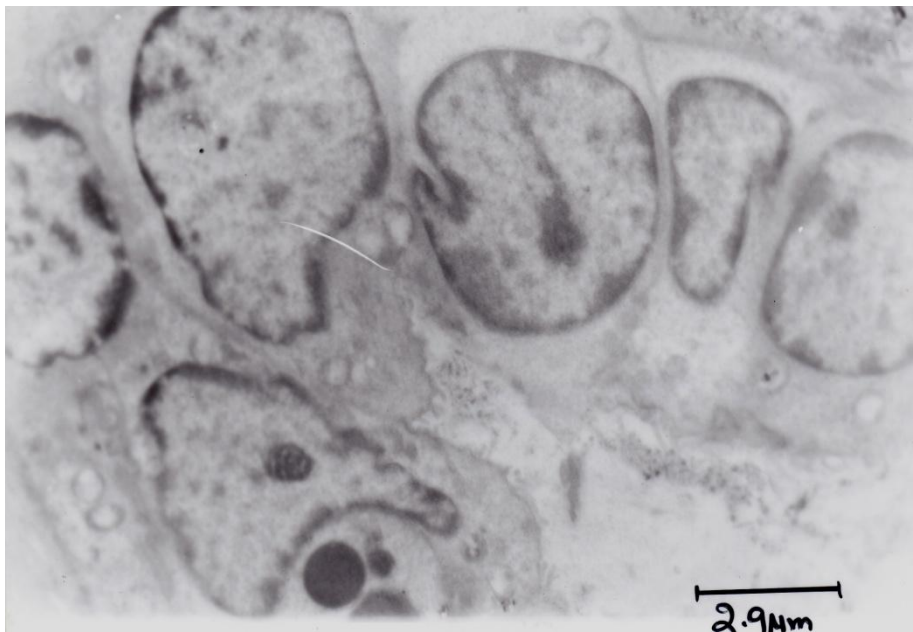


Figure 81. Mammary tumor, rat - CNMA prophylactic - TEM showing marked pleomorphism of nuclei (x3.5 K)

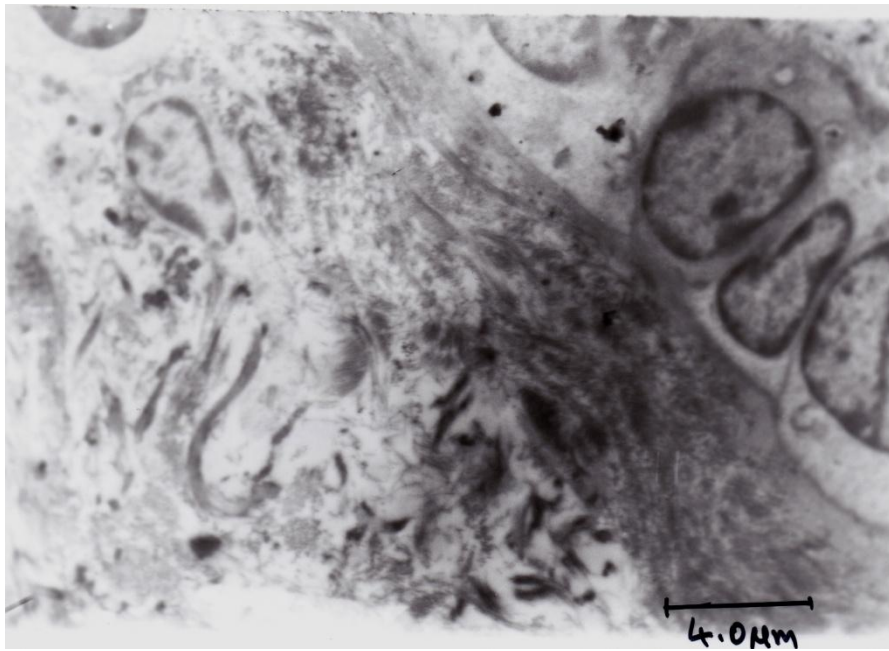


Figure 82. Mammary tumor, rat - Tamoxifen control - TEM showing connective tissue proliferation (x2.5 K)

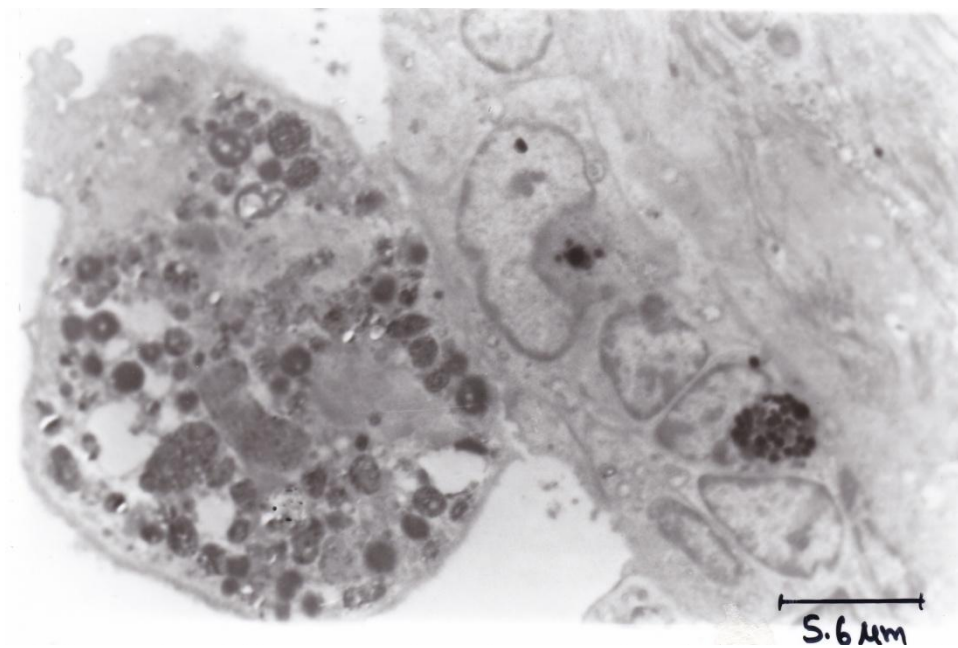


Figure 83. Mammary tumor, rat - Tamoxifen control - TEM showing numerous centrally placed electron translucent bodies, cellular components were lost, large vesicles are present. Pleomorphic cells showing different stages of nuclei (x1.8 K)

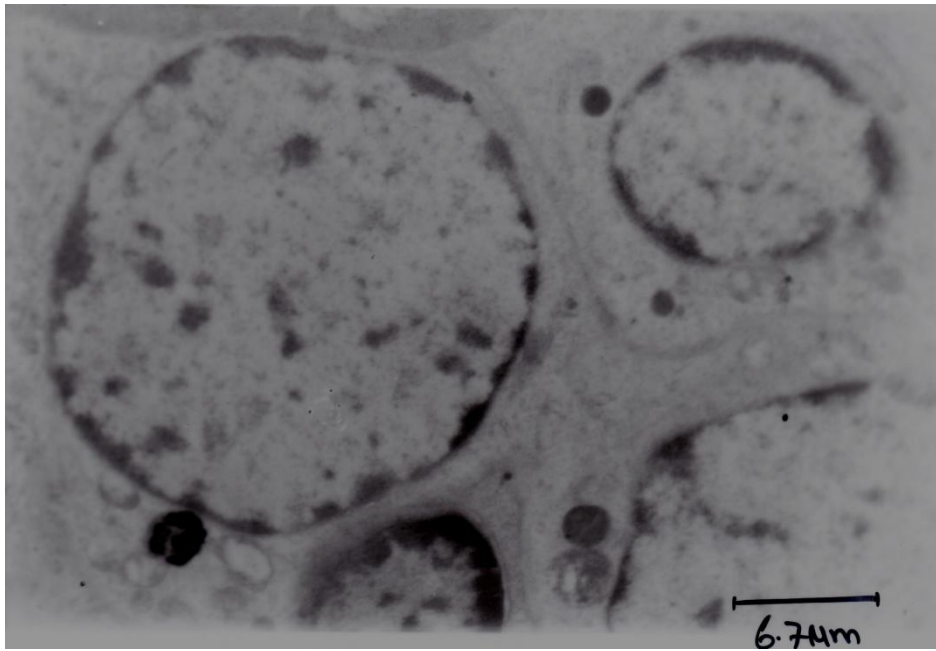


Figure 84. Mammary tumor, rat - Tamoxifen control - TEM showing cells with various stages of nuclei showing severe to moderate swelling (x5 K)

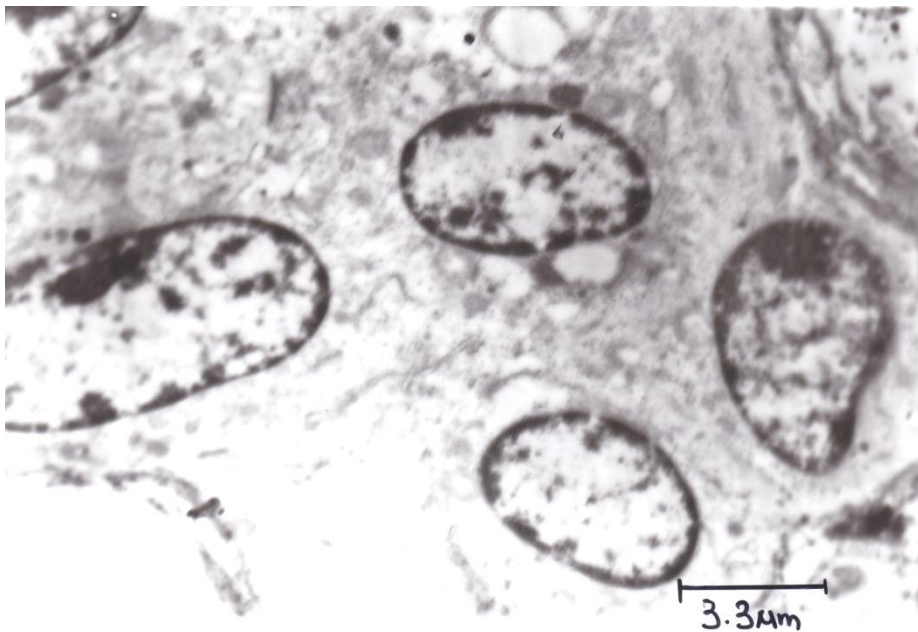


Figure 85. Mammary tumor, rat - CNMA treatment - TEM showing perinuclear vesicles in cytoplasm of a cell (x3 K)

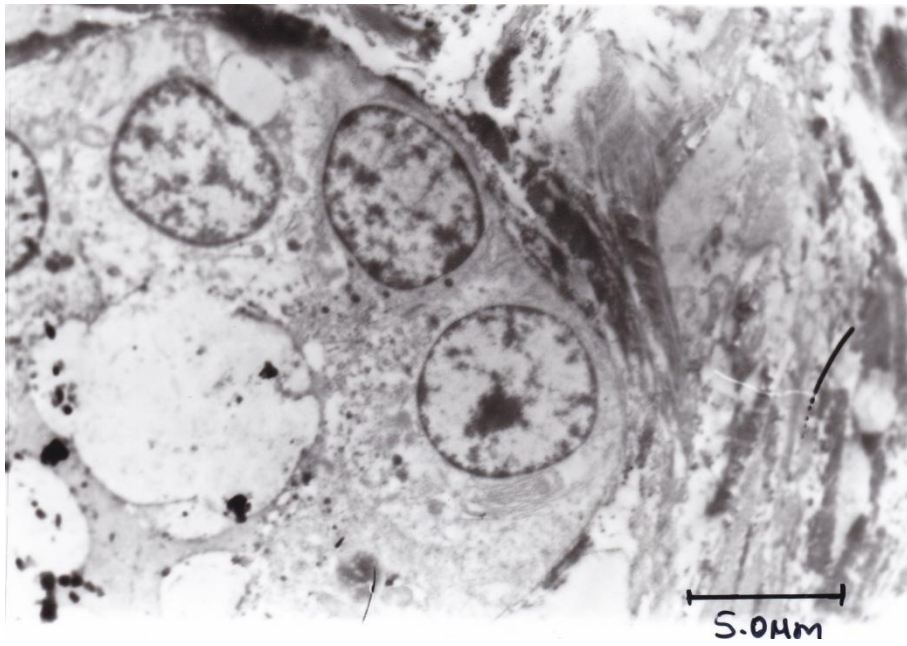


Figure 86. Mammary tumor, rat - CNMA treatment - TEM showing perivascular fibrous tissue proliferation (x2 K)

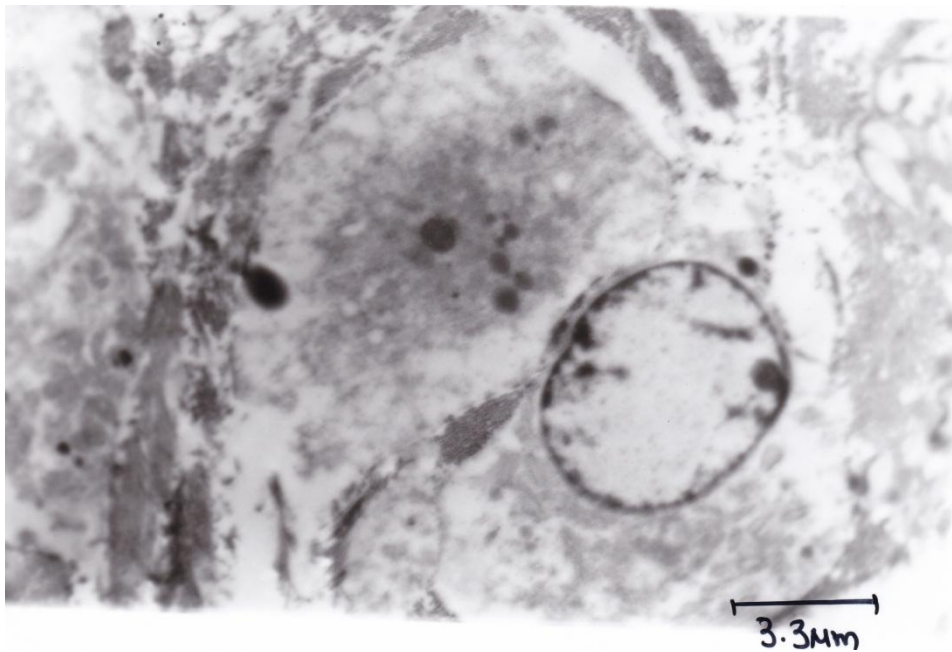


Figure 87. Mammary tumor, rat - CZN treatment - TEM showing pericellular fibroblast proliferation (x3 K)

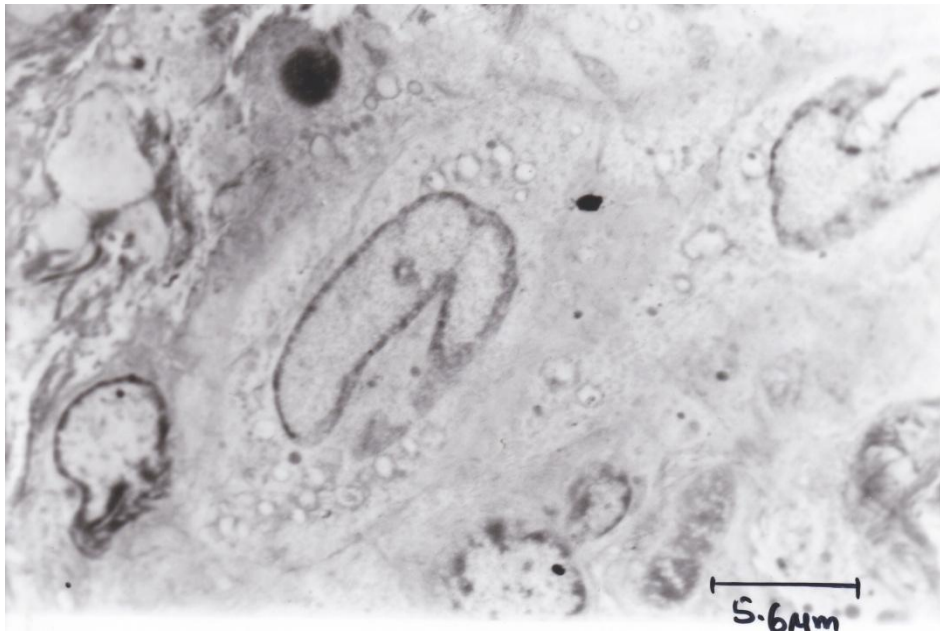


Figure 88. Mammary tumor, rat - CZN treatment - TEM showing nuclear pleomorphism and a dividing nucleus in one of the cells (x1.8 K)

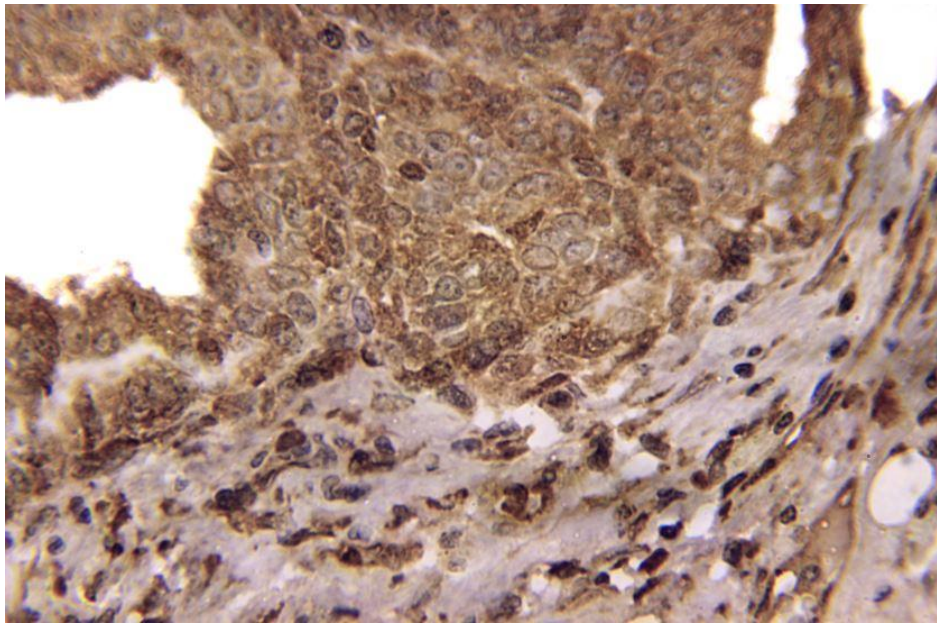


Figure 89. DMBA induced rat mammary gland adenocarcinoma showing abundant ER alpha expression in the nuclei of the glandular epithelium. Immunoperoxidase/ DAB substrate/ Mayer's haematoxylin counter stain (x400)

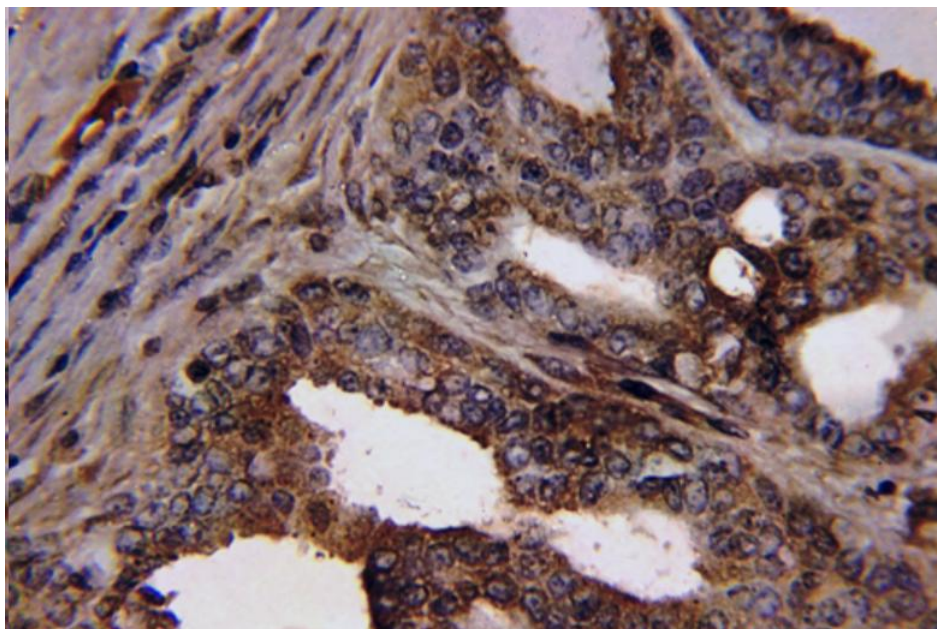


Figure 90. DMBA induced rat mammary gland adenocarcinoma showing abundant PR expression in the nuclei of the glandular epithelium. Immunoperoxidase/ DAB substrate/ Mayer's haematoxylin counter stain (x400)

CHAPTER V

DISCUSSION

Among the cancers, breast cancer is the most common cancer in women (Bener *et al.*, 2008; Saadat, 2008; El Hajj and Hamid, 2011). Worldwide, breast cancer represents the second leading cause of cancer death among women (National Breast Cancer Foundation, Inc[®]. 2016). According to the World Health Organization breast cancer accounts for 16% of all types of cancer deaths globally.

Anti cancer activity of *trans*-cinnamaldehyde and its nano zinc preparation was evaluated in virgin female Sprague-Dawley (SD) rats, with induced mammary tumors. A chemical carcinogen dimethylbenz(a)anthracene (DMBA) was used to induce tumors. In addition acute oral toxicity and pharmacokinetic behaviour of CNMA were also studied.

Zinc oxide nanoparticles were prepared using the phyto-chemical *trans*-cinnamaldehyde and the preparation was characterized by means of dynamic light scattering technique, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Analysis of SEM revealed the formation of spherical shaped zinc nano particles in electron dense matrix. From the TEM analysis it was evident that synthesized nanoparticles were of different shape and size and surrounded by homogenous translucent thin membrane. The average, surface potential and size of CZN

particles were -6.6 mv and 70 nm respectively. Similar characteristics for ZnO NPs were reported by Iravani (2011), Rajendran and Sengodan (2017). Gauthami *et al.* (2015) reported that nanoparticles having zeta potential within the range of -30 mv to +30 mv considered to be more stable. Green synthesis of nanoparticles makes use of environmental friendly, non-toxic and safe reagents. Plant extracts are used for the bio-reduction of metal ions to form nanoparticles. Nanoparticles synthesised using green technology have diverse nature, with greater stability and appropriate dimensions since they are synthesised using a one-step procedure. It has been demonstrated that plant metabolites like sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins play an important role in metal ion reduction into nanoparticles (Parveen *et al.*, 2016). Flavonoids are a large group of polyphenolic compounds comprising several classes: anthocyanins, isoflavonoids, flavnols, chalcones, flavones and flavnones, which can actively chelate and reduce metal ions into NPs (Praveen *et al.*, 2016).

Several plants have been successfully used for synthesis of gold and silver nanoparticles. Leaf extracts of *Cinnamomum camphora* (Huang *et al.*, 2007), *Azadirachta indica* (Shankar *et al.*, 2004), *Aloe vera* (Chandran *et al.*, 2006) and fruit extract of *Embllica officinalis* (Ankamwar *et al.*, 2005) have proven potential in reducing Au(III) ions to form gold nanoparticles Au(0) and silver nitrate to form silver nanoparticles Ag(0). The advantage of using nanoparticles for the drug delivery results from their small size. Nanoparticles can penetrate through the smaller capillaries and are also taken up by cells which allow efficient drug accumulation at the target sites. The use of biodegradable material for nanoparticle preparation allows sustained release within the target site over the period of days or even weeks (Shinde *et al.*, 2012). Retention of the drug at the target site results in longer clearance time. Thus nanoparticles increase the therapeutic efficiency as well as bioavailability.

Acute oral toxicity studies revealed that the body weight gain of rats treated with CNMA and CZN did not differ significantly from control group rats. The relative weights of vital organs viz. liver, lung, heart, kidney, brain and spleen and different haematological parameters like haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte count, neutrophil count and monocyte count in acutely treated rats with CNMA and CZN did not differ significantly from control rats. Among the biochemical parameters, blood urea nitrogen, AST and ALP levels were found elevated in CZN treated rats when compared to control group while in CNMA group they were comparable to control. Histopathology of liver, kidney and stomach revealed congestive and degenerative changes in both the treatments with an additional neutrophil infiltration was evinced in nano zinc cinnamaldehyde treated rats, while the heart and brain showed areas of congestion in both the treatment groups.

Acute oral toxicity of ZnO nanoparticles is dependent on the dose and duration of exposure. In a study by Wang *et al.* (2008) submicron sized ZnO NPs at a dose rate of 2000 mg/kg b.wt significantly reduced the body weight gain in females, while nano sized ZnO did not affect the weight gain. Cho *et al.* (2013) and Saman *et al.* (2013) reported reduced body weight gain with repeated administration of nano ZnO at much smaller doses. Similarly dose and duration dependent effect of nano ZnO on relative organ weights, serum biochemical and haematological profile were reported by Hong *et al.* (2014), Ko *et al.* (2015), Pasupuleti *et al.* (2012) and Wang *et al.* (2016). Mild pathological changes caused by nano ZnO that were dose, duration of treatment and size dependent were reported by Wang *et al.* (2008), Pasupuleti *et al.* (2012) and Ko *et al.* (2015).

To know the pharmacokinetic behaviour of *trans*-cinnamaldehyde (CNMA), rats were administered an oral dose of 500 mg of CNMA. Plasma concentration of CNMA and its major metabolite cinnamic acid (CA) were estimated at various predetermined

time intervals and non compartmental pharmacokinetic parameters were calculated. Area under plasma concentration versus time curve (AUC) is an important parameter and is needed for calculating the bioavailability and to determine the clearance of a compound from the body. The AUC_{0-t} values obtained in this study for CNMA and CA were 28.1315 ± 1.7439 and 15588.9 ± 869.325 $\mu\text{g}\cdot\text{min}/\text{ml}$ respectively. Mean MRT (min.) and clearance ($\mu\text{g}/\text{ml}/\text{min}$) values of CNMA were 123.373 ± 5.6078 was 18.0406 ± 1.0803 , respectively. Apparent volume of distribution at study state and elimination rate constant obtained for CNMA were 2206.35 ± 84.1316 $\mu\text{g}/\text{ml}/\text{min}$ and 0.00818 ± 0.00039 min^{-1} . Thus it can be concluded that cinnamaldehyde is rapidly converted to cinnamic acid, as evidenced from the plasma concentration of cinnamic acid. Another interesting finding is the contrast between the apparent volume of distribution of two compounds, which was high for CNMA and low for CA. Rather than due to re-location of the drug from the plasma, this may be due to increased conversion of CNMA to CA. This is supported by the high value of clearance, which normally should reduce the volume of distribution. Moreover, the conversion of CNMA to CA appears to be reversible to some extent, as seen in the abrupt rise and fall of the plasma concentrations over time, which may be another contributory factor for the above. It can also be noted that though the clearance is high for CNMA, the $t_{1/2}$ and MRT are less, indicating a back conversion to CNMA from CA. In a study by Zhao *et al.* (2014) in male SD rats using GC-MS, the AUC_{0-t} (ng.h/ml), MRT (h) and $t_{1/2}$ (h) values were 1984 ± 531 , 7.6 ± 0.6 and 6.7 ± 1.5 , respectively following cinnamaldehyde (500 mg/kg, P.O) administration. In a study conducted by Yuan *et al.* (1992) rats dosed with 500 mg CNMA/kg b.wt resulted in a prolonged and poorly defined elimination phase of CNMA. The variations observed might be due to the differences in gender, method of detection and chromatographic conditions. AUC_{0-t} for CA was greater than the parent compound as approximately 60% cinnamaldehyde is oxidized to cinnamic acid rapidly (Zhao *et al.*,

2014). Yuan *et al.* (1992) detected a relatively high cinnamic acid concentration immediately after intravenous dosing of cinnamaldehyde indicating a rapid oxidation of CNMA to CA. When rats were administered with higher doses of CNMA, cinnamic acid was detected in blood at concentrations greater than 10 µg/ml (Yuan *et al.*, 1992). In the present study volume of distribution and clearance for cinnamic acid were very low. Low volume of distribution and rapid clearance were recorded in a toxicokinetic study of CA using F344 rats administered with an intravenous dose of 14.2 mg/kg b.wt (Yuan *et al.*, 1992). The difference in the clearance may be due to difference in route of administration. Fluctuations in plasma concentration-time profile of cinnamaldehyde and cinnamic acid can be explained by several factors such as enterohepatic circulation, fractionated gastric emptying and separated absorption windows (Deng *et al.*, 2008; Zhu *et al.*, 2010 and Ding *et al.*, 2012).

For evaluation of anticancer activity of cinnamaldehyde and nano zinc cinnamaldehyde, mammary tumors were induced by administering 7, 12-dimethylbenz(a)anthracene (DMBA) at an oral dose of 20 mg in corn oil to 50 day old Sprague-Dawley rats. As a standard treatment for mammary cancer, tamoxifen citrate was used as positive control in this study. Tamoxifen, a nonsteroidal triphenylethylene derivate and selective ER modulator, has been used as a single agent in the treatment of ER- α -positive breast cancer (Rajput *et al.*, 2013). Fisher and Costantino (2006) opined that clinical response to tamoxifen is associated with both decreased proliferation and increased apoptosis. Studies have revealed that tamoxifen is also effective in the treatment of ER- α -negative neoplasia including breast cancer, malignant gliomas, pancreatic carcinomas and melanoma (Perry *et al.*, 1995; Salami and Karami-Tehrani, 2003). The apoptotic inducible effect of tamoxifen is not reversible by addition of estrogens, suggesting that ER- α independent induction of apoptosis could be a central mechanism of action in ER- α -negative breast cancer (Mandlekar and Kong 2001;

Weng *et al.*, 2008). In addition, Haran *et al.* (1994) reported that tamoxifen has been shown to cause tumor necrosis and regression via inhibition of angiogenesis in MCF-7 breast tumor xenografts. It has been reported that tamoxifen and its more active metabolite 4-hydroxytamoxifen, due to their high partition into membranes, induce multiple cellular effects including antioxidant actions (Wiseman *et al.*, 1990).

Experimental models of chemically induced tumors have been widely used to understand the potential of dietary factors in the induction, promotion and/or prevention of mammary carcinogenesis (Dias *et al.*, 1999). DMBA induced rat mammary gland tumor model has been widely used for the development and screening of chemopreventive drugs intended for use in human breast cancer (Mehta, 2000). Mammary glands of several strains, most notably Sprague-Dawley rats are susceptible to neoplastic transformation by chemical carcinogens (Russo *et al.*, 1990). According to Grubbs *et al.* (1986) and Rose *et al.* (1980) the susceptibility of the mammary gland to DMBA or N-methylnitrosourea (NMU), carcinogenesis is strongly age-dependent, being maximal when the drug is administered to rats between the age of 45 and 60 days, at which the sexual maturity begins. Moi *et al.* (2012) induced breast cancer with DMBA in SD rats with a single oral dose of 20 mg while Hollingsworth *et al.* (1998) administered 5 mg DMBA to SD rats for four successive weeks amounting to a total dose of 20 mg. Purushothaman *et al.* (2012) administered 25 mg DMBA orally for induction of mammary tumors.

In, DMBA only administered group palpable growths were first detected 70 days after administration of DMBA, while in cinnamaldehyde prophylactic (CNMA-P) group the onset of palpable growths were noticed 90 days post DMBA administration. By the end of 90 day post DMBA administration 80 percent rats in DMBA alone treated and 71 percent rats in CNMA-P group developed growths in the mammary gland region.

In cinnamaldehyde prophylactic group (CNMA-P), CNMA-T and CZN-T groups the tumor volume was significantly ($P < 0.05$) reduced when compared to DMBA control (DMBA-C) group and was almost similar to tamoxifen treatment (TAM) group. Tumor burden also followed the same trend. Tumor multiplicity was highest in DMBA-C group when compared to all other groups. Samy *et al.* (2006) reported that DMBA alone treated rats showed highest tumor incidence, tumor multiplicity and tumor weight. In the multistage carcinogenesis tumor initiation and progression were irreversible stages, whereas tumor promotion is reversible especially at earlier stage (Barile, 2010). According to Surh *et al.* (2003) phytochemicals having chemopreventive properties can block initiation or reverse the promotion stage of multistep carcinogenesis. They can also retard the progression of precancerous cells into malignant state. Significant opportunities in nutrition and cancer prevention exist in the early stages of initiation and promotion prior to clonal expansion of heterogeneous populations (Milner and Romagnolo, 2010). Luteolin, hydroxylated flavone derivative, when administered alone to tumor bearing rats showed moderate anti-tumor activity (Samy *et al.* 2006). In a study by Verma *et al.* (1988) prophylactic dietary quercetin, at both 2% and 5% levels inhibited the incidence and multiplicity of mammary tumors in rats. Garlic powder supplementation significantly delayed the onset of mammary tumors induced by N-methyl-N-nitrosourea (NMU) and reduced the tumor incidence and total tumor number compared to rats receiving the un-supplemented diet (Schaffer *et al.*, 1996). Arroyo-Acevedo *et al.* (2015) reported that *Piper aduncum* capsule showed protective effects in terms of reduced incidence of adenocarcinomas and lymph node metastasis in DMBA induced rat breast cancer. The importance of timing of nutrient exposure in cancer prevention is highlighted by experimental evidence showing that supplementation with the isoflavone genistein during the prepubertal and prepubertal plus adult periods protected against mammary carcinogenesis (Lamartiniere *et al.*,

2002). The reduction in mammary tumorigenesis was associated with increased expression of antioxidant genes and lower expression of genes that stimulate cell proliferation (Olivo-Marston *et al.*, 2008). Resveratrol increased tumor latency and reduced the number of tumors in an NMU-induced mammary cancer model (Bhat *et al.*, 2001), and more recently long-term administration of resveratrol (since birth) was shown to increase the differentiation of lobular structures in the mammary gland and reduce proliferative activity, whilst reducing numbers and increasing the latency of DMBA-induced tumors (Whitsett *et al.*, 2006). Damianaki *et al.* (2000) opined that a regular intake of flavonoids, such as polyphenols and quercetin, was linked to lower rates of stomach, pancreatic, lung and possibly of breast cancer.

Most chemical carcinogens need activation by body enzymes to be transformed to a species that readily binds to genetic DNA to form DNA adducts (Abbadessa *et al.*, 2006). Carcinogen-DNA adducts can be repaired by body enzymes. The unrepaired adducts will be fixed after one cell cycle and lead to mutations and consequent breast cancer development. Therefore, preventing carcinogen-DNA adduct formation is a key step in breast cancer prevention at the initiation step of carcinogenesis (Dipple, 1984). DMBA is a polycyclic aromatic hydrocarbon used to induce breast cancer. This potent carcinogen cause DNA damage in the cell, through its reactive metabolite DMBA-3,4-dihydrodiol-1,2-epoxide (DMBA-DE) that adds adenine and guanine residues to DNA. The conversion of genotoxic metabolites as DMBA-DE is promoted by the action of cytochrome P450 family. CYP1A1 and CYP1B1 are identified as enzymes that metabolize DMBA to produce DMBA-DE (Thompson and Singh, 2000).

Wattenberg (1985) categorized chemopreventive agents into three classes: i) *inhibitors of carcinogen formation* that prevent the formation of carcinogens from precursor substances, ii) *blocking agents* that inhibit carcinogen-induced mutations by preventing metabolic activation of carcinogens, enhancing detoxification systems, or by

trapping reactive oxygen species? (ROS) before they interact with critical target sites, and iii) *suppressing agents* that prevent tumor progression by influencing cell proliferation, differentiation, senescence and/or apoptosis. A large number of phytochemicals inhibit carcinogenesis by one or more of these mechanisms (Guilford and Pezzuto, 2008).

Compounds that are high in antioxidant potential are efficient interceptors of DNA-reactive elements (Malone, 1991). Plant phenolics prevent DNA adduction, presumably by presenting alternative targets for attack by carcinogens (Newmark, 1985; Tell and Castonguay, 1992). Another mechanism by which plant components can prevent cancer in the early phases of carcinogenesis is through the modulation of carcinogen metabolism. Fruits and vegetables can impact the cancer process by modifying the behaviour of cancer cells (Wargovich, 2000).

The body weights were found significantly ($P < 0.05$) lowered in all DMBA administered rats compared to normal control and nano control group rats. However a significant increase in body weight was observed in various CNMA and CZN treated groups compared to only DMBA treated group. Decreased body weight were observed in breast cancer induced rats (Ouhtit *et al.* 2014; Krishnamoorthy and Sankaran, 2016) and mice (Ojeswi *et al.* 2010). MacDonald (2007) stated that the presenting feature of cancer was anorexia and reduced food intake. Further Muscaritoli *et al.* (2006) opined that tumor growth deranges metabolism of protein, carbohydrate and lipid, thereby exacerbating the nutritional effects of the caloric deficit induced by anorexia and simultaneously reducing the efficiency of protein-energy supplementation. Anorexia and reduced food intake contribute to weight loss in cancer. In particular, muscle wasting is exacerbated because of increased proteolysis and reduced anabolism (Laviano *et al.*, 2007). Consistent experimental and clinical data indicate that increased oxidative stress is involved in the pathogenesis of anorexia and cachexia of cancer.

Barreiro *et al.* (2005) showed that in the muscles of tumor bearing animals, oxidative stress was increased when compared with that in control animals. Ethanolic extract of *Thuja occidentalis* (10 mg/kg b.wt) improved the body weight in breast cancer induced mice with DMBA (Ojeswi *et al.*, 2010). From his study on DMBA induced rat mammary cancer Samy *et al.* (2006) concluded that luteolin (a hydroxylated flavones derivative) treatment non-significantly ($P > 0.05$) increased the body weight of rats.

Tumor markers are naturally occurring or modified molecules that can be measured in serum, plasma or other body fluids and the changes in their concentration can be used for early detection and predicting the therapeutic response and prognosis of cancer (Schauer, 1982; Duffy, 2013). Shamberger (1984) reported that an elevation in the concentration of serum total sialic acid has been observed in different cancer types. Rapid increase of sialic acid levels in breast cancer was related to enhanced activity of sialidase enzyme (Sonmez *et al.*, 1999). Keeping in view total sialic acid (TSA) levels were estimated as a tumor associated marker. Statistically significant ($P < 0.05$) increase in TSA levels were observed in all DMBA administered groups when compared to control (C) and nano cinnamaldehyde (CZN) control group. Among all DMBA administered groups, rats in CNMA-P group showed lowest TSA levels followed by TAM, CZN-T, CNMA-T and DMBA-C. Increased sialylation in tumor tissue is reflected as increased sialic acid levels in serum and other body fluids (Dadhich *et al.*, 2014). Studies carried out on human subjects by Correale *et al.* (1992) and Sreeramulu and Venkata Ramana (2014) revealed that all groups of investigated breast cancer patients have mean values of sialic acid higher than healthy controls. Similar findings were reported by Abd El-Aziz *et al.* (2005) and Hamdy *et al.* (2012) from their study on mammary carcinoma. In some studies, the TSA content has been observed to increase in highly metastatic cells compared with non metastatic cells (Skipski *et al.*, 1981; Yogeeshwaran and Salk, 1981). Raval *et al.* (1997) reported that total sialic acid and lipid

associated sialic acid could be used for assessment of response to anticancer treatment in breast cancer patients. Elevation in serum levels of sialic acid and sialyl transferase in breast cancer was negatively associated with response to anticancer treatment (Raval *et al.*, 2004). Administration of genistin (Hamdy *et al.*, 2012) and *Nigella sativa* (Abd El-Aziz *et al.*, 2005) to rats with breast cancer significantly reduced the TSA levels. Boshra and Hussein (2016) reported that cranberry extract significantly reduced the plasma sialic acid levels in N-methyl-N-nitrosourea (MNU) induced breast cancer rats. However, Monti *et al.* (1998) reported that measurement of sialic acid was not of clinical benefit in breast cancer.

Generally, antioxidants are involved directly in the conversion of ROS to less-reactive species. Halliwell (1996) stated that the harmful effects of ROS are balanced by the antioxidant action of non-enzymatic-antioxidants in addition to antioxidant enzymes. It has been reported by McEligot *et al.* (2005) that, in many human cancers, altered levels of antioxidant enzymes (SOD, catalase, glutathione peroxidase) and non-enzymatic antioxidants (GSH, Vit-C, thioredoxin) and changes in the related signal pathways were observed. According Valko *et al.* (2006) Mn-SOD was one of the most effective antioxidant enzymes with anti-tumor activity. Hence antioxidant (super oxide dismutase, SOD and reduced glutathione, GSH) and peroxidation (thiobarbituric acid reactive substances, TBARS) activity levels were also estimated in selected tissues and plasma to know the antioxidant potential of cinnamaldehyde and its nano preparation against the oxidative stress caused by carcinogenesis. In the present study, significantly ($P < 0.05$) decreased SOD levels were observed in liver, heart, kidney, mammary tissue and plasma of DMBA-C group. But in CNMA-P, CNMA-T and CZN-T groups, improved SOD levels were observed in liver, kidney and plasma of rats bearing DMBA induced mammary tumors. Heart homogenates of CNMA-T, nano cinnamaldehyde (CZN-T) and tamoxifen (TAM) treatment groups showed non-significant ($P > 0.05$)

increase in SOD activity when compared to DMBA-C group. Comparable SOD levels were found in lung and brain tissues of all the experimental groups including tumor control. Glutathione (GSH) was a multifunctional intracellular non-enzymatic antioxidant and considered to be the major thiol-disulphide redox buffer of the cell (Masella *et al.*, 2005). GSH activity in different organs was decreased in all DMBA administered groups when compared to control. But the decrease was not significant ($P > 0.05$) in heart homogenates of CNMA-P. Similar trend was observed in case of brain tissue homogenates of CNMA-P and CNMA-T group. The extent of lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) called MDA (Zhang *et al.*, 2006). Increased MDA levels were observed in all tissue homogenates of DMBA treated rats when compared to control. But prophylactic treatment with cinnamaldehyde significantly ($P < 0.05$) reversed the elevation of TBARS levels in all tissue homogenates and plasma. Except tamoxifen, other treatment protocols significantly ($P < 0.05$) lowered the lipid peroxidation produced by DMBA in mammary tissue of rats. From these results it is evident that cinnamaldehyde and its nano preparation showed favourable effect in terms of SOD, GSH and TBARS content in various tissues of tumor bearing rats.

Reactive oxygen species (ROS) are seemingly involved in variety of important pathophysiological conditions including mutagenesis and carcinogenesis (Aruoma, 1994). According to Ren *et al.*, (2003) it is generally believed that the formation of growth promoting oxidants (reactive oxygen species, ROS) is a major “catalyst” of the tumor promotion and progression stages, which follow the initiation stage (carcinogen metabolic activation to mutagens). ROS cause strand breaks, alterations in guanine and thymine bases, and sister chromatid exchanges (Wiseman and Halliwell, 1996). This may inactivate additional tumor suppressor genes with in tumor cells, or further increases expression of proto-oncogenes. Genetic instability due to persistent carcinoma

cell oxidative stress will therefore increase the malignant potential of the tumor (Szatrowski and Nathan, 1991). Free radicals play an important role in tumor promotion by direct chemical reaction or alteration of cellular metabolic processes (Cerutti, 1985), while their scavengers (SOD, catalase, reduced glutathione, GPx) represent inhibitors at different stages of carcinogenesis (Sun, 1990). These antioxidants are involved in the biotransformation and detoxification of carcinogens (Wilkinson and Clapper, 1997). Reactive oxygen species (ROS) cause injury to cells and intracellular membranes resulting in lipid peroxidation and may lead to cellular destruction and subsequently, cell death (Halliwell, 2006).

Toxic manifestation of DMBA is associated with its oxidative metabolism, leading to the formation of reactive metabolites (epoxides and quinines) capable of generating free radicals (Giri *et al.*, 1995). The resultant oxidative stress from the excessive production of ROS, exhausts antioxidant defences (Hussein *et al.*, 2005). Previous studies also reported that rats treated with DMBA displayed significant elevation in MDA levels (Jang and Kim, 2002; Moselhy and Al mslmani, 2008; Hamdy *et al.*, 2012). Masella *et al.* (2005) opined that protective role of glutathione against oxidative stress is partly due to direct scavenging of hydroxyl radicals and singlet oxygen and also detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase (GPx). Antioxidants that scavenge ROS protect against carcinogenesis by one or a combination of the following mechanisms: inhibition of procarcinogen activation, carcinogen inactivation, blocking DNA adduct formation, enhancing DNA repair enzymes, inhibition of cell proliferation, invasion and angiogenesis, upregulating gap junctional communication, reducing protooncogene activation and mutant p53 expression, apoptosis induction, and modulation of transcription factors and proteins involved in tumor progression (Bhuvaneswari and Nagini, 2005; Khan *et al.*, 2008; Nagini, 2008; Oyagbemi *et al.*, 2009).

Many phytochemicals function as potent antioxidants and inhibit the pro-inflammatory mediators COX-2 and LOX by suppressing the transcription factors NF- κ B and activator protein-1 (AP-1) and by inhibiting nitric oxide synthase induction (Kundu and Sureh, 2005; Oyagbemi *et al.*, 2009; Neergheen *et al.*, 2010).

However, protection produced by antioxidant therapy against cancer depends on the stage of cancer. When used during the progression stage of cancer, it might actually stimulate growth of tumors through enhanced survival of tumor cells (Valko *et al.*, 2006). Recent investigations demonstrated that the antioxidant properties of plants can be correlated with the defence mechanism against oxidative stress and different human diseases including cancer and arteriosclerosis and aging processes (Rolo and Palmeira, 2006). Oxidative stress plays an important role in both the treatment response of the patient and clinical outcome in general (Ozben, 2007).

Schroeter *et al.* (2002) opined that antioxidant phenolic compounds act as terminators of free radical chains and as chelators of redox-active metal ions that are capable of catalysing lipid peroxidation. According to Valko *et al.* (2006) Phenolic antioxidants (PhOH) interfere with the oxidation of lipids and other molecules by the rapid donation of hydrogen atom to radicals $\text{ROO}^\bullet + \text{PhOH} \longrightarrow \text{ROOH} + \text{PhO}^\bullet$, and these phenoxy radical intermediates are relatively stable so they do not propagate further reactions.

Numerous *in vitro* studies conducted earlier with cinnamaldehyde and nano zinc revealed their anticancer activity. Anti-proliferative and pro-apoptotic properties of cinnamaldehyde were demonstrated in various cancer cell lines (Moon and Pack, 1983; Kwon *et al.*, 1998; Jeong *et al.*, 2003; Lee *et al.*, 2007). From his study using human hepatoma PLC/PRF/5 cells, Wu *et al.* (2005) ascribed the apoptotic activity of cinnamaldehyde to its stimulatory action on Bax and Bid pro-apoptotic proteins and mitogen – activated protein kinases (MAPK) signalling pathways. Duessel *et al.* (2008)

demonstrated that cinnamaldehyde displayed significant anti-proliferative effects on human colon cancer cells in concentration and kinetic dependent manners. Treatment with zinc oxide (ZnO) nanorods resulted in up regulation of cell-cycle check point protein p53 and pro-apoptotic protein Bax and also induced caspase -3 and 9 in human alveolar adenocarcinomas cells (Ahamed *et al.*, 2011). Studies have shown that ZnO NPs induce cytotoxicity in a cell specified and proliferation dependent manner, with rapidly dividing cancer cells being the most susceptible, and quiescent cells being the least sensitive (Hanley *et al.*, 2008; Ostrovsky *et al.*, 2009; Premanathan *et al.*, 2011).

These results suggest that the protective role of plant phenolic, cinnamaldehyde and its metabolite cinnamic acid could be due to their antioxidant properties. Several previous studies postulated that the administration of cinnamaldehyde would decrease oxidative stress, lipid abnormalities and inflammatory markers in the liver and the muscles of fructose-fed rats (Mang *et al.*, 2006; Mahfouz *et al.*, 2009; El-Bassossy *et al.*, 2011). From the study of Kumar *et al.* (2012) it was evident that treatment with cinnamaldehyde and cinnamomum tamala oil significantly reduced the plasma MDA levels and improved the plasma GSH levels on streptozotocin induced diabetes mellitus in rats. Subash babu *et al.* (2014) reported that administration of cinnamaldehyde significantly reduced the generation of lipid peroxides and increased the activities of antioxidant enzymes such as SOD, CAT and GPx in streptozotocin induced diabetic rats. Cinnamic acid and cinnamic-aldehyde, showed cardio protective effects against isoproterenol induced acute ischemic myocardial injury, by improving the SOD and NO levels and decreasing the TNF- α and IL-6 levels in the serum and also reducing the MDA levels in myocardium (Song *et al.*, 2013). Patra *et al.* (2012) demonstrated that cinnamic acid protects mice from cyclophosphamide induced hepato-toxicity and myelo-toxicity by detoxifying the free radicals and thereby preventing the depletion of GSH levels. Sova (2012) reported that many cinnamic acid derivatives, especially those

with the phenolic hydroxyl group, are well known antioxidants and are supposed to have several health benefits due to their strong free radical scavenging properties. Cinnamic acid administration significantly improved the lipid peroxidation in the mice induced by cisplatin as manifested by the decreased MDA level, accompanied by the increased GSH content (Tohamy *et al.*, 2016), which can be attributed to the potential antioxidant effect of cinnamic acid (Dai *et al.*, 2012). On the contrary Gowder and Devaraj (2006) reported time and dose dependent negative effect of cinnamaldehyde on the antioxidant status of rat kidney. Similarly cinnamaldehyde when administered at a daily dose of 73.5 mg/kg b.wt for 90 days, induce prooxidant state (Devaraj *et al.* 1992), glutathione depletion and lipid peroxidation in rat liver (Raveendran *et al.*, 1993).

It is well known that Zn is a powerful antioxidant metal; it is the core constituent of antioxidant enzymes such as SOD and a recognised protector of sulfhydryl groups; it is also thought to impair lipid peroxidation by displacing transition metals such as iron and copper from catalytic sites (Aitken and Roman, 2008). ZnO nano particles are able to protect cell membrane integrity against oxidative stress damage, increase antioxidant enzyme levels, and decrease MDA level (Afifi *et al.*, 2015). It can improve antioxidant activity, enhance the activities of antioxidant enzymes and decrease the levels of free radicals (Badkoobeh *et al.*, 2013). Akhtar *et al.*, (2012) reported that ZnO NPs significantly increased ROS generation and lipid peroxidation, while significantly lowering the GSH concentration and the activity of antioxidant enzymes SOD, CAT and GPx. The essential oil of *Cinnamomum zeylanicum* containing almost 98% cinnamaldehyde, was found to protect against alloxan-induced renal damage in a dose dependent manner (Mishra *et al.*, 2010). Afifi *et al.* (2015) reported that ZnO NPs alone or in combination with insulin improved the antioxidant status of the testicular tissue. ZnO NPs improved the activity and mRNA expression levels of SOD, CAT and GSH and decreased the MDA levels in brain tissue of diabetic rats (Afifi and Abdelazim, 2015).

In the present study, no alterations were observed in plasma glucose and cholesterol, while total protein levels were significantly ($P < 0.05$) decreased in DMBA control and CNMA-T groups, whereas albumin levels were significantly decreased in DMBA-C, CNMA-P, CNMA-T, CZN-T and TAM groups when compared to control. Total bilirubin levels were found increased only in DMBA-C and tamoxifen (TAM) group when compared to normal control.

To know the effect of cinnamaldehyde and nano zinc cinnamaldehyde on the liver and kidney, the functional markers like ALT, AST, ALP, BUN and creatinine, levels were estimated in the plasma. Marked increase in the levels of ALT and AST was observed in DMBA-C group alone, but a significant ($P < 0.05$) raise in ALP levels was noticed in all tumor produced groups when compared to control. Blood urea nitrogen was significantly ($P < 0.05$) increased in DMBA-C, CZN-T and TAM groups while creatinine levels were found increased in DMBA-C and TAM groups when compared to control. Mhammad *et al.*, (2015) reported cinnamon extract significantly improved the kidney and liver functional markers in alloxan induced diabetic rats.

Light microscopic examination of mammary tumors in DMBA control group rats showed different types of morphologies viz. tubular adenocarcinoma, papillary adenocarcinoma, cribriform carcinoma and comedo carcinoma. Histopathological studies revealed the protective effect of various treatments against DMBA induced mammary cancer. Tumors from CNMA-P and CZN-T groups revealed near normal alveolar and ductal structures and most of the alveoli were lined by uniform single layer of epithelial cells. Apart from this increased number of alveoli and some alveoli were found dilated in tumors from rats treated with nano zinc cinnamaldehyde. Whereas the CNMA-T group mammary tumors was almost similar to DMBA control group. However, under microscopic examination tamoxifen treated rats showed favourable regressive changes like desquamation of epithelium, presence of multinucleated cells,

and connective tissue proliferation to a higher extent and most of the alveoli were lined by a single epithelial layer. The ultra structure of cancerous tissue from DMBA-C rats revealed nuclear pleomorphism and nuclear indentation. Transmission electron microscopy of tumor tissues from different protective treatment groups showed presence of cytoplasmic vesicles/vacuoles and pleomorphic nuclei (CNMA-P), fibrous tissue proliferation, presence of perinuclear vesicles and fragmentation of nuclei (CNMA-T and CZN-T), ultra sections of rat tumors from tamoxifen treated rats revealed connective tissue proliferation, existence of cytoplasmic vacuoles and pleomorphic cells with enlarged nuclei. Immunohistochemical analysis of tumor tissues exposed the presence of estrogen and progesterone receptors.

Mammary gland tumors of rodent models are estrogen dependent, aggressive and locally invasive and also similar to most frequently diagnosed breast cancer in women in terms of tumor histology and hormone dependence (Gullino *et al.*, 1975; Masso-Welch *et al.*, 2000; Russo and Russo, 2000). Rat mammary tumors arise in the epithelium of the terminal end buds, which are comparable structures to the terminal ductal lobular units in the human breast (Grubbs *et al.*, 1986). According to Russo *et al.* (1990) most of highly malignant tumors in rats show some common features with intraductal and infiltrating ductal carcinomas in humans. According to Clarke (1997) most of the lesions found in rats have corresponding lesions in humans, allowing the translation of basic research in rats into the clinic.

Depending on the state of development of the mammary tissue at the time of initiation, multiple histological pathways of tumorigenesis can be identified (Russo and Russo, 1987). Carcinogen exposure to mammary lobules results in the lobular hyperplasia. Less mature mammary tissues containing alveolar buds progress to hyperplastic alveolar nodules and tubular adenomas, a form of non-invasive mammary pathology. Immature mammary tissues containing terminal end buds progress to

intraductal hyperplasia (IDP), carcinoma in situ (CIS) and finally invasive carcinoma (INV). Because rodents have dissynchronous mammary development, more than one pathway may be identified in an individual animal after carcinogen exposure (Heffeling *et al.*, 2000).

From the study of Currier *et al.* (2005) on FVB mouse the most common histologies of the DMBA- induced mammary tumors were squamous or adenosquamous carcinomas, scirrhous tubular carcinoma, spindle cell tumor and papillary carcinoma. Apoptosis is a type of cell death and agents with the ability to induce apoptosis in tumors have the potential to be used for anti-tumor therapy. Apoptosis inducing activities of flavonoids have been identified in several previous studies (Chen *et al.*, 2003; Shen *et al.*, 2003). Previous studies also reported apoptotic activity for cinnamaldehyde (Ka *et al.*, 2003, Huang *et al.*, 2007, Lin *et al.*, 2013) and cinnamic acid (de Oliveira Niero and Machado-Santelli, 2013; Qi *et al.*, 2016). Debnath *et al.* (2002) opined that apart from autophagic death and para-apoptosis cytoplasmic vacuolation is a characteristic of alternative death process. The study by Ouhtit *et al.* (2014) and Chang *et al.* (2017), on rat mammary tumors, revealed ultra structural changes like desquamation of the mammary epithelia, pyknotic nuclei, marked peripheral nuclear chromatin condensation, cytoplasmic vacuolation in breast tumors upon dietary treatment with spirulina and *Kappaphycus alvarezii*. Similar protective ultra structural findings were also reported by Amin *et al.* (2005) with *Trigonella foenum graecum* in rats bearing DMBA induced mammary tumors. Amin *et al.*, (2005) reported that expression of PR was less evident and restricted to ductal side of the branching cells in DMBA induced rat mammary tumor.

Prophylactic treatment with cinnamaldehyde decreased the risk of breast tumor occurrence and also reduced the severity in terms of tumor number and size. Mammary tumor histology also revealed favourable alterations in rats received cinnamaldehyde

prophylactically. Therapeutic treatment with cinnamaldehyde and its nano formulation reduced the tumor progression. Histologically, tumors from nano zinc cinnamaldehyde treated rats showed more favourable regressive changes when compared to cinnamaldehyde treated rats. From the study it was evident that, though treatment with cinnamaldehyde and its nano preparation showed favourable results, cinnamaldehyde can reduce the breast cancer incidence if given prior to DMBA administration for tumor induction.

CHAPTER VI

SUMMARY

Breast cancer is the most common cancer affecting women which accounts for highest morbidity and mortality. Although the chemotherapeutic agents are effective on the tumor cells, their toxicity to normal tissues and development of drug resistance remains the major obstacles for clinical use. *trans*- Cinnamaldehyde, also known as cinnamaldehyde, is the major component of cinnamon bark oil, with sweet taste and yellowish appearance. Nanotechnology has prompted new and improved materials for biomedical applications with particular emphasis in therapy and diagnostics.

Hence the study was planned to know the anticancer potential of cinnamaldehyde and its nano zinc preparation against chemically induced mammary cancer in Sprague-Dawley rats. In addition cinnamaldehyde disposition kinetics and acute oral toxicity studies of both the test compounds were also conducted.

Acute oral toxicity studies were conducted as per OECD-425 guidelines. Rats dosed with cinnamaldehyde and nano zinc cinnamaldehyde at the rate of 2000 mg/kg b.wt., showed no morbidity and mortality during the 14 day study period. Though body weights were found decreased in both treatment groups, the change was not significant when compared to control. Further no change was observed in relative organ weights and haematology. However, plasma biochemistry revealed significant ($P < 0.05$) elevation in the levels of BUN, AST and ALP of nano zinc cinnamaldehyde

treated rats when compared to control. Light microscopic examination revealed pathological changes like congestion and degenerative changes in liver, congestion and haemorrhage in heart, mild degenerative changes in stomach and kidney and congestion in brain of rats treated with both compounds.

Plasma concentration and other pharmacokinetic parameters were calculated for cinnamaldehyde (CNMA) and cinnamic acid (CA) in rats administered with cinnamaldehyde at a dose rate of 500 mg/kg b.wt by nancompartmental modelling. The AUC_{0-t} values obtained in this study for CNMA and CA were 28.13 ± 1.74 and 15588.9 ± 869.325 $\mu\text{g}\cdot\text{min}/\text{ml}$ respectively. Mean MRT (min.) and clearance ($\mu\text{g}/\text{ml}/\text{min}$) values of CNMA were 123.373 ± 5.607 and 18.04 ± 1.08 respectively. Apparent volume of distribution and elimination rate constant obtained for CNMA were 2206.35 ± 84.13 $\mu\text{g}/\text{ml}/\text{min}$ and 0.00818 ± 0.00039 min^{-1} . The mean half life ($t_{1/2}$) values were found 85.49 ± 3.88 minutes for cinnamaldehyde and 52.47 ± 0.95 minutes for cinnamic acid. The experiment indicates that most of the cinnamaldehyde was converted into cinnamic acid before entering into the systemic circulation as evidenced from the plasma concentration of cinnamic acid. Rather than relocation of CNMA from the plasma in to peripheral compartments, increased conversion of CNMA to CA in central compartment results in decreased cinnamic acid volume of distribution. This is supported by the high value of clearance, which normally should reduce the volume of distribution. Moreover, the conversion of CNMA to CA appears to be reversible to some extent, as seen in the abrupt rise and fall of the plasma concentrations over time, which may another contributory factor. It can also be noted that though the clearance is high for CNMA, the $t_{1/2}$ and MRT are less, indicating a back conversion of cinnamic acid to cinnamaldehyde.

Anticarcinogenic effect of cinnamaldehyde and nano zinc cinnamaldehyde were studied against DMBA induced rat mammary cancer. It was found that prophylactic treatment with cinnamaldehyde, increased the time required to develop tumor after

DMBA administration. Tumor volume, tumor burden, tumor multiplicity and decrease in animal body weights were found reduced in different cinnamaldehyde and nano zinc cinnamaldehyde administered groups. The tumorigenicity marker, total sialic acid levels were found significantly lowered in different groups of protected rats with a highest reduction in cinnamaldehyde prophylactic group.

To know the effect of cinnamaldehyde and nano zinc cinnamaldehyde on antioxidant and peroxidation status SOD, GSH and TBARS levels were measured in liver, lung, heart, kidney, brain, mammary gland tissues and plasma. SOD levels were significantly ($P < 0.05$) lowered in liver, heart, kidney, mammary gland and plasma of DMBA control group. Improved SOD levels were observed in liver, kidney and plasma of CNMA-P, CNMA-T and CZN-T group rats. Heart SOD levels in CNMA-T, CZN-T and TAM groups were comparable to DMBA-C. Glutathione (GSH) levels in different organs were found decreased in all tumor induced groups but, the decrease was not significant in heart and brain tissue of CNMA-P group and brain tissue of CNMA-T group. TBARS levels in liver, heart, brain and plasma were significantly ($P < 0.05$) increased in all DMBA administered groups when compared to normal control. Whereas improved peroxidation levels of lung and kidney homogenates were found only in rats received cinnamaldehyde prophylactically. Mammary gland TBARS levels were increased significantly ($P < 0.05$) in DMBA- C and TAM groups when compared to control.

The plasma biochemical analysis revealed that the liver functional markers like ALT and AST was significantly increased in tumor control group, while ALP levels was significantly ($P < 0.05$) increased in all DMBA administered groups when compared to normal control. The kidney functional marker BUN was significantly increased in DMBA-C, CZN-T and TAM group whereas, creatinine levels were found increased in DMBA-C and TAM groups when compared to control group (C). Total

protein levels were significantly decreased in DMBA-C and CNMA-T groups when compared to control. Albumin levels were significantly decreased in all DMBA treated groups. Total bilirubin levels were significantly elevated only in DMBA-C and TAM group.

It was found that DMBA administration induced different types of mammary tumors characterized by proliferation of epithelial cells, invasion of neoplastic cells in to the stroma, alveoli lined with multilayered cells, growth of neoplastic cells in to the lumen with different morphologies like tubular adenocarcinoma, papillary adenocarcinoma, cribriform carcinoma and comedo carcinoma. However tumor bearing rats treated with tamoxifen showed greater regressive changes when compared to cinnamaldehyde prophylactic and nano zinc cinnamaldehyde treated rats. But these regressive changes were found minimum in tumor bearing rats that received cinnamaldehyde as treatment and almost resembled DMBA-C group tumor histology. Transmission electron microscopy study of tumors from protected rats revealed different changes viz. connective tissue proliferation, cytoplasmic vacuolation, cellular and nuclear pleomorphism. Immunohistochemistry of DMBA induced mammary tumors revealed the presence of estrogen and progesterone receptors.

Hence it can be concluded from the study that, of the three treatment protocols, prophylactic administration of cinnamaldehyde produced most favourable results followed by nano zinc cinnamaldehyde treatment and cinnamaldehyde treatment

- Abbadessa G, Spaccamiglio A, Sartori M L, Nebbia C, Dacasto M, Di Carlo F and Racca S (2006) The aspirin metabolite, salicylate, inhibits 7, 12-dimethylbenz[a]anthracene-DNA adduct formation in breast cancer cells. *International Journal of Oncology* 28 : 1131–1140.
- Abd El-Aziz M A, Hassan H A, Mohamed M H, Meki A M A, Abdel-Ghaffar S K H and Hussein M R (2005) The biochemical and morphological alterations following administration of melatonin, retinoic acid and *Nigella sativa* in mammary carcinoma: an animal model. *International Journal of Experimental Pathology* 86 : 383-396.
- Abdullaev F I (2001) Plant-derived agents against cancer. pp 345-354, In *Pharmacology and therapeutics in the new millennium* (ed S K Gupta) Narosa Publishing House, New Delhi, India.
- Abdullaev F I (2002) Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). *Experimental Biology and Medicine* 227 : 20-25.
- Abercrombie M and Ambrose E J (1962) The surface properties of cancer cells: a review. *Cancer Research* 22 : 525-48.
- Afifi M and Abdelazim A M (2015) Ameliorative effect of zinc oxide and silver nanoparticles on antioxidant system in the brain of diabetic rats. *Asian Pacific Journal of Tropical Biomedicine* 5 : 874-877.
- Afifi M, Almaghrabi O A and Kadasa N M (2015) Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin-induced diabetic rat testes. *BioMed Research International* ID 153573: 1-6.
- Aggarwal B B and Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. *Biochemical Pharmacology* 71 : 1397-1421.
- Ahamed M, Akhtar M J, Raja M, Ahmad I, Siddiqui M K J, AlSalhi M S and Alrokayan S A (2011) ZnO nanorod-induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: role of oxidative stress. *Nanomedicine* 7 : 904-913.
- Ahamed M, Kams M, Goodson M, Rowe J, Hussain S M, Schlager J J and Hong Y (2008) DNA damage response to different surface chemistry of silver

nanoparticles in mammalian cells. *Toxicology and Applied Pharmacology* 233 : 404-410

Aitken R J and Roman S D (2008) Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity* 1 : 15-24.

Akhtar M J, Ahamed M, Kumar S, Khan M M, Ahmad J and Alrokayan S A (2012) Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. *International Journal of Nanomedicine* 7 : 845.

Ali S M, Khan A A, Ahmed I, Musaddiq M, Ahmed K S, Polasa H, Rao L V, Habibullah C M, Sechi L A and Ahmed N (2005) Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. *Annals of Clinical Microbiology and Antimicrobials* 4 : 20.

Allred D C, Brown P and Medina D (2004) The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer. *Breast Cancer Research* 6 : 240.

Al-Rimawi F, Rishmawi S, Ariqat S H, Khalid M F, Warad I and Salah Z (2016) Anticancer Activity, Antioxidant Activity, and Phenolic and Flavonoids Content of Wild *Tragopogon porrifolius* Plant Extracts. *Evidence-Based Complementary and Alternative Medicine* ID 9612490 : 1-7.

Amaral S, Mira L, Nogueira J M F, da Silva A P and Florencio M H (2009) Plant extracts with anti-inflammatory properties-A new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. *Bioorganic and Medicinal Chemistry* 17 : 1876–1883.

Amati B and Land H (1994) Myc-Max-Mad: A transcription factor network controlling cell cycle progression, differentiation and death. *Current Opinion in Genetics and Development* 4: 102-108.

Ames B N and Gold L S (1992) Animal cancer tests and cancer prevention. *Journal of the National Cancer Institute Monographs* 12 : 125-132.

Amin A, Alkaabi A, Al-Falasi S and Daoud S A (2005) Chemopreventive activities of *Trigonella foenum graecum* (Fenugreek) against breast cancer. *Cell Biology International* 29 : 687-94.

- Ankamwar B, Damle C, Ahmad A and Sastry M (2005) Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. *Journal of Nanoscience and Nanotechnology* 5 :1665-1671.
- Arroyo-Acevedo J, Chávez-Asmat R J, Anampa-Guzmán A, Donaires R and Ráez-González J (2015) Protective effect of *Piper aduncum* capsule on DMBA-induced breast cancer in rats. *Breast Cancer: Basic and Clinical Research* 9 : 41.
- Aruoma O I (1994) Nutrition and health aspects of free radicals and antioxidants. *Food and Chemical Toxicology* 32 : 671-685.
- Babu P S, Prabuseenivasan S and Ignacimuthu S (2007) Cinnamaldehyde - a potential antidiabetic agent. *Phytomedicine* 14 : 15-22.
- Badkoobeh P, Parivar K, Kalantar S M, Hosseini S D and Salabat A (2013) Effect of nano-zinc oxide on doxorubicin-induced oxidative stress and sperm disorders in adult male Wistar rats. *Iranian Journal of Reproductive Medicine* 11 : 355-364.
- Balasubramanian K A, Manohar M and Mathan V I (1988) An unidentified inhibitor of lipid peroxidation in intestinal mucosa. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism* 962 : 51-58.
- Barile F A (2010) Carcinogenicity and mutagenicity testing *in vivo*. pp 144, In *Clinical toxicology: principles and mechanisms*, 2nd edition, New York, London: Informa Healthcare Publishers.
- Barreiro E, de la Puente B, Busquets S, López-Soriano F J, Gea J and Argilés J M (2005) Both oxidative and nitrosative stress are associated with muscle wasting in tumour-bearing rats. *FEBS Letters* 579 : 1646-1652.
- Barros A C, Muranaka E N, Mori L J, Pelizon C H, Iriya K, Giocondo G and Pinotti J A (2004) Induction of experimental mammary carcinogenesis in rats with 7, 12-dimethylbenz (a) anthracene. *Revista do Hospital das Clínicas* 59 : 257-61.
- Bagchi M, Moriyama H and Shahidi F (2012) *Bio-Nanotechnology: A revolution in food, biomedical and health science* (ed D Bagchi) John Wiley & Sons.
- Beato M and Sánchez-Pacheco A (1996) Interaction of steroid hormone receptors with the transcription initiation complex. *Endocrine Reviews* 17 : 587-609.

- Bener A, Ayub H, Kakail R and Ibrahim W (2008) Patterns of cancer incidence among the population of Qatar: a worldwide comparative study. *Asian Pacific Journal of Cancer Prevention* 9 : 19-24.
- Bhat K P, Lantvit D, Christov K, Mehta R G, Moon R C and Pezzuto J M (2001). Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. *Cancer Research* 61 : 7456-7463.
- Bhuvaneswari V and Nagini S (2005) Lycopene: a review of its potential as an anticancer agent. *Current Medicinal Chemistry-Anticancer Agents* 5 : 627-635.
- Black D M (1994) The genetics of breast cancer. *European Journal of Cancer* 30 : 1957-1961.
- Boshra S A and Hussein M A (2016) Cranberry extract as a supplemented food in treatment of oxidative stress and breast cancer induced by n-methyl-n-nitrosourea in female virgin rats. *International Journal of Phytomedicine* 19 : 217-27.
- Boyd M T, Hildebrandt R H and Bartow S A (1996) Expression of the estrogen receptor gene in developing and adult human breast. *Breast Cancer Research and Treatment* 37 : 243-251.
- Bozzola J J and Russell L D (1999) pp 670, In *Electron Microscopy: Principles and Techniques for Biologist*, Jones and Bartlett Learning, Sudbury, Massachusetts.
- Bray F, Jemal A, Grey N, Ferlay J and Forman D (2012) Global cancer transitions according to the human development index (2008–2030): A population-based study. *The Lancet Oncology* 13 : 790-801.
- Brunner N, Bronzert D, Vindelov L L, Rygaard K, Spang-Thomsen M and Lippman M E (1989) Effect on growth and cell cycle kinetics of estradiol and tamoxifen on MCF-7 human breast cancer cells grown in vitro and in nude mice. *Cancer Research* 49 : 1515-1520.
- Cabello C M, Bair W B, Lamore S D, Ley S, Bause A S, Azimian S and Wondrak G T (2009) The cinnamon-derived Michael acceptor cinnamic aldehyde impairs melanoma cell proliferation, invasiveness, and tumor growth. *Free Radical Biology and Medicine* 46 : 220-231.

- Carthew P, Lee P N, Edwards R E, Heydon R T, Nolan B M and Martin E A (2001) Cumulative exposure to Tamoxifen: DNA adducts and liver cancer in the rat. *Archives of Toxicology* 75 : 375-380.
- Cato A C, Nestl A and Mink S (2002) Rapid actions of steroid receptors in cellular signaling pathways. *Sciences STKE*. 138: 1-11.
- Cerutti P A (1985) Prooxidant states and tumor promotion. *Science* 227 : 375-381.
- Chandran S P, Chaudhary M, Pasricha R, Ahmad A and Sastry M (2006) Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnology Progress*. 22 : 577-583.
- Chang S T, Chen P F and Chang S C (2001) Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. *Journal of Ethnopharmacology* 77 : 123-127.
- Chang V S, Okechukwu P N and Teo S S (2017) The properties of red seaweed (*Kappaphycus alvarezii*) and its effect on mammary carcinogenesis. *Biomedicine & Pharmacotherapy* 87 : 296-301.
- Chao L K, Chang W T, Shih Y W and Huang J S (2010) Cinnamaldehyde impairs high glucose-induced hypertrophy in renal interstitial fibroblasts. *Toxicology and Applied Pharmacology* 244 : 174-180.
- Chao L K, Hua K F, Hsu H Y, Cheng S S, Lin I F, Chen C J, Chen S T, Chang S T (2008) Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. *Food and Chemical Toxicology* 46 : 220-231.
- Chen Y C, Shen S C and Lin H Y (2003) Rutin at C7 attenuates the apoptosis inducing activity of flavonoids. *Biochemical Pharmacology* 66 : 1139-1150.
- Chen Y F, Wang Y W, Huang W S, Lee M M, Wood W G, Leung Y M and Tsai H Y (2016) Trans-cinnamaldehyde, an essential oil in cinnamon powder, ameliorates cerebral ischemia-induced brain injury via inhibition of neuroinflammation through attenuation of iNOS, COX-2 expression and NF κ -B signaling pathway. *Neuromolecular Medicine* 18 : 322-333.

- Cheung C, Hotchkiss S A, Pease C K (2003) Cinnamic compound metabolism in human skin and the role metabolism may play in determining relative sensitisation potency. *Journal of Dermatological Science* 31 : 9-19.
- Chew E H, Nagle A A, Zhang Y, Scarmagnani S, Palaniappan P, Bradshaw T D, Holmgren A and Westwell A D (2010) Cinnamaldehydes inhibit thioredoxin reductase and induce Nrf2: potential candidates for cancer therapy and chemoprevention. *Free Radical Biology and Medicine* 48 : 98-111.
- Cho K, Wang X U, Nie S and Shin D M (2008) Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research* 14 : 1310-1316.
- Cho W S, Kang B C, Lee J K, Jeong J, Che J H and Seok S H (2013) Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. *Particle and Fibre Toxicology* 10 : 9.
- Chougule M, Patel A R, Sachdeva P, Jackson T and Singh M (2011) Anticancer activity of Noscapine, an opioid alkaloid in combination with Cisplatin in human non-small cell lung cancer. *Lung Cancer* 71 : 271-282.
- Clarke R (1997) Issues in experimental design and endpoint analysis in the study of experimental cytotoxic agents in vivo in breast cancer and other models. *Breast Cancer Research and Treatment* 46 : 255-278.
- Cocchiara J, Letizia C S, Lalko J, Lapczynski A and Api A M (2005) Fragrance material review on cinnamaldehyde. *Food and Chemical Toxicology* 43 : 867-923.
- Correale M, Abbate I, Gargano G, Catino A, Musci M D, Dragone C D and De Lena M (1992) The determination of sialic acid in cancer patients. *Minerva Medica* 83 : 427-431.
- Cummings S R, Tice J A, Bauer S, Browner W S, Cuzick J, Ziv E, Vogel V, Shepherd J, Vachon C, Smith-Bindman R and Kerlikowske K (2009) Prevention of breast cancer in postmenopausal women: approaches to estimating and reducing risk. *Journal of the National Cancer Institute* 101 : 384-398.

- Currier N, Solomon S E, Demicco E G, Chang D L, Farago M, Ying H, Dominguez I, Sonenshein G E, Cardiff R D, Xiao Z X and Sherr D H (2005) Oncogenic signaling pathways activated in DMBA-induced mouse mammary tumors. *Toxicologic Pathology* 33 : 726-737.
- Dadhich M, Prabhu V, Pai V R, D'Souza J, Harish S and Jose M (2014) Serum and salivary sialic acid as a biomarker in oral potentially malignant disorders and oral cancer. *Indian Journal of Cancer* 51 : 214.
- Dai A H, Nie Y X, Yu B, Li Q, Lu L Y and Bai J G (2012) Cinnamic acid pretreatment enhances heat tolerance of cucumber leaves through modulating antioxidant enzyme activity. *Environmental and Experimental Botany* 79 : 1-10.
- Dai J and Mumper R J (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15 : 7313-7352.
- Damianaki A, Bakogeorgou E, Kampa M, Notas G, Hatzoglou A, Panagiotou S, Gemetzi C, Kouroumalis E, Martin P M and Castanas E (2000) Potent inhibitory action of red wine polyphenols on human breast cancer cells. *Journal of Cellular Biochemistry*. 78 : 429-441.
- Debersac P, Heydel J M, Amiot M J, Goudonnet H, Artur Y, Suschetet M and Siess M H (2001) Induction of cytochrome P450 and/or detoxication enzymes by various extracts of rosemary: description of specific patterns. *Food AND Chemical Toxicology* 39 : 907-918.
- De Jong W H and Borm P J A (2008) Drug delivery and nanoparticles: Applications and hazards. *International Journal of Nano Medicine* 3 : 133-149.
- de Oliveira Niero E L and Machado-Santelli G M (2013) Cinnamic acid induces apoptotic cell death and cytoskeleton disruption in human melanoma cells. *Journal of Experimental & Clinical Cancer Research* 32 : 31.
- Debnath J, Mills K R, Collins N L, Reginato M J, Muthuswamy S K and Brugge J S (2002) The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini. *Cell* 111 : 29-40.
- Deng Y, Liao Q, Li S, Bi K, Pan B and Xie Z (2008) Simultaneous determination of berberine, palmatine and jatrorrhizine by liquid chromatography–tandem mass

spectrometry in rat plasma and its application in a pharmacokinetic study after oral administration of coptis–evodia herb couple. *Journal of Chromatography B* 863 : 195-205.

Deorukhkar A, Krishnan S, Sethi G and Aggarwal B B (2007) Back to the basics: how natural products can provide the basis for new therapeutics. *Expert Opinion on Investigational Drugs* 16 : 1753-1773.

Devaraj H, Niranjali S and Raveendran M (1992) Effect of food flavor cinnamaldehyde on liver microsomal cytochrome P-450 in rats. *Bulletin of Environmental Contamination and Toxicology* 49 : 306-311.

Dewanto V, Wu X, Adom K K and Liu R H (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* 50 : 3010-3014.

Dias M F, Sousa E, Cabrita S, Patricio J and Oliveria C F (2000) Chemoprevention of DMBA - induced mammary tumors in rats by a combined regimen of alpha - tocopherol, selenium and ascorbic acid. *The Breast Journal* 16 : 14-19.

Dias M, Cabrita S, Sousa E, Franca B, Patricio J and Oliveira C F (1999) Benign and malignant mammary tumors induced by DMBA in female Wistar rats. *European Journal of Gynaecol Oncology* 20 : 285-288.

Ding H, Liu Y, Zeng Z, Si H, Liu K, Liu Y, Yang F, Li Y and Zeng D (2012) Pharmacokinetics of mequindox and one of its major metabolites in chickens after intravenous, intramuscular and oral administration. *Research in Veterinary Science* 93 : 374-377.

Ding Y, Wang S, Qiu L, Zhao G, Xu J and Wang S (2010) Influence of cinnamaldehyde on viral myocarditis in mice. *The American Journal of Medical Sciences* 340 : 114-120.

Dipple A, Moschel R C and Bigger C A H (1984) Polynuclear aromatic carcinogens. pp 41-163, In *Chemical Carcinogens* (ed C E Searle). ACS monograph, American Chemical Society, Washington, D.C.

- Doisneau-Sixou S F, Sergio C M, Carroll J S, Hui R, Musgrove E A and Sutherland R L (2003) Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. *Endocrine-Related Cancer* 10 : 179-186.
- Domadia P, Swarup S, Bhunia A, Sivaraman J and Dasgupta D (2007) Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. *Biochemical Pharmacology* 74 : 831-840.
- Dragland S, Senoo H, Wake K, Holte K and Blomhoff R (2003) Several culinary and medicinal herbs are important sources of dietary antioxidants. *The Journal of Nutrition* 133 : 1286–1290.
- Dubik D and Shiu R P (1992) Mechanism of estrogen activation of *c-myc* oncogene expression. *Oncogene* 7 : 1587-1594.
- Duessel S, Heuertz R M and Ezekiel U R (2008) Growth inhibition of human colon cancer cells by plant compounds. *Clinical Laboratory Science* 21 : 151-157.
- Duffy M J (2013) Tumor markers in clinical practice: a review focusing on common solid cancers. *Medical Principles and Practice* 22 : 4-11.
- Dugoua J J, Seely D, Perri D, Cooley K, Forelli T, Mills E and Koren G (2007) From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark This article is one of a selection of papers published in this special issue (part 1 of 2) on the Safety and Efficacy of Natural Health Products. *Canadian Journal of Physiology and Pharmacology* 85: 837-847.
- Earnshaw W C (1995) Nuclear changes in apoptosis. *Current Opinion in Cell Biology* 7 : 337-343.
- Edenharder R, Frangart J and Hager M (1998) Protective effects of fruits and vegetables against in vivo clastogenicity of cyclophosphamide or benzo(α)pyrene in mice. *Food and Chemical Toxicology* 36 : 637-645.
- El Hajj M S and Hamid Y (2011) Breast cancer health promotion in Qatar: a survey of community pharmacists interests and needs. *International Journal of Clinical Pharmacy* 33 : 70-79.

- El-Bassossy H M, Fahmy A and Badawy D (2011) Cinnamaldehyde protects from the hypertension associated with diabetes. *Food and Chemical Toxicology*. 49: 3007-3012.
- Elegbede J A, Elson C E, Qureshi A, Tanner M A and Gould M N (1984) Inhibition of DMBA induced mammary cancer by the monoterpene d-limonene. *Carcinogenesis* 5 : 661-664.
- Fang S H, Rao Y K and Tzeng Y M (2004) Cytotoxic effect of trans-cinnamaldehyde from *Cinnamomum osmophloeum* leaves on Human cancer cell lines. *International Journal of Applied Science and Engineering* 2 : 136-47.
- Farnebo M, Bykov V J and Wiman K G (2010) The p53 tumor suppressor: a master regulator of diverse cellular processes and therapeutic target in cancer. *Biochemical and Biophysical Research Communications* 396 : 85-89.
- Fenk C J, Kaufman N and Gerbig Jr D G (2007) A new colorimetric assay of tabletop sweeteners using a modified biuret reagent. *Journal of Chemical Education* 84 : 1676.
- Fisher B and Costantino J P (2006) RESPONSE Re: Tamoxifen for the prevention of breast cancer: Current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *Journal of the National Cancer Institute* 98 : 643-644.
- Foti M C, Daquino C and Geraci C (2004) Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH• radical in alcoholic solutions. *The Journal of Organic Chemistry* 69 : 2309-2314.
- Gauthami M, Srinivasan N, M Goud N, Boopalan K and Thirumurugan K (2015) Synthesis of silver nanoparticles using *Cinnamomum zeylanicum* bark extract and its antioxidant activity. *Nanoscience & Nanotechnology-Asia* 5 : 2-7.
- Gerloff K, Albrecht C, Boots A W, Forster I and Schins R P (2009) Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. *Nanotoxicology* 3 : 355-364.
- Gill A O and Holley R A (2004) Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. *Applied and Environmental Microbiology* 70 : 5750-5755.

- Giri U, Sharma S D, Abdulla M and Athar M (1995) Evidence that *in situ* generated reactive oxygen species act as a potent stage-I tumor promoter in mouse skin. *Biochemical and Biophysical Research Communications* 209 : 698-705.
- Gledhill BL and Mauro F (1991) *New horizons in biological dosimetry*. Wiley-Liss., New York pp. 373-386.
- Gowder S J and Devaraj H (2006) Effect of the food flavour cinnamaldehyde on the antioxidant status of rat kidney. *Basic & Clinical Pharmacology & Toxicology* 99 : 379-382.
- Grubbs C J, Juliana M M, Hill D L and Whitaker L M (1986) Suppression by pregnancy of chemically induced preneoplastic cells of the rat mammary gland. *Anticancer Research* 6 : 1395-1400.
- Guilford J M and Pezzuto J M (2008) Natural products as inhibitors of carcinogenesis. *Expert Opinion on Investigational Drugs* 17 : 1341-1352.
- Gullino P M, Pettigrew H M and Grantham F H (1975) N-nitrosomethylurea as mammary gland carcinogen in rats. *Journal of the National Cancer Institute* 54 : 401-414.
- Gutzeit H O and Ludwig-Müller J (2014) *Plant Natural Products: Synthesis, Biological Functions and Practical Applications*. John Wiley & Sons.
- Guyton K Z and Kensler T W (1993) Oxidative mechanisms in carcinogenesis. *British Medical Bulletin* 49 : 523-544.
- Haag J D, Lindstrom M J and Gould M N (1992) Limonene-induced regression of mammary carcinomas. *Cancer Research* 52 : 4021-4026.
- Halliwell B (1994) Free radicals, antioxidants and human disease: curiosity, cause or consequence? *Lancet* 344 : 721-724.
- Halliwell B (1996) Antioxidants in human health and disease. *Annual Review of Nutrition* 16 : 33-50.
- Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology* 141 : 312-322.

- Hamdy S M, Latif A K, Drees E A and Soliman S M (2012) Prevention of rat breast cancer by genistin and selenium. *Toxicology and Industrial Health* 28 : 746–757.
- Han D C, Lee M Y, Shin K D, Jeon S B, Kim J M, Son K H, Kim H C, Kim H M and Kwon B M (2004) 2'-benzoyloxycinnamaldehyde induces apoptosis in human carcinoma via reactive oxygen species. *Journal of Biological Chemistry* 279 : 6911-6920.
- Han X and Liehr J G (1994) 8-Hydroxylation of guanine bases in kidney and liver DNA of hamsters treated with estradiol: role of free radicals in estrogen-induced carcinogenesis. *Cancer Research* 54 : 5515-5517.
- Hanahan D and Weinberg R A (2011) Hallmarks of cancer: the next generation. *Cell* 144 : 646-674.
- Hanley C, Layne J, Punnoose A, Reddy K M, Coombs I, Coombs A, Feris K and Wingett D (2008) Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles. *Nanotechnology* 19 : 295103.
- Haran E F, Maretzek A F, Goldberg I, Horowitz A and Degani H (1994) Tamoxifen enhances cell death in implanted MCF7 breast cancer by inhibiting endothelium growth. *Cancer Research* 54 : 5511-5514.
- Harris T J, von Maltzahn G and Bhatia S N (2006) Multifunctional nanoparticles for cancer therapy. pp 59-75, In *Nanotechnology for Cancer Therapy*.
- Hassan M, Watari H, AbuAlmaaty A, Ohba Y and Sakuragi N (2014) Apoptosis and molecular targeting therapy in cancer. *BioMed Research International* ID 150845 : 1-23.
- Hayashi K, Imanishi N, Kashiwayama Y, Kawano A, Terasawa K, Shimada Y and Ochiai H (2007) Inhibitory effect of cinnamaldehyde, derived from Cinnamomi cortex, on the growth of influenza A/PR/8 virus in vitro and in vivo. *Antiviral Research* 74 : 1-8.
- Hebert C D, Yuan J and Dieter M P (1994) Comparison of the toxicity of cinnamaldehyde when administered by microencapsulation in feed or by corn oil gavage. *Food and Chemical Toxicology* 32 : 1107-1115.

- Heffelfinger S C, Gear R B, Taylor K, Miller M A, Schneider J, LaDow K, and Warshawsky D (2000) DMBA-induced mammary pathologies are angiogenic in vivo and in vitro. *Laboratory Investigation* 80 : 485-492.
- Heng B C, Zhao X, Xiong S, Ng K W, Boey F Y and Loo J S (2010) Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress. *Food and Chemical Toxicology* 48 : 1762-1766.
- Hollingsworth A B, Lerner M R, Lightfoot S A, Wilkerson K B, Hanas J S, McCay P B and Brackett D J (1998) Prevention of DMBA-induced rat mammary carcinomas comparing leuprolide, oophorectomy, and tamoxifen. *Breast Cancer Research and Treatment* 47 : 63-70.
- Hollman P C and Katan M B (1999) Dietary flavonoids: intake, health effects and bioavailability. *Food and Chemical Toxicology* 37 : 937-942.
- Hong J S, Park M K, Kim M S, Lim J H, Park G J, Maeng E H, Shin J H, Kim M K, Jeong J, Park J A and Kim J C (2014) Prenatal development toxicity study of zinc oxide nanoparticles in rats. *International Journal of Nanomedicine* 9 : 159.
- Hooth M J, Sills R C, Burka L T, Haseman J K, Witt K L, Orzech D P, Fuciarelli A F, Graves S W, Johnson J D and Bucher J R (2004) Toxicology and carcinogenesis studies of microencapsulated trans-cinnamaldehyde in rats and mice. *Food and Chemical Toxicology* 42 : 1757-1768.
- Howe L R and Dannenberg A J (2002) A role for cyclooxygenase-2 inhibitors in the prevention and treatment of cancer. In *Seminars in Oncology* 29 : 111-119
- Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes J F, Hocht-Boes G, Houghton J and Locker G Y (2005) Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years adjuvant treatment for breast cancer. *Lancet* 365 : 60-62.
- Huang J, Wang S, Luo X, Xie Y and Shi X (2007) Cinnamaldehyde reduction of platelet aggregation and thrombosis in rodents. *Thrombosis Research* 119 : 337-342.
- Hughes G A (2005) Nanostructure - mediated drug delivery. *Nanomedicine* 1 : 22-30.

- Hussein M R (2005) Ultraviolet radiation and skin cancer: molecular mechanisms. *Journal of Cutaneous Pathology* 32 : 191-205.
- Imai T, Yasuhara K, Tamura T, Takizawa T, Ueda M, Hirose M and Mitsumori K (2002) Inhibitory effects of cinnamaldehyde on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung carcinogenesis in rasH2 mice. *Cancer Letters* 175 : 9-16.
- Iravani S (2011) Green synthesis of metal nanoparticles using plants. *Green Chemistry*. 13 : 2638-2650.
- Jang T J and Kim D K (2002) Inducible nitric oxide synthase expression of tumor and stromal cells is associated with the progression of 7,12-dimethylbenz[a]anthracene-induced rat mammary tumors. *Cancer Letters* 182 : 121–126
- Jayakumar R, Kanthimathi M S (2012) Dietary spices protect against hydrogen peroxide-induced DNA damage and inhibit nicotine-induced cancer cell migration. *Food Chemistry* 134 : 1580-1584.
- Jemal A, Bray F, Center M M, Ferlay J, Ward E and Forman D (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians* 61 : 69-90.
- Jenner P M, Hagan E C, Taylor J M, Cook E L and Fitzhugh O G (1964) Food flavourings and compounds of related structure I. Acute oral toxicity. *Food and Cosmetics Toxicology* 2 : 327-43.
- Jensen E V and Jordan V C (2003) The estrogen receptor. *Clinical Cancer Research*. 9 : 1980-1989.
- Jeong H W, Han D C, Son K H, Han M Y, Lim J S, Ha J H, Lee C W, Kim H M, Kim H C and Kwon B M (2003) Antitumor effect of the cinnamaldehyde derivative CB403 through the arrest of cell cycle progression in the G2/M phase. *Biochemical Pharmacology* 65 : 1343-1350.
- Jin T, Sun D, Su J Y, Zhang H and Sue H J (2009) Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonella Enteritidis*, and *Escherichia coli* O157: H7. *Journal of Food Science* 74 : 46-52.

- Jordan V C and Murphy C S (1990) Endocrine pharmacology of antiestrogens as antitumor agents. *Endocrine Reviews* 11 : 578-610.
- Ka H, Park H J, Jung H J, Choi J W, Cho K S, Ha J and Lee K T (2003) Cinnamaldehyde induces apoptosis by ROS-mediated mitochondrial permeability transition in human promyelocytic leukemia HL-60 cells. *Cancer Letters* 196 : 143-152.
- Kaefer C M and Milner J A (2008) The role of herbs and spices in cancer prevention. *The Journal of Nutritional Biochemistry* 19 : 347-361.
- Kalidas M and Brown P (2005) Aromatase inhibitors for the treatment and prevention of breast cancer. *Clinical Breast Cancer* 6 : 27-37.
- Kang S M, Maeda K, Onoda N, Chung Y S, Nakata B, Nishiguchi Y and Sowa M (1997) Combined analysis of p 53 and vascular endothelial growth factor expression in colorectal carcinoma for determination of tumor vascularity and liver metastasis. *International Journal of Cancer* 74 : 502-507.
- Katzenellenbogen B S (1980) Dynamics of steroid hormone receptor action. *Annual Review of Physiology* 42 : 17-35.
- Kaur G, Athar M and Alam M S (2010) Eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis. *Molecular Carcinogenesis*. 49 : 290-301.
- Kawashima Y (2001) Nanoparticulate systems for improved drug delivery. *Advanced Drug Delivery Reviews* 47 : 1-2.
- Khan N, Afaq F and Mukhtar H (2008) Cancer chemoprevention through dietary antioxidants: progress and promise. *Antioxidants & Redox Signalling* 10 : 475-510.
- Kim B H, Lee Y G, Lee J, Lee J Y and Cho J Y (2010) Regulatory effect of cinnamaldehyde on monocyte/macrophage-mediated inflammatory responses. *Mediators of Inflammation* ID 529329 : 1-9
- Klaunig, J E, Xu Y, Isenberg J S, Bachowski S, Kolaja K L, Jiang J, Stevenson D E and Walborg Jr E F (1998) The role of oxidative stress in chemical carcinogenesis. *Environmental Health Perspectives* 106 : 289-295.

- Klug W S, Cummings M R, Spencer C A and Palladino M A (2010). pp 522, in Essentials of genetics, 7th ed., Pearson Education Inc., San Francisco.
- Ko J W, Hong E T, Lee I C, Park S H, Park J I, Seong N W, Hong J S, Yun H I and Kim J C (2015) Evaluation of 2-week repeated oral dose toxicity of 100 nm zinc oxide nanoparticles in rats. *Laboratory Animal Research* 31 : 139-147.
- Krishnamoorthy D and Sankaran M (2016) Modulatory effect of *Pleurotus ostreatus* on oxidant/antioxidant status in 7, 12-dimethylbenz (a) anthracene induced mammary carcinoma in experimental rats-A dose-response study. *Journal of Cancer Research and Therapeutics* 12 : 386-394.
- Kumar S, Vasudeva N and Sharma S (2012) GC-MS analysis and screening of antidiabetic, antioxidant and hypolipidemic potential of *Cinnamomum tamala* oil in streptozotocin induced diabetes mellitus in rats. *Cardiovascular Diabetology* 11 : 95.
- Kundu J K and Surh Y J (2005) Breaking the relay in deregulated cellular signal transduction as a rationale for chemoprevention with anti-inflammatory phytochemicals. *Mutation Research* 591 : 123-146.
- Kwon B M, Lee S H, Choi S U, Park S H, Lee C O, Cho Y K, Sung N D and Bok S H (1998) Synthesis and in vitro cytotoxicity of cinnamaldehydes to human solid tumor cells. *Archives of Pharmacal Research* 21 : 147-152.
- Kwon K H, Barve A, Yu S, Huang M T and Kong A N T (2007) Cancer chemoprevention by phytochemicals: potential molecular targets, biomarkers, and animal models. *Acta Pharmacologica Sinica* 28 : 1409-1421.
- Laidlaw I J, Clarke R B, Howell A, Owen A W, Potten C S and Anderson E (1995) The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone. *Endocrinology* 136 : 164-171.
- Lamartiniere C A, Cotroneo M S, Fritz W A, Wang J, Mentor-Marcel R, and Elgavish A (2002) Genistein chemoprevention: Timing and mechanisms of action in murine mammary and prostate. *Journal of Nutrition* 132 : 552-558.

- Landel C C, Kushner P J and Greene G L (1994) The interaction of human estrogen receptor with DNA is modulated by receptor-associated proteins. *Molecular Endocrinology* 8 : 1407-1419.
- Lanone S and Boczkowski J (2006) Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. *Current Molecular Medicine* 6 : 651-663.
- Laviano A, Meguid M M, Preziosa I and Fanelli F R (2007) Oxidative stress and wasting in cancer. *Current Opinion in Clinical Nutrition & Metabolic Care* 10 : 449-56.
- Lee C W, Lee S H, Lee J W, Ban J O, Lee S Y, Yoo H S, Jung J K, Moon D C, Oh K W and Hong J T (2007) 2-hydroxycinnamaldehyde inhibits SW620 colon cancer cell growth through AP-1 inactivation. *Journal of Pharmacological Sciences* 104 : 19-28.
- Lee J H, Huh Y M, Jun Y W, Seo J W, Jang J T, Song H T, Kim S, Cho E J, Yoon H G, Suh J S and Cheon J (2007) Artificially engineered magnetic nanoparticles for ultra-sensitive molecular imaging. *Nature Medicine* 13 : 95-99.
- Lee J, Park E Y and Lee J (2014) Non-toxic nanoparticles from phytochemicals: preparation and biomedical application. *Bioprocess and Biosystems Engineering* 37 : 983-989.
- Leroueil P R, Hong S, Mecke A, Baker J R Jr, Orr B G and Holl M M (2007) Nanoparticle interaction with biological membranes: does nanotechnology present a Janus face?. *Accounts of Chemical Research* 40 : 335-342.
- Leung B S and Potter A H (1987) Mode of estrogen action on cell proliferation in CAMA- 1 cells: II. Sensitivity of G1 phase population. *Journal of Cellular Biochemistry* 34 : 213-225.
- Li S D and Huang L (2008) Pharmacokinetic and biodistribution of nanoparticles. *Molecular Pharmacology* 5 : 496-504.
- Liao B C, Hsieh C W, Liu Y C, Tzeng T T, Sun Y W and Wung B S (2008) Cinnamaldehyde inhibits the tumor necrosis factor- α -induced expression of cell adhesion molecules in endothelial cells by suppressing NF- κ B activation:

Effects upon I κ B and Nrf2. *Toxicology and Applied Pharmacology* 229 : 161-171.

Liao J C, Deng J S, Chiu C S, Hou W C, Huang S S, Shie P H and Huang G J (2012) Anti-inflammatory activities of *Cinnamomum cassia* constituents in vitro and in vivo. *Evidence-Based Complementary and Alternative Medicine* ID 429320 : 1-12.

Lin C C, Wu S J, Chang C H and Ng L T (2003) Antioxidant activity of *Cinnamomum cassia*. *Phytotherapy Research* 17 : 726-730.

Lin L T, Tai C J, Chang S P, Chen J L, Wu S J and Lin C C (2013) Cinnamaldehyde-induced apoptosis in human hepatoma PLC/PRF/5 cells involves the mitochondrial death pathway and is sensitive to inhibition by cyclosporin A and z-VAD-fmk. *Anti-Cancer Agents in Medicinal Chemistry* 13 : 1565-1574.

Lippman M, Bolan G and Huff K (1976) The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Research* 36 : 4595-4601.

Lippman S M and Brown P H (1999) Tamoxifen prevention of breast cancer: an instance of the fingerpost. *Journal of the National Cancer Institute* 91 : 1809-1819.

Liu J, He C, Zhou K, Wang J and Kang J X (2009) Coptis extracts enhance the anticancer effect of estrogen receptor antagonists on human breast cancer cells. *Biochemical and Biophysical Research Communications* 378 : 174-178.

Liu R H (2003) Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *The American Journal of Clinical Nutrition* 78 : 517-520.

Lockshin R A and Williams C M (1965) Programmed cell death—V. Cytolytic enzymes in relation to the breakdown of the intersegmental muscles of silkworms. *Journal of Insect Physiology* 11 : 831-44.

Lorenzo H K and Susin S A (2004) Mitochondrial effectors in caspase- independent cell death. *Federation of European Biochemical Societies Letters* 557 : 14-20.

- Luna L G (1968) Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. Blakiston, McGraw-Hill.
- MacDonald N (2007) Cancer cachexia and targeting chronic inflammation: a unified approach to cancer treatment and palliative/supportive care. *Journal of Supportive Oncology* 5 : 157-162.
- Madesh M and Balasubramanian K A (1998) Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry & Biophysics* 35 : 184-188.
- Mahfouz M H, Ghanem H M and Mohamed M A (2009) Therapeutic effect of L-carnitine on sialic acid, soluble Fas (sFas) and other biochemical variables in hyperinsulinemic rats. *Life Science Journal* 6 : 76-82.
- Malone W F (1991) Studies evaluating antioxidants and beta-carotene as chemopreventives. *The American Journal of Clinical Nutrition* 53 : 305-313.
- Malvia S, Bagadi S A, Dubey U S and Saxena S (2017) Epidemiology of breast cancer in Indian women. *Asia- Pacific Journal of Clinical Oncology* 13 : 289-295.
- Mandlekar S and Kong A N (2001) Mechanisms of tamoxifen-induced apoptosis. *Apoptosis* 6 : 469-477.
- Mang B, Wolters M, Schmitt B, Kelb K, Lichtinghagen R, Stichtenoth D O and Hahn A (2006) Effects of a cinnamon extract on plasma glucose, HbA1c, and serum lipids in diabetes mellitus type 2. *European Journal of Clinical Investigation* 36 : 340-344.
- Marino M, Galluzzo P and Ascenzi P (2006) Estrogen signaling multiple pathways to impact gene transcription. *Current Genomics* 7 : 497-508.
- Masella R, Di Benedetto R, Vari R, Filesi C and Giovannini C (2005) Novel mechanisms of natural compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *The Journal of Nutritional Biochemistry* 16 : 577-586.
- Masood S (1992) Estrogen and progesterone receptors in cytology: a comprehensive review. *Diagnostic Cytopathology* 8 : 475-91.

- Masso-Welch P A, Darcy K M, Stangle-Castor N C and Ip M M (2000) A developmental atlas of rat mammary gland histology. *Journal of Mammary Gland Biology and Neoplasia* 5 : 165-185.
- McDonnell D P and Norris J D (2002) Connections and regulation of the human estrogen receptor. *Science* 296 : 1642-1644.
- McEligot A J, Yang S, Meyskens F L Jr. (2005) Redox regulation by intrinsic species and extrinsic nutrients in normal and cancer cells. *Annual Review of Nutrition* 25 : 261-95
- McPherson K, Steel C and Dixon J M (2000) ABC of breast diseases: breast cancer-epidemiology, risk factors, and genetics. *British Medical Journal* 321 : 624-628.
- McTiernan A (2008) Mechanisms linking physical activity with cancer. *Nature Reviews Cancer* 8 : 205-211.
- Mehta R G (2000) Experimental basis for the prevention of breast cancer. *European Journal of Cancer* 36 : 1275-1282
- Mhammad H A, Jubrail A M and Najeeb M K (2015) Impact of cinnamon extract on hyperlipidemic and diabetic rats. *International Journal of Chemical and Biomolecular Sciences* 1 : 96-106.
- Milner J A and Romagnolo D F (2010) Cancer biology and Nutrigenomics. pp 25. In, *Bioactive Compounds and Cancer Nutrition and Health*, Springer Science & Business Media.
- Mirvish S S (1981) Ascorbic acid inhibition of N-nitroso compound formation in chemical, food, and biological systems. pp 101-126, In *Inhibition of Tumor Induction and Development*. Springer US.
- Mishra A, Bhatti R, Singh A and Ishar M P (2010) Ameliorative effect of the cinnamon oil from *Cinnamomum zeylanicum* upon early stage diabetic nephropathy. *Planta Medica* 76 : 412-417.
- Mobley J A and Brueggemeier R W (2004) Estrogen receptor-mediated regulation of oxidative stress and DNA damage in breast cancer. *Carcinogenesis* 25 : 3-9.

- Modak B, Contreras M L, Gonzalez-Nilo F and Torres R (2005) Structure-antioxidant activity relationships of flavonoids isolated from the resinous exudate of *Heliotropium sinuatum*. *Bioorganic and Medicinal Chemistry Letters* 15 : 309-312.
- Moi L L, Flågeng M H, Gjerde J, Madsen A, Røst T H, Gudbrandsen O A, Lien E A and Mellgren G (2012) Steroid receptor coactivators, HER-2 and HER-3 expression is stimulated by tamoxifen treatment in DMBA-induced breast cancer. *BMC Cancer* 12 : 247.
- Mollard S, Mousseau Y, Baaj Y, Richard L, Cook-Moreau J, Monteil J, Funalot B and Sturtz F G (2011) How can grafted breast cancer models be optimized?. *Cancer Biology & Therapy* 12 : 855-864.
- Monti M, Catania S, Locatelli E, Scazzoso A, Calzaferri G and Cuniatti E (1988) Sialic acid, ferritin and CEA levels in peripheral blood and blood draining from the tumor in breast cancer. *The International Journal of Biological Markers* 3 : 243-248.
- Moon K H and Pack M Y (1983) Cytotoxicity of cinnamic aldehyde on leukemia L1210 cells. *Drug and Chemical Toxicology* 6 : 521-35.
- Morrissey P A and O'brien N M (1998) Dietary antioxidants in health and disease. *International Dairy Journal* 8 : 463-472.
- Moselhy S S, Al mslmani M A B (2008) Chemopreventive effect of lycopene alone or with melatonin against the genesis of oxidative stress and mammary tumors induced by 7,12 dimethyl(a) benzantracene in sprague dawely female rats. *Molecular and Cellular Biochemistry* 319 : 175-180
- Muscaritoli M, Bossola M, Aversa Z, Bellantone R and Fanelli F R (2006) Prevention and treatment of cancer cachexia: new insights into an old problem. *European Journal of Cancer* 42 : 31-41.
- Nagini S (2008) Cancer chemoprevention by garlic and its organosulfur compounds- panacea or promise? *Anti-cancer Agents in Medicinal Chemistry* 8 : 313-321.
- National Breast Cancer Foundation, Inc.[®] (2016) <http://www.nationalbreastcancer.org>.

- Neergheen V S, Bahorun T, Taylor E W, Jen L S and Aruoma O I (2010) Targeting specific cell signalling transduction pathways by dietary and medicinal plant bioactive compounds in cancer chemoprevention. *Toxicology* 278 : 229-241.
- Nel A, Xia T, Madler L and Li N (2006) Toxic potential of materials at the nanolevel. *Science* 311 : 622-627.
- Newmark H L (1985) A hypothesis for dietary components as blocking agents of chemical carcinogenesis: plant phenolics and pyrrole pigments. *Nutrition and Cancer* 6 : 58-70.
- Ng L T and Wu S J (2011) Antiproliferative activity of *Cinnamomum cassia* constituents and effects of pifithrin- α on their apoptotic signaling pathways in Hep G2 cells. *Evidence-Based Complementary and Alternative Medicine* ID 492148 : 1-6
- Nostro A, Scaffaro R, D'Arrigo M, Botta L, Filocamo A, Marino A and Bisignano G (2012) Study on carvacrol and cinnamaldehyde polymeric films: mechanical properties, release kinetics and antibacterial and antibiofilm activities. *Applied Microbiology and Biotechnology* 96 : 1029-1038.
- Ohgaki M, Kizuki T, Katsura M and Yamashita K (2001) Manipulation of selective cell adhesion and growth by surface charges of electrically polarized hydroxyapatite. *Journal of Biomedical Materials Research* 57 : 366-373.
- Ojeswi B K, Khoobchandani M, Hazra D K and Srivastava M M (2010) Protective effect of *Thuja occidentalis* against DMBA-induced breast cancer with reference to oxidative stress. *Human & Experimental Toxicology* 29 : 369-375.
- Olivo-Marston S E, Zhu Y, Lee R Y, Cabanes A, Khan G, Zwart A, Wang Y, Clarke R and Hilakivi-Clarke L (2008) Gene signaling pathways mediating the opposite effects of prepubertal low-fat and high-fat n-3 polyunsaturated fatty acid diets on mammary cancer risk. *Cancer Prevention Research* 1 : 532-545.
- Ostrovsky S, Kazimirsky G, Gedanken A and Brodie C (2009) Selective cytotoxic effect of ZnO nanoparticles on glioma cells. *Nano Research* 2 : 882-890.
- Ott M, Gogvadze V, Orrenius S and Zhivotovsky B (2007) Mitochondria, oxidative stress and cell death. *Apoptosis* 12 : 913-922.

- Ouhtit A, Ismail M F, Othman A, Fernando A, Abdraboh M E, El-Kott A F, Azab Y A, Abdeen S H, Gaur R L, Gupta I and Shanmuganathan S (2014) Chemoprevention of rat mammary carcinogenesis by spirulina. *The American Journal of Pathology* 184 : 296-303.
- Oyagbemi A A, Azeez O I and Saba A B (2009) Interactions between reactive oxygen species and cancer: the roles of natural dietary antioxidants and their molecular mechanisms of action. *Asian Pacific Journal of Cancer Prevention* 10 : 535-544.
- Ozben T (2007) Oxidative stress and apoptosis: impact on cancer therapy. *Journal of Pharmaceutical Sciences* 96 : 2181-2196.
- Panchal R G (1998) Novel therapeutic strategies to selectively kill cancer cells. *Biochemical Pharmacology* 55 : 247-252.
- Papo N, Shahar M, Eisenbach L and Shai Y (2003) A novel lytic peptide composed of DL-amino acids selectively kills cancer cells in culture and in mice. *Journal of Biological Chemistry* 278 : 21018-21023.
- Parveen K, Banse V and Ledwani L (2016) Green synthesis of nanoparticles: Their advantages and disadvantages. In *AIP Conference Proceedings* 1724 (020048) : 1-7.
- Pasupuleti S, Alapati S, Ganapathy S, Anumolu G, Pully N R and Prakhya B M (2012) Toxicity of zinc oxide nanoparticles through oral route. *Toxicology and Industrial Health* 28 : 675-686.
- Patra K, Bose S, Sarkar S, Rakshit J, Jana S, Mukherjee A, Roy A, Mandal D P and Bhattacharjee S (2012) Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid. *Chemico-Biological Interactions* 195 : 231-239.
- Perry R R, Kang Y and Greaves B (1995) Effects of Tamoxifen on Growth and Apoptosis of Estrogen-Dependent and Estrogen-Independent Human Breast Cancer Cells. *Annals of Surgical Oncology* 2 : 238-245.
- Pike M C, Spicer D V, Dahmouch L and Press M F (1993) Estrogens progestogens normal breast cell proliferation and breast cancer risk. *Epidemiologic reviews* 15 : 17-35.

- Plucinsky M C, Michael Riley W, Prorok J J and Alhadeff J A (1986) Total and lipid- associated serum sialic acid levels in cancer patients with different primary sites and differing degrees of metastatic involvement. *Cancer* 58 : 2680-2685.
- Powles T J (2003) Anti-oestrogenic chemoprevention of breast cancer - the need to progress. *European Journal of Cancer* 39 : 572-579.
- Prall O W, Rogan E M and Sutherland R L (1998) Estrogen regulation of cell cycle progression in breast cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology* 65 : 169-174.
- Premanathan M, Karthikeyan K, Jeyasubramanian K and Manivannan G (2011) Selective toxicity of ZnO nanoparticles toward Gram positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine: Nanotechnology, Biology and Medicine* 7 : 184-192.
- Purushothaman A Y, Nandhakumar E L and Sachdanandam P A (2012) Anticancer effect of Shemamruthaa (a phytochemical formulation) on 7, 12-dimethylbenz (a) anthracene induced mammary carcinoma in rats. *Asian Journal of Pharmaceutical and Clinical Research* 5 : 101-107.
- Qi G, Chen J, Shi C, Wang Y, Mi S, Shao W, Yu X, Ma Y, Ling J and Huang J (2016) Cinnamic Acid (CINN) Induces Apoptosis and Proliferation in Human Nasopharyngeal Carcinoma Cells. *Cellular Physiology and Biochemistry* 40 : 589-96.
- Rajendran S P and Sengodan K (2017) Synthesis and Characterization of Zinc Oxide and Iron Oxide Nanoparticles Using *Sesbania grandiflora* Leaf Extract as Reducing Agent. *Journal of Nanoscience* ID 8348507 : 1-7
- Rajkumar L, Balasubramanian K, Arunakaran J, Govindarajulu P and Srinivasan N (2006) Influence of estradiol on mammary tumor collagen solubility in DMBA-induced rat mammary tumors. *Cell Biology International* 30 : 164-8.
- Rajput S, Kumar B P, Sarkar S, Das S, Azab B, Santhekadur P K, Das S K, Emdad L, Sarkar D, Fisher P B and Mandal M (2013) Targeted apoptotic effects of thymoquinone and tamoxifen on XIAP mediated Akt regulation in breast cancer. *PLoS One* 8 : e61342.

- Rasmussen J W, Martinez E, Louka P and Wingett D G (2010) Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opinion on Drug Delivery* 7 : 1063-1077.
- Raval G N, Parekh L J, Patel D D, Jha F P, Sainger R N and Patel P S (2004) Clinical usefulness of alterations in sialic acid, sialyl transferase and sialoproteins in breast cancer. *Indian Journal of Clinical Biochemistry* 19 : 60-71.
- Raval G N, Parekh L J, Patel M M, Patel P S, Rawal R M, Balar D B and Patel D D (1997) Role of sialic acid and alkaline DNase in breast cancer. *The International Journal of Biological Markers* 12 : 61-67.
- Raveendran M, Thanislass J, Maheswari G U and Devaraj H (1993) Induction of prooxidant state by the food flavor cinnamaldehyde in rat liver. *The Journal of Nutritional Biochemistry* 4 : 181-183.
- Ren W, Qiao Z, Wang H, Zhu L and Zhang L (2003) Flavonoids: promising anticancer agents. *Medicinal Research Reviews* 23 : 519-534.
- Rice-Evans C A, Miller N J and Paganga G (1996) Structure – antioxidant activity relations of flavonoids and phenolic compounds. *Free Radical Biology and Medicine* 20 : 933-956.
- Rich I N, Worthington-White D, Garden O A and Musk P (2000) Apoptosis of leukemic cells accompanies reduction in intracellular pH after targeted inhibition of the Na⁺/H⁺ exchanger. *Blood* 95 : 1427-1434.
- Rolo A P and Palmeira C M (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicology and Applied Pharmacology* 212 : 167-78.
- Rose D P, Pruitt B, Stauber P, Ertürk E and Bryan G T (1980) Influence of dosage schedule on the biological characteristics of N-nitrosomethylurea-induced rat mammary tumors. *Cancer Research* 40 : 235-239.
- Russo J and Russo I H (1987) Development of the human mammary gland. pp 67-93, In *The Mammary Gland: Development, Regulation and Function* (ed Neville M C, Daniels C W) New York, Plenum Press.

- Russo J and Russo I H (2000) Atlas and histologic classification of tumors of the rat mammary gland. *Journal of Mammary Gland Biology and Neoplasia* 5 : 187-200.
- Russo J, Gusterson B A, Rogers A E, Russo I H, Wellings S R and Van Zwieten M J (1990) Comparative study of human and rat mammary tumorigenesis. *Laboratory Investigation* 62 : 244-278.
- Russo J, Hu Y F, Yang X and Russo I H (2000) Chapter 1: Developmental, cellular, and molecular basis of human breast cancer. *Journal of the National Cancer Institute Monographs* 27 : 17-37.
- Russo J, Wilgus G and Russo I H (1979) Susceptibility of the mammary gland to carcinogenesis: I Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. *The American Journal of Pathology* 96 : 721-736.
- Saadat S (2008) Can we prevent breast cancer?. *International Journal of Health Sciences* 2 : 167-170.
- Salami S and Karami-Tehrani F (2003) Biochemical studies of apoptosis induced by tamoxifen in estrogen receptor positive and negative breast cancer cell lines. *Clinical Biochemistry* 36 : 247–253.
- Saman S, Moradhaseli S, Shokouhian A and Ghorbani M (2013) Histopathological effects of ZnO nanoparticles on liver and heart tissues in wistar rats. *Advances in BioResearch* 4 : 83-8.
- Samy R P, Gopalakrishnakone P and Ignacimuthu S (2006) Anti-tumor promoting potential of luteolin against 7, 12-dimethylbenz (a) anthracene-induced mammary tumors in rats. *Chemico-Biological Interactions* 164 : 1-4..
- Schaffer E M, Liu J Z, Green J, Dangler C A and Milner J A (1996) Garlic and associated allyl sulfur components inhibit N-methyl-N-nitrosourea induced rat mammary carcinogenesis. *Cancer Letters* 102 : 199-204.
- Schauer R (1982) Chemistry, metabolism, and biological functions of sialic acids. *Advances in Carbohydrate Chemistry and Biochemistry* 40 : 131-234.

- Schroeter H, Boyd C, Spencer J P, Williams R J, Cadenas E and Rice-Evans C (2002) MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. *Neurobiology of Aging* 23 : 861-880.
- Schulte-Hermann R, Timmermann-Trosiener I, Barthel G and Bursch W (1990) DNA synthesis, apoptosis, and phenotypic expression as determinants of growth of altered foci in rat liver during phenobarbital promotion. *Cancer Research* 50 : 5127–5135.
- Sedlak J and Lindsay R H (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* 25 : 192-205.
- Shamberger R J (1984) Serum sialic acid in normals and in cancer patients. *Clinical Chemistry and Laboratory Medicine* 22 : 647-652.
- Shan B, Cai Y Z, Brooks J D and Corke H (2007) Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. *Journal of Agricultural and Food Chemistry* 55 : 5484-5490.
- Shankar S S, Rai A, Ahmad A and Sastry M (2004) Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of Colloid and Interface Science* 275 : 496-502.
- Sharma M, Jain T, Singh S and Pandey O P (2012) Photocatalytic degradation of organic dyes under UV–visible light using capped ZnS nanoparticles. *Solar Energy* 86 : 626–633.
- Sharma V, Shukla R K, Saxena N, Parmar D, Das M and Dhawan A (2009) DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicology Letters* 185 : 211-218.
- Shen S C, Chen Y C, Hsu F L and Lee W R (2003) Differential apoptosis- inducing effect of quercetin and its glycosides in human promyeloleukemic HL- 60 cells by alternative activation of the caspase 3 cascade. *Journal of Cellular Biochemistry* 89 : 1044-1055.
- Sherr C J (2004) Principles of tumor suppression. *Cell* 116 : 235-246.

- Shinde S B, Fernandes C B and Patravale V B (2012) Recent trends in in-vitro nanodiagnostics for detection of pathogens. *Journal of Controlled Release* 159 : 164-180.
- Shrode L D, Tapper H and Grinstein S (1997) Role of intracellular pH in proliferation, transformation, and apoptosis. *Jouranl of Bioenergetics and Biomembranes* 29 : 393-399
- Siegel R L, Miller K D and Jemal A (2015) Cancer statistics, 2015. *CA: A Cancer Journal for Clinicians* 65 : 5-29.
- Silberstein G B, Van Horn K A, Shyamala G and Daniel C W (1994) Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens. *Endocrinology* 134 : 84-90.
- Silva M M, Santos M R, Caroco G, Rocha R, Justino G and Mira L (2002) Structure-antioxidant activity relationships of flavonoids: a re-examination. *Free Radical Research* 36 : 1219-1227
- Singh A, Khan S A, Choudhary R and Bodakhe S H (2016) Cinnamaldehyde attenuates cataractogenesis via restoration of hypertension and oxidative stress in fructose-fed hypertensive rats. *Journal of Pharmacopuncture* 19:137.
- Singh G, Maurya S and Catalan C A (2007) A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food and Chemical Toxicology* 45 : 1650-1661.
- Singh N, Manshian B, Jenkins G J, Griffiths S M, Williams P M, Maffeis T G, Wright C J, and Doak S H (2009) NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials* 30 : 3891-3914.
- Skipski V P, Carter S P, Terebus-Kekish O I, Podlaski F J Jr, Peterson R H F and Stock C C (1981) Ganglioside profiles of metastases and of metastasizing and nonmetastasizing rat primary mammary carcinomas. *Journal of the National Cancer Institute* 67 : 1251-1258.
- Smith I and Chua S (2006) Medical treatment of early breast cancer I: adjuvant treatment. *British Medical Journal* 7 : 34-37.

- Song F, Li H, Sun J and Wang S (2013) Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats. *Journal of Ethnopharmacology* 150 : 125-30.
- Sonmez H, Suer S, Gungor Z, Baloglu H and Kokoglu E (1999) Tissue and serum sialidase levels in breast cancer. *Cancer Letters* 136 : 75-78.
- Sova M (2012) Antioxidant and antimicrobial activities of cinnamic acid derivatives. *Mini Reviews in Medicinal Chemistry* 12 : 749-767.
- Sporn A, Dinu I and Stanciu V (1965) Investigation of the toxicity of cinnamic aldehyde. *Igiena* 14 : 339-346.
- Spurr A R (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26 : 31-43.
- Sreeramulu V and Venkata Ramana Ch (2014) Evaluation of serum total and lipid associated sialic acid as a tumor marker in breast malignancy. *IOSR Journal of Pharmacy and Biological Sciences* 9 : 14-17.
- Storer R D, Kraynak A R, McKelvey T W, Elia M C, Goodrow T L and DeLuca J G (1997) The mouse lymphoma L5178Y Tk+/- cell line is heterozygous for a codon 170 mutation in the p53 tumor suppressor gene. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 373 : 157-165.
- Subash babu P, Alshatwi A A and Ignacimuthu S (2014) Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin-induced pancreatic β -cells damage in wistar rats. *Biomolecules & Therapeutics* 22 : 47-54.
- Sun Y (1990) Free radicals, antioxidant enzymes and carcinogenesis. *Free Radical Biology and Medicine* 8 : 583-599.
- Surh Y J (2003) Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer* 3 : 768-780.
- Surh Y J, Kundu J K, Na H K and Lee J S (2005) Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. *The Journal of Nutrition* 135 : 2993-3001.

- Suzi S A and El-Makawy A (2007) Could cinnamaldehyde be harmful? histological, cytogeneticaland biochemical studies on its effect on some organs of mice. The Egyptian Journal of Histology 30 : 447-464.
- Szatrowski T P and Nathan C F (1991) Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Research 51 : 794-798.
- Tang D, Kang R, Cheh C W, Livesey K M, Liang X, Schapiro N E, Benschop R, Sparvero L J, Amoscato A A, Tracey K J and Zeh H J (2010) HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. Oncogene 29 : 5299-5310.
- Taraphdar A K, Roy M and Bhattacharya R K (2001) Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. Current Science 10 : 1387-1396.
- Teel R W and Castonguay A (1992) Antimutagenic effects of polyphenolic compounds. Cancer Letters 66 : 107-113.
- Thompson H J and Singh M (2000) Rat models of premalignant breast disease. Journal of Mammary Gland Biology and Neoplasia 5 : 409-420.
- Tohamy A A, Aref A M, Moneim A E and Sayed R H (2016) Cinnamic acid attenuates cisplatin-induced hepatotoxicity and nephrotoxicity. Journal of Basic and Environmental Sciences 3 : 1-9.
- Trichopoulos D, MacMahon B and Cole P (1972) Menopause and Breast Cancer Risk 2. Journal of the National Cancer Institute 48 : 605-613.
- Tsai M and O'Malley B W (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annual Review of Biochemistry 63 : 451-486.
- Tsai P J, Tsai T H, Yu C H and Ho S C (2007) Evaluation of NO-suppressing activity of several Mediterranean culinary spices. Food and Chemical Toxicology 45 : 440-447.
- Valko M, Rhodes C, Moncol J, Izakovic M M and Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 160 : 1-40.

- Van de Vijver M J (1993) Molecular genetic changes in human breast cancer. *Advances in Cancer Research* 61 : 25-56.
- Van Erk M J, Teuling E, Staal Y C, Huybers S, Van Bladeren P J, Aarts J M and Van Ommen B (2004) Time and dose dependent effects of curcumin on gene expression in human colon cancer cells. *Journal of Carcinogenesis* 3 : 8.
- Venkatesh S and Lipper R A (2000) Role of the development scientist in compound lead selection and optimization. *Journal of Pharmaceutical Sciences* 89 : 145-154.
- Verma A K, Jhonson J A, Gould M N and Tanner M A (1988) Inhibition of 7,12 dimethylbenz(a)anthracene and N-nitrosomethylurea induce rat mammary cancer by dietary flavonol quercetin. *Cancer Research* 48 : 5754-5758.
- Visaria R K, Griffin R J, Williams B W, Ebbini E S, Paciotti G F, Song C W and Bischof J C (2006). Enhancement of tumor thermal therapy using gold nanoparticle–assisted tumor necrosis factor- α delivery. *Molecular Cancer Therapeutics* 5 : 1014-1020.
- Vogelstein B, Lane D and Levine A J (2000) Surfing the p53 network. *Nature* 408 : 307-310
- Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M, Ouyang H, Shi J, Zhang F, Zhao Y and Chai Z (2008) Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice. *Journal of Nanoparticle Research* 10 : 263-276.
- Wang C, Lu J, Zhou L, Li J, Xu J, Li W, Zhang L, Zhong X and Wang T (2016). Effects of long-term exposure to Zinc oxide nanoparticles on development, Zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice. *PloS One* 11 : e0164434.
- Wang H, Wingett D, Engelhard M H, Feris K, Reddy K M, Turner P, Layne J, Hanley C, Bell J, Tenne D and Wang C (2009) Fluorescent dye encapsulated ZnO particles with cell-specific toxicity for potential use in biomedical applications. *Journal of Materials Science: Materials in Medicine* 20 : 11-22.

- Wang L, Liu Y, Li W, Jiang X, Ji Y, Wu X, Xu L, Qiu Y, Zhao K, Wei T, Li Y, Zhao Y and Chen C (2011) Selective targeting of gold nanorods at the mitochondria of cancer cells: implications for cancer therapy. *Nano Letters*. 11 : 772-780.
- Wang S Y, Chen P F and Chang S T (2005) Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. *Bioresource Technology* 96 : 813-818.
- Wang T T and Phang J M (1995) Effects of estrogen on apoptotic pathways in human breast cancer cell line MCF-7. *Cancer Research* 55 : 2487-2489.
- Wang Z L (2008) Splendid one-dimensional nanostructures of zinc oxide: a new nanomaterial family for nanotechnology. *American Chemical Society Nano* 2 : 1987-1992.
- Wargovich M J (2000) Anticancer properties of fruits and vegetables. *HortScience* 35 : 573-575.
- Wattenberg L W (1985) Chemoprevention of cancer. *Cancer Research* 45 : 1-8.
- Wattenberg L W (1992) Inhibition of carcinogenesis by minor dietary constituents. *Cancer Research* 52 : 2085-2091.
- Weng S C, Kashida Y, Kulp S K, Wang D, Brueggemeier R W, Shapiro C L and Chen C S (2008) Sensitizing estrogen receptor-negative breast cancer cells to tamoxifen with OSU-03012, a novel celecoxib-derived phosphoinositide-dependent protein kinase-1/Akt signaling inhibitor. *Molecular Cancer Therapeutics* 7 : 800–808.
- Whitsett T G, Carpenter D M and Lamartiniere C A (2006) Resveratrol, but not EGCG, in the diet suppresses DMBA-induced mammary cancer in rats. *Journal of Carcinogenesis* 5 : 15.
- Wilkinson J I V and Clapper M (1997) Detoxification enzymes and chemoprevention. *Proceedings of the Society for Experimental Biology and Medicine* 216 : 192-200.
- Wiseman H and Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemical Journal* 313 : 17.

- Wiseman H, Laughton MJ, Arnstein H R V, Cannon M and Halliwell B (1990) The antioxidant action of Tamoxifen and its metabolites inhibition of lipoperoxidation. *FEBS Letters* 236 : 192-194.
- Wu S J and Ng L T (2007) MAPK inhibitors and pifithrin- α block cinnamaldehyde-induced apoptosis in human PLC/PRF/5 cells. *Food and Chemical Toxicology* 45 : 2446-53.
- Wu S J, Ng L T and Lin C C (2005) Cinnamaldehyde-induced apoptosis in human PLC/PRF/5 cells through activation of the proapoptotic Bcl-2 family proteins and MAPK pathway. *Life Sciences* 77 : 938-951.
- Wu Y N, Chen D H, Shi X Y, Lian C C, Wang T Y, Yeh C S, Ratnac K R, Thordarson P, Braet F and Shieh D B (2011) Cancer-cell-specific cytotoxicity of non-oxidized iron elements in iron core-gold shell NPs. *Nanomedicine: Nanotechnology, Biology and Medicine* 7 : 420-427.
- Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh J I, Zink J I and Nel A E (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *American Chemical Society Nano* 2 : 2121-2134.
- Xu P, Van Kirk E A, Zhan Y, Murdoch W J, Radosz M and Shen Y (2007) Targeted Charge- Reversal Nanoparticles for Nuclear Drug Delivery. *Angewandte Chemie International Edition* 46 : 4999-5002.
- Yadav P and Jaroli D P (2010) Breast cancer: awareness and risk factors in college-going younger age group women in Rajasthan. *Asian Pacific Journal of Cancer Prevention* 11 : 319-322.
- Yamamoto S, Sobue T, Kobayashi M, Sasaki S and Tsugane S (2003) Soy, isoflavones, and breast cancer risk in Japan. *Journal of the National Cancer Institute* 17 :1881-1882.
- Yen T B and Chang S T (2008) Synergistic effects of cinnamaldehyde in combination with eugenol against wood decay fungi. *Bioresource Technology* 99 : 232-236.

- Yin H, Casey P S, McCall M J and Fenech M (2010) Effects of surface chemistry on cytotoxicity, genotoxicity, and the generation of reactive oxygen species induced by ZnO nanoparticles. *Langmuir* 26 : 15399-15408.
- Yip N C, Fombon I S, Liu P, Brown S, Kannappan V, Armesilla A L, Xu B, Cassidy J, Darling J L and Wang W (2011) Disulfiram modulated ROS–MAPK and NFκB pathways and targeted breast cancer cells with cancer stem cell-like properties. *British Journal of Cancer* 104 : 1564-1574.
- Yogeeswaran G and Salk P L (1981) Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines. *Science* 212 : 1514-1516.
- Youn H S, Lee J K, Choi Y J, Saitoh S I, Miyake K, Hwang D H and Lee J Y (2008) Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization. *Biochemical Pharmacology* 75 : 494-502.
- Yu C, Liu S L, Qi M H, Zou X (2014) Cinnamaldehyde/chemotherapeutic agents interaction and drug-metabolizing genes in colorectal cancer. *Molecular Medicine Reports* 9 : 669-676.
- Yuan J H, Dieter M P and Bucher J R (1992) Toxicokinetics of cinnamaldehyde in F344 rats. *Food Chemical Toxicology* 30 : 997-1004.
- Yuan Q, Hein S and Misra R D (2010) New generation of chitosan-encapsulated ZnO quantum dots loaded with drug: synthesis, characterization and in vitro drug delivery response. *Acta Biomaterialia* 6 : 2732-2739.
- Zaitsev A N and Rakhmanina N L (1974) Some data on the toxic properties of phenylethyl and cinnamyl alcohol derivatives. *Voprosy pitaniia* 5 : 48-53.
- Zhang H, Chen B, Jiang H, Wang C, Wang H and Wang X (2011) A strategy for ZnO nanorod mediated multi-mode cancer treatment. *Biomaterials* 32 : 1906-1914.
- Zhang Y, Huo M, Zhou J and Xie S (2010) PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine* 99 : 306-314.
- Zhang X Y, Tan Y L, Cao L Y, Wu G Y, Xu Q, Shen Y and Zhou D F (2006) Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia

treated with typical and atypical antipsychotics. *Schizophrenia Research* 81 : 291-300.

Zhao H, Xie Y, Yang Q, Cao Y, Tu H, Cao W and Wang S (2014) Pharmacokinetic study of cinnamaldehyde in rats by GC–MS after oral and intravenous administration. *Journal of Pharmaceutical and Biomedical Analysis* 89 : 150-157.

Zhu Z, Zhao L, Liu X, Chen J, Zhang H, Zhang G and Chai Y (2010) Comparative pharmacokinetics of baicalin and wogonoside by liquid chromatography–mass spectrometry after oral administration of Xiaochaihu Tang and *Radix scutellariae* extract to rats. *Journal of Chromatography B* 878 : 2184-2190.