SUB-ACUTE ORAL TOXICITY EVALUATION OF *Jatropha curcas* Linn. LEAF EXTRACT COATED SILVER NANOPARTICLES IN WISTAR RATS.

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

BY

BELE SANGEETA SAKHARAM

Enrolment No: - V/14/017

College of Veterinary and Animal Sciences, Parbhani

MAHARASHTRA ANIMAL AND FISHERY SCIENCES

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled, **"SUB-ACUTE ORAL TOXICITY EVALUATION OF *Jatropha curcas* Linn. LEAF EXTRACT COATED SILVER NANOPARTICLES IN WISTAR RATS",** or part thereof has not been submitted for any other degree or diploma of any University, not the data have been derived from any thesis publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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BELE SANGEETA SAKHARAM
Enrolment No: V/14/017

Dr. N. D. Jadhav
Chairman
Advisory Committee with date

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Miss. **BELE SANGEETA SAKHARAM** has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, **"SUB-ACUTE ORAL TOXICITY EVALUATION OF *Jatropha curcas* Linn. LEAF EXTRACT COATED SILVER NANOPARTICLES IN WISTAR RATS."** submitted by her is the result of original research work is sufficient to warrant its presentation to the examination in the subject of **VETERINARY PHARMACOLOGY AND TOXICOLOGY** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

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Place :
Date : / /

Dr. N. D. Jadhav
Advisor / Guide
Assistant Professor,
Dept. of Veterinary Pharmacology and
Toxicology COVAS, Parbhani

Advisory Committee

	Name and Designation	Signature
1	**Dr. S. R. Rajurkar** Professor and Head Dept. of Veterinary Pharmacology, and Toxicology, COVAS Parbhani	_____
2	**Dr. B. M. Kondre** Assistant Professor, Department of Veterinary Pathology, COVAS Parbhani	_____
3	**Dr. G. M. Chigure** Assistant Professor, Department of Veterinary Parasitology, COVAS Parbhani	_____
4	**Dr. P. B. Ghorpade** Assistant Professor, Dept. of Veterinary Biochemistry, COVAS Parbhani	_____

CERTIFICATE

This is to certify that the thesis entitled, **"SUB-ACUTE ORAL TOXICITY EVALUATION OF *Jatropha curcas* Linn. LEAF EXTRACT COATED SILVER NANOPARTICLES IN WISTAR RATS"** submitted by Miss. **BELE SANGEETA SAKHARAM** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

Name and Signature of External Examiner	**Dr. S. R. Rajurkar** Professor and Head Department of Veterinary Pharmacology and Toxicology	**Dr. N. D. Jadhav** Advisor/Guide Assistant Professor Department of Veterinary Pharmacology and Toxicology

Advisory Committee

	Name and Designation	**Signature**
1	**Dr. S. R. Rajurkar** Professor and Head Dept. of Veterinary Pharmacology and Toxicology, COVAS Parbhani	_____
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4	**Dr. P. B. Ghorpade** Assistant Professor, Dept. of Veterinary Biochemistry, COVAS Parbhani	_____

Associate Dean,
COVAS, Parbhani

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***Place :** Parbhani* *(Bele Sangeeta Sakharam)*

Date : *Enroll No. V/14/017*

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ABBREVATION

%	Percent
°C	Degree Celsius
µg	Microgram
Kv	Kilovolt
Hz	Hertz
pH	Potential of hydrogen
AgNO3	Silver nitrate
AgNPs	Silver nanoparticles
ALT	Alanine transaminase
AST	Aspartate transaminase
B.wt,	Body weight
BUN	Blood urea nitrogen
BOD	Biochemical oxygen demand
CPCSEA	Committee for the purpose of control and supervision of experiments on animals
CRD	Completely Randomized Block Design
DLC	Differential leucocyte count
DNA	Deoxyribo Necleic Acid
CD	Critical Difference
Cm	Centimeter
et al	*et* alii (masculine), *et*aliae (feminine), et alia (neuter)-plural form
etc.	et cetera
EDS	Energy dispersive spectrophotometer
EDX	Energy Dispersive X-ray Analysis
Fig.	Figure
ELISA	Enzyme Linked Immunosorbant Assay
FRBD	Factorial randomized block design
FTIR	Fourier transform infrared spectroscopy
g/dl	Gram/decillitre
g/kg	Gram per kilogram
Gm or g	Gram
Hb	Haemoglobin
HS	Highly significant
i.e.	That is
I.P.	Intraperitoneal
IAEC	Institutional animal ethical committee
IU/L	International units per litre
J.	*Jatropha*
JcLE	*Jatropha curcas* leaf extract
JCSNP	*Jatropha curcas* silver nanoparticle
k.g.	Kilogram
L	Liter
LD50	Median lethal dose
LC50	Medial lethal concentration
M	Molar

MAFSU	Maharashtra Animal And Fishery Science University
MCHC	Mean Corpuscular Haemoglobin Concentration
MCH	Mean Corpuscular Haemoglobin
MTT	3[4,5-diamethylthiazol-20yl]-2,5-diphenyltetrazolium bromide.
Mg	Milligram
mg/dl	Milligram per decilitre
mg/kg	Milligram perkilogram
Ml	Mililiter
Mm	Milimeter
Nm	Nanometer
MWCNT	Multi -walled carbon nanotubes
NS	Not significant
NACMA	Nanoparticles Associated Cytotoxicity Microscopy Analysis
OECD	Organization for Economic Cooperation and Development.
RBCs	Red Blood Cell
S	Significant
Spp.	Species
SE	Standard Error
SEM	Scanning Electron Microscopy
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamate pyruvate transaminase
TEC	Total Erythrocyte Count
TEM	Transmission Electron Microscope
TLC	Total Leucocyte Count
TP	Total Protein
U	Unit
UV-Vis	Ultraviolet-Visible
XRD	X-ray diffraction
µl	Micro liter
W	Watt
WASP	Web Based Agriculture Statistical Software
WBC	White Blood Cells

Introduction

INTRODUCTION

In recent years, nanoparticles have been identified as having a critical role to play in medicine, science, and a variety of biotechnological sectors. Nanoparticles are potentiated for a variety of biomedical applications, including target drug delivery, imaging and biosensors, diagnosis, and disease therapy (Wang and Wang 2014). Nanotechnology has experienced phenomenal growth as a result of its numerous applications in sectors such as material science, chemistry, medicine and bio-nanotechnology. Nanoparticles (NPs) have attracted a lot of attention because of their high surface-to-volume ratio and extremely small size, which results in differences in physical and chemical properties when compared to bulk materials with the same composition (Brust *et al*., 1995). It is emerging as a rapidly expanding field with applications in science and technology for the purpose of creating new nanoscale materials (Albrecht *et al*., 2006).

The therapeutic properties of silver have been known for almost 2,000 years. Since the nineteenth century, silver-based compounds have been used in antibacterial applications. Nanoparticles have been used for a wide range of applications. Silver nanoparticles are being used as antimicrobial agents (Prabhu and Poulose, 2012).

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties viz. optical, electrical, thermal, as well as strong electrical conductivity and biological qualities, are among them (Mukherjee *et al*., 2001., Li *et al*., 2010 and Gurunathan *et al*., 2015).

Many researchers have attempted to develop novel, effective antimicrobial reagents free of resistance and cost in response to the rise and spread of microbial organisms resistant to various antibiotics, as well as the continued emphasis on health-care expenses. Such issues and requirements have prompted a renaissance in the use of silver nanoparticles-based antiseptics, which may be connected to broad-spectrum activity and a lesser proclivity to cause infection. Antibiotics are less likely to cause germ resistance (kim *et al*., 2007)

Nanoparticles are now employed commercially for a wide range of coating applications. Electronics, energy contact actions and pharmaceuticals are only a few examples. AgNPs are widely used in commercial applications. These nanoparticles are used in the pharmaceutical and other industries and medical sciences (Abdelghany *et al*., 2017).

Colorimetric sensors, surface-enhanced raman spectrometry sensors, fluorescence sensors, and chemiluminescence sensors are all examples of sensors that use AgNPs (Jouyban *et al*., 2020). These sensors detect pollutants such as ammonia, heavy metals, and pesticides that are released into the environment. Similarly, the treatment of diseases such as cancer is delayed due to late detection of the sickness; AgNPs have aided in early detection. (Hernandez-Arteaga *et al*., 2017 and Yang *et al*., 2018) Besides, AgNPs are less toxic to mammalian cells than other metal nanoparticles (Zhao and Stevens,1998) and They can easily permeate the cell membrane and function as a possible antimicrobial agent due to their small size. AgNPs tend to agglomerate due to their high surface energy, lowering their antibacterial potential. This can be avoided by putting AgNPs on a solid support structure. (Nie *et al*., 2018).

A variety of techniques are available for the synthesis of silver nanoparticles, including reducing the number of solutions, reverse micelle chemical and photochemical reactions, silver complex heat decomposition, radiation assisted, electrochemical, green chemistry approach, sonochemical, microwave aided process, and most recently via green chemistry route.The use of environmentally friendly materials for the synthesis of silver nanoparticles, such as plant leaf extract, bacteria, fungi, and enzymes, has numerous advantages in terms of environmental friendliness and compatibility for pharmaceutical and other biomedical applications, because toxic materials are not used in the synthesis protocol (Jain *et al*., 2009).The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods (Varghese *et al*., 2015).

Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international market place. These herbs have the

advantage of being readily available, easy to cultivate, inexpensive, and effective (Firenzuoli *et al*., 2008). The use of herbs to treat a variety of diseases has long been a characteristic of traditional practitioners (Pendota *et al*., 2010). Among these herbs *Jatropha curcas* has been touted as a wonder plant. It is a plant that produces seeds with high oil content that can be used as biodiesel (Griner *et al*., 2007 and Gudeta *et al*., 2016). *Jatropha* is a genus of Euphorbiaceae family. Africa, North America, and the Caribbean are all native to these plants. *Jatropha* has been used as a therapeutic herb in many ancient medical systems around the world. This plant is prevalent in the Indian folklores with tremendous ethnobotanical significance (Thomas *et al*., 2008).

Jatropha oil is a clean fuel that minimises greenhouse gas emissions, improves lubricity, and actually reduces on engine wear. Biodiesel made from jatropha is naturally non-toxic (Islam *et al*., 2011). Most parts of *J. curcas* have been widely used for veterinary purposes. The seeds are highly effective against *Strongyloides papillosus* infection in goats (Adam and Magzoub, 1975).

Plant *Jatropha curcas* traditionally used in the treatment of scaticia, dropsy, paralysis, rheumatism, dysentery, diarrohea and various skin diseases. Seeds are used to treat arthritis, jaundice and contractives. Nuts are used as contraceptives. Bark is used as fish poison. Latex is used to inhibit watermelon mosaic virus. Shrub is used in hepatotoxicity and antiobesity. Tender/twig/stem are used in toothache, gum inflammation, gum bleeding, pyorrhoea. Plant sap is used in dermatomucosal disease. Plant extract is used in the treatment of allergies, burns, cuts, wound inflammation, leprosy, leucoderma and smallpox. The water extract of branches used to treat human immunodeficiency virus infection (HIV) and tumor. Plant extract is used to treat wound healing (Sharma *et al*., 2012). The plant also possesses disinfectant, antiparasitic, wound healing, insecticidal, antidiarrhoeal effect, anti-inflammatory activity, antioxidant activity, antimicrobial, anticancer activity, antiviral activity, antidiabetic activity, analgesic activity, hepatoprotective activity, wound healing activity, anticoagulant and procoagulant activity, antifertility activity (abortifacient activity) (Laxane *et al*.,

(2013), Abdelgadir and Van Staden (2013). *Jatropha curcas* leaves exert anti-arthritic activity (Baroroh *et al*., (2014).

The unique physico-chemical properties of silver nanoparticles (Ag NPs) have attracted the scientific community's interest. (Beyene *et al*., 2017) due to their high thermal conductivity, plasmonic properties, chemical stability and antibacterial ability (Chen *et al*., 2008). The usage of Ag is not new; it dates back to Hippocrates' time, when it was used as an antibiotic to treat ulcers (Alexander, 2009). Many commercial products now include AgNPs, including soaps, plastics, food, textiles, catheters, and bandages. Around 383 items are estimated to be based on nano Ag globally, representing for around 24% of all nano-products in use. As a result, scientists are attempting to understand the negative effects on living organisms. Numerous *in vitro* and *in vivo* studies have shown that their toxicity is caused by disruptions in cellular pathways (Bhattacharya *et al*., 2011); nevertheless, their mechanism of action is still unclear. A lot of factors such as size, shape, morphology, surface chemistry, charge, coating/capping agents, agglomeration, purity influence the biological activity of AgNPs and as consequence the adverse effects are different in different cell types (Jo *et al*., 2015). Considering the toxicity of silver nanoparticles, the present research was conducted to evaluate the Sub-acute oral toxicity of *Jatropha curcas* Linn. Leaf extract coated silver nanoparticles in wistar rats (OECD- Guidelines 407) with following objective.

11. Objectives:

1. To synthesize the silver nanoparticles using biological method and coatings of silver nanoparticles with *Jatropha curcas* Linn. aqueous extract.
2. To study sub-acute oral toxicity of *Jatropha curcas* coated silver nanoparticles in wistar rats.
3. To study biochemical and haematological alteration in wistar rats following Sub-acute exposure to *Jatropha curcas* coated silver nanoparticles.
4. To assess adverse effects of long term of exposure (28 days) of *Jatropha curcas* coated silver nanoparticles in liver, kidney and heart in wistar rats.

Review of Literature

REVIEW OF LITERATURE

2.1 Nanoparticles and history of silver nanoparticles:

The prefix nano comes from the Greek word ‘nanos,' which means ‘dwarf,' and refers to objects that are one billionth in size. Richard Feynman, an American physicist renowned as the "Father of Nanotechnology". Nanotechnology field offers us the power of manipulating the atoms or molecules and transforming them into structures having desired geometry and properties. It has both environmental and health applications which includes effective drug delivery and applications in harvesting solar energy etc. It also helps in reducing the use of industrial chemicals and making the environment healthier, safer and worth living. (Sharma, *et al*., 2019)

Nanotechnology has applications in a variety of sectors, including traditional chemical processes, as well as therapeutic and environmental technologies. Drug delivery, ointments, nanomedicine, chemical sensing, data storage, cell biology, agriculture, cosmetics, textiles, the food industry, photocatalytic organic dye–degradation activity, antioxidants, and antimicrobial agents are just a few of the applications in which AgNPs have made significant contributions. Despite the contradictory reports on AgNPs' toxicity, its importance as a disinfectant and antibacterial agent has been recognized (Ahmad *et al*., 2019).

Silver nanoparticles are one of the most important in the fields of nanotechnology and nanomedicine. These materials are outstanding and indispensable due to their unique size-dependent features. Silver nanoparticles have unacceptably harmful effects on human health and the environment, in addition to their antibacterial activity. In addition to argyria and argyrosis, soluble silver compounds can cause liver and kidney damage, eye, skin, respiratory, and intestinal irritation, and blood cell abnormalities. (Panyala *et al*., 2008)

Nanoparticles offer an appealing platform for a wide range of biological applications. *In vitro* and *in vivo*, nanoparticles have already been used for a variety of applications. However, achieving their full potential will necessitate

addressing a number of unresolved concerns, such as the acute and long-term health consequences of nanomaterials, as well as scalable, repeatable manufacturing methods and trustworthy characterization measures for these materials. (De *et al*., 2008)

Herodotus, the father of history, accounts that no persianking, including cirrus, would drink water that was not transported in silver containers, which kept the water fresh for years. This was particularly important in military conflicts, where fresh water from natural sources was not readily available (Grier, 1968).

The ancient Phoenicians, Greeks, Romans, Egyptians, and others also were recorded to have used silver in one for more another to preserve food and water, and this was practiced through World War II. The application of silver plates to achieve better wound healing was used by the Macedonians, perhaps the first attempt to prevent or treat surgical infections. Hippocrates used silver preparations for the treatment of ulcers and to promote wound healing. It is likely that silver nitrate also was used medically because it was mentioned in a pharmacopeia published in Rome in 69 B.C.E. (Hill, 1939).

Alexander (2009), review the historical use of silver nitrate and he exposure that the first record of silver nitrate being used as a medical agent was reported by Gabor in 702–705, and Avicenna used silver filings as a blood purifier in 980 A.D. and also to prevent palpitations of the heart and to treat offensive breath. In this review he recorded that silver nitrate used as a caustic for the treatment of wounds, a practice that continues today. He also written as silver nitrate can be used internally as a counter irritant, as a purgative, and for the treatment of brain infections. On the basis of his review, he concluded that, silver has been a major therapeutic agent in medicine, especially in infectious disease, including surgical infections. Its risk: benefit ratio is advantageous.

Mathur *et al*., (2018) reviewed that, silver has been most broadly studied and used since early times to fight against infections and hinder spoilage in compares onto other antimicrobial agents. Silver is also found to be non-toxic to humans in minute concentrations. Varied applications of silver nanoparticles were

found in the form of wound dressings, coatings for medical devices, silver nanoparticles impregnated textile fabrics, etc., as there is uninterrupted release of silver ions and the devices can be coated by both the outer and inner side hence, alleviating its antimicrobial efficacy. On the whole, the silver nanoparticles due to their unique properties of silver and nano size appear to be promising in pharmaceutical, biomedical and allied fields provided safety data is generated to prove their safety and simultaneously ruling out their toxicity.

2.2 Methods of synthesis of silver nanoparticles:

Three methods of synthesis of silver nanoparticles:

a. Physical

b. Chemical

c. Biological

Silver nanoparticles are generally synthesized by two approaches, (i) “top to bottom” approach and (ii) bottom to top approach. In the top-down approach, suitable bulk material is broken down into smaller fine particles by size reduction using various techniques such as grinding, milling, sputtering, thermal/laser ablation, etc. Thus the "top-down" method consists of mechanical grinding of bulk metals with subsequent stabilisation using colloidal protecting agents, whereas in the bottom-up approach, nanoparticles are synthesised using chemical and biological methods by self-assembly of atoms to new atoms which grow into nano size particles viz. chemical reduction, electrochemical and sono-decomposition (Ahmed *et al*., 2016 and Zhang *et al*., 2016).The most significant benefit of the "bottom to top" approach method is the ability to produce a high number of nanoparticles in a short amount of time.

Chemical procedures have a large yield advantage over physical ones, which have a poor yield. Nanoparticles are typically manufactured using the evaporation condensation procedure in a tube furnace at atmospheric pressure in the "top to bottom" approach. The basic material, which is centred at the furnace, is vaporised into a carrier gas within a boat in this process. The defects in the

surface structure of the product, as well as the other physical qualities of nanoparticles, are strongly dependent on the surface structure in relation to surface chemistry, which is one of the method's main constraints. As a result, a cost-effective and environmentally friendly alternative synthetic approach was unavoidable, resulting in green synthesis (Zhang *et al*., 2016).

2.2.1 Physical method

Tsuji *et al*., (2002) prepared silver nanoparticles in water by using 355, 532, and 1064 nm laser ablation at a relatively high fluence of 36 J/cm2. Nd:YAG laser (Spectra Physics GCR-200) was used for ablation. (Potassium dihydrogen phosphate) KDP crystals were used to create light with wavelengths of 355 and 532 nm. Silver targets (99.99 % Nilaco) were placed in a glass cell with 5 mL deionized water. A quartz lens (f 14 100 mm) was used to focus laser light on the targets' surfaces. A magnetic stirrer was used to stir the solution during ablation. A TEM (JEM-200CX) operating at 200 kv was used to observe colloidal particles. With decreasing the laser wavelength, the mean particle size reduces from 29 to 12 nm, by TEM was observed This could be explained in terms of particle fragmentation caused by self-absorption. He also found that particle size of colloids prepared by laser ablation can also be controlled by changing laser wavelength from 1064 to 355 nm. This technique will be useful to prepared desired size colloids in solutions.

El supikhe *et al*., (2015) synthesized the Ag-NPs by reducing AgNO3 using ultrasonic waves in the presence of κ-carrageenan. By adding 10 ml of 0.1 M AgNO3 to 40 ml of carrageenan. The concentrations of -carrageenan employed were 0.1, 0.15, 0.20, 0.25, and 0.3 wt%, respectively. The solutions were agitated for 1 hour, to get AgNO3/-carrageenan. Ultrasound irradiation was performed using ultrasonic liquid processors immersed directly in the reaction solution (Hielscher ultrasound UP-400S, Teltow, Brandenburg, Germany, 50/100 Hz). The nanoparticles were precipitated then dried at 40°C under vacuum overnight to obtain the Ag-NPs. Result found that the κ-carrageenan and AgNO3 were a colourless suspension; when the suspension was exposed to ultrasonic irradiation at amplitude 50% for 90 min at room temperature, the colour changed from

colourless to dark brown indicating the formation of Ag-NPs in the κcarrageenan suspensions.

2.2.2 Chemical method

Guzman *et al*., (2009) used Sodium Dodecyl Sulphate (SDS) and sodium citrate as the stabilizing compounds to synthesised silver nanoparticles. As reducing agents, hydrazine hydrate solution with a concentration range from 2,0 mM to 12 mM and sodium citrate solution (1,0 mM to 2,0 mM) were utilized. Citrate of sodium was also used as stabilizing agent at room temperature. When sodium citrate was added as a stabilising agent, the translucent colourless solution was transformed into the typical light yellow and pale red colour. The creation of silver nanoparticles was indicated by the appearance of colour. Result indicates that the colloidal solution turned pale brown, pale yellow and pale red confirms the synthesis of silver nanoparticles.

Suriati *et al*., (2014) prepared silver nano particles by a simple chemical reduction method. Silver nitrate (AgNO3) was used as a starting material while trisodium citrate (C6H5O7Na3) and ascorbic acid (C6H8O6) were used as the reducing agent and surfactant, respectively. The reductant (C6H5O7Na3) immediately reduced Ag+ to create metallic Ag atoms throughout the production process. The generated Ag atoms subsequently acted as nucleation centres, catalyzing the reduction of the solution's remaining metal ions. Metal clusters are formed when atoms coalesce, and they are usually stabilised by ligands, surfactants, or polymers. On the basis of result, he concluded that fine dispersion and narrow size distribution of silver nanoparticles can be acquired by a simple, and partly green chemical reduction method. It is believed that ascorbic acid played a significant role in dispersing the particles whilst lowering the degree of particle agglomeration.

2.2.3. Biological method: Green Synthesis of Silver nanoparticles:

Bar *et al*., (2009) conducted research on silver nanoparticles and noted that silver nanoparticles were effectively produced from AgNO3 utilising the latex of *Jatropha curcas* as a reducing and capping agent in a simple green process. 1 ml

crude latex was diluted to 300 ml with triply distilled de-ionized water to make it 3 %, and 20 ml of this latex solution was combined with 20 ml 5 × 10−3 M aqueous silver nitrate solution in a conventional reaction process. Result was found that the silver nanoparticles were progressively formed after heating the mixture at 85°C for 4 hours in an oil bath with steady stirring. The same reactions were carried out with different concentrations of AgNO3 and latex.

Jain *et al*., (2009) synthesized silver nanoparticles using papaya fruit extract by green or biological method. The aqueous extract was made from green unripe papaya fruits, fully ripe papaya fruits and green leaves. For the synthesis of silver nanoparticles, a 1mM aqueous solution of silver nitrate (AgNO3) was prepared. For reduction into Ag+ ions, 10 ml of papaya fruit extract was added to 90 ml of aqueous solution containing 1 mM silver nitrate and maintained at room temperature for 5 hours. Result was found that the papaya fruit extract was added to the silver ion complex aqueous solution, the colour changed from watery to yellowish brown due to the reduction of silver ions, indicating the formation of silver nanoparticles.

Awwad *et al*., (2012) carried out research to evaluate the green synthesis of silver nanoparticles by mulberry leaves extract. After adding 5 mL of mulberry leaves extract to 50 mL of 1x10-3 M aqueous AgNO3 solution at room temperature for 60 minutes, the solution turned grey-black, confirming AgNP formation. AgNO3 solution and mulberry leaves extract concentrations were also changed from 1 to 4 mM and 5 to 10% by volume, respectively. The production of silver nanoparticles was demonstrated by a prominent surface plasmon resonance (SPR) band at 425 nm in the UV-vis spectra.

Lalitha *et al*., (2013) synthesised silver nanoparticles from leaf extract *Azhadirachta indica* to study its anti-bacterial and antioxidant property. Neem leaves weighing 25 g were carefully washed with distilled water for 5 minutes, dried, cut into fine pieces, and heated for 15 minutes in a 500 ml Erlenmeyer flask with 100 ml sterile distilled water and then filtered. In a water solution of 1mM Silver Nitrate, 10 ml of neem extract was added. The sample was then incubated for 24 hours in the dark. UV-visible spectrophotometry was used to determine the

sample's maximum absorbance after 24 hours. The results showed that the leaf extract of *Azhadirachta indica* useful for synthesis of silver nanoparticles.

Namratha and Monica, (2013) discussed the green synthesis of Silver Nanoparticles using *Azadirachta indica* (Neem) extract at room temperature. In a water bath, 25 g of the leaves were added to 100 mL of deionized water and cooked for one hour. The mixture was filtered to yield 20% concentration aqueous extract. Accumulated 100 mL volume in 250 mL Erlenmeyer flask for synthesis of silver nanoparticles, the produced neem leaf broth was mixed with 0.01 M AgNO3 solution at a 1:4 mixing ratio. Ammonium hydroxide (NH4OH) was used to maintain the pH of the reaction mixture in the range of 8. The flask was kept for 4 h in rotary shaker at 80 rpm to achieve homogenous reaction. The dry powders of the silver nanoparticles were obtained after 4 h of reaction period. The colour change from silver nitrate solution to reduced silver nanoparticles is *indica*ted by its change in colour. On the basis of results, she concluded that the shape and size of the nanoparticles produced through bio reduction by Neem leaves extract were strongly dependent on the process parameters like neem broth concentration, mixing ratio of neem extract to AgNO3 solution, interaction time and pH of the solution. She also reported that the minimum interaction time was about 4 hours to obtain the nanoparticles with nearly spherical shape of size below 50 nm.

Husain *et al*., (2015) biosynthesized the silver nanoparticles (AgNP) using 30 cyanobacteria. Cultures were kept in conical flasks (5–5000 ml) with BG-11 growth media and 20 W fluorescent tubes producing a light intensity of 2000 200 lux for 12:12 h light and dark cycles to screen cyanobacterial extracts. For large-scale biomass production, sub-culturing was done at regular intervals. Before lypholization, Arthrospira and Spirulina were harvested by filtration, while the rest of the strains were harvested by centrifugation with distilled water. Prior to freeze drying for lyophilization, the biomass was thoroughly washed with distilled water and crushed in a pestle and mortar for powder preparation. Silver nitrate (1 mg/2 ml) was added to the aforementioned mixture at 30°C to produce AgNP while maintaining a 1 mM solution in the presence of 2000 lux light. In addition,

a 1 mM AgNO3 solution without cyanobacterial biomass extracts was preserved under the same conditions as the control. The formation of nanoparticles from cyanobacterial extracts was detected by a change in the colour of the solution (pale yellow to blackish brown). After 24 hours, suitable aliquots were taken out and UV–VIS spectroscopy in the 300–700 nm range was used to confirm AgNP production.

Ahmed *et al*., (2016) prepared green synthesis of silver nanoparticles using aqueous leaf extract of *Azadirachta indica*. The plant extract serves as both a reducing and capping agent. In an Erlenmeyer flask, a 100 mL, 1 mM silver nitrate solution was prepared. Then, independently, 1, 2, 3, 4, and 5 mL of plant extract were added to 10 mL of silver nitrate solution at a concentration of 1 mM. Variable concentrations of AgNO3 (1 mMe5 mM) were also used to make silver nanoparticles while keeping the extract concentration constant (1 mL). To avoid photo-activation of silver nitrate at ambient temperature, this set-up was incubated in a dark environment. The colour from colourless to brown confirmed the reduction of Ag+ to Ag0. UV-Visible spectroscopy was also used to validate its production.

Al-Shmgani *et al*., (2017) carried out research on biosynthesis of silver nanoparticles (AgNPs) from *Catharanthus roseus* leaf extract and their characterization, as well as antioxidant, antimicrobial, and wound-healing activities were evaluated. The leaf extract was made using the Soxhlet extraction method using methanol as the solvent. An aqueous solution of 2 mM AgNO3 was prepared. At 70°C, the aqueous solution (90 mL) was mixed with 10 mL of leaf extract and magnetically agitated for 3 minutes at 1000 rpm was done. The colour changing from yellowish to reddish brown after adding 2 mM AgNO3 and exposing to heat at 70 °C for 3 min indicates the synthesis of silver nanoparticles.

2.3 Characterization of Silver Nanoparticles:

Amin *et al*., (2012) investigated the green synthesis route for the production of silver nanoparticles using methanol extract from *Solanum xanthocarpum* berry extract (SXE). Synthesized silver nanoparticles were

characterized using UV–visible spectroscopy, powdered X-ray diffraction, and transmission electron microscopy (TEM). Pharmaspec UV-1700 (Shimadzu Corporation, Tokyo, Japan) was used to record the UV-Vis absorption spectra of AgNps. The samples were scanned between 300 and 800 nm, with a resolution of 1 nm and a scan speed of 300 nm/min. The findings revealed that the reaction duration, temperature, and volume ratio of SXE to AgNO3 may all alter the AgNps size and shape, as well as the rate of Ag+ reduction. The nanoparticles were discovered to be around 10 nm in size, monodispersed, and spherical in form.

Banerjee *et al*., (2014) investigated an efficient and long-term method for preparing AgNP from 1 mM aqueous AgNO3 using leaf extracts from *Musa balbisiana* (banana), *Azadirachta indica* (neem), and *Ocimum tenuiflorum* (black tulsi). The characterization of silver nanoparticles was done by UV-visible (vis) spectrophotometer, particle size analyzer (Dynamic light scattering), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive spectroscopy (EDS). On the basis of results obtained he concluded that AgNPs have been appropriately characterized using UV-vis spectroscopy, SEM, TEM and EDS analysis. Results denoted banana leaf extract to be a better reducing agent in comparison to neem and tulsi leaf extracts. FTIR analysis revealed the efficient capping and stabilization properties of these AgNPs.

Banala *et al*., (2015) examined the characterization of Carica papaya leaf extract coated silver nanoparticles by using Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). The spectrometric measurement of biosynthesized silver nanoparticles was performed using an ELICO SL-159 UV–visible spectrophotometer (Andhra Pradesh, INDIA). At 200–800 nm, the decrease of silver was observed on a regular basis. CPL-AgNPs (Carica papaya leaf extract) had absorbance spectra at 470 nm, indicating bio-reduction of silver nitrate into silver nanoparticles, according to UV absorption spectrometric measurement. The particle size was between 50 and 200 nm, and the average size of silver

nanoparticles was between 5 and 40 nm, with a spherical morphology, by SEM and TEM method. On his study he concluded that the characterization of CPL-AgNPs confirmed by UV–visible spectrophotometer, FTIR, SEM, TEM, XRD and EDX techniques. The nanoparticles appeared to be spherical in shape with smooth surface and the size of the particle varied from 5 to 50 nm, but amongst them most of the particles obtained were sized in between 5and 15 nm.

Ibrahim, (2015) synthesised and characterization silver nanoparticles of banana peel extract. The UV-Vis spectrophotometer (Helios Gamma, Thermo Corporation, England) with a resolution of 0.5 nm. SEM with EDX, FESEM, and TEM were used to determine the shape and size of silver nanoparticles. The UV-Vis spectrum of silver nanoparticles revealed a characteristic surface plasmon resonance (SPR) peak at 433 nm and were characterized. X ray diffraction revealed their crystalline nature. Scanning electron microscope and field emission scanning electron microscope showed spherical shaped and monodispersed nanoparticles. Transmission electron microscope confirmed the spherical nature and the crystallinity of nanoparticles. The average size of nanoparticles was 23.7 nm as determined by dynamic light scattering. Energy dispersive X-ray spectroscopy analysis showed the peak in silver region confirming presence of elemental silver. Fourier transform infrared spectroscopy affirmed the role of BPE as a reducing and capping agent of silver ions.

Ahmed *et al.,* (2016) conducted research to synthesis silver nanoparticles utilising *Azadirachta indica* aqueous leaf extract. For characterization UV-Vis spectral analysis was done by using Shimadzu UV-visible spectrophotometer (UV-1800, Japan). UV-Visible absorption spectrophotometer with a resolution of 1nm between 200 and 800nm was used. FT-IR spectra of were recorded on Perkin Elmer 1750 FTIR spectrophotometer. The particle size and surface morphology were analysed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 120kV. The UV-vis spectra recorded, implied that most rapid bio-reduction was achieved using *Azadirachta indica* leaf extract as reducing agent. The UV-vis spectra and visual observation revealed that formation of silver nanoparticles occurred rapidly within 15min. Transmission

electron microscopy (TEM) reveals that the silver nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be having structures of irregular shape. The nano-particles are homogeneous and spherical which conforms to the shape of SPR band in the UV-visible spectrum. The particle size agrees with that calculated from dynamic light scattering histogram with average diameter of around 34nm.

Raza *et al*., (2016) noted that the Scanning electron microscopy (SEM), UV-visible spectroscopy (UV-VIS), and X-ray diffraction (XRD) techniques were used to examined the shape, size, and structural features of the nanoparticles. The diameters of spherical AgNPs were determined to be in the range of 15 to 90 nm, whereas the lengths of the edges of the triangular particles were around 150 nm, as shown by SEM. As measured by UV-VIS, the distinctive surface plasmon resonance (SPR) peaks of several spherical silver colloids occurred in the wavelength range of 397 to 504 nm, whereas triangular particles showed two peaks, one at 392 nm and the other at 789 nm. The XRD spectra of the produced samples revealed that metallic AgNPs have a face-centered cubic crystalline structure.

Al-Shmgani *et al*., (2017) characterized the synthesized AgNPs by using Hitachi U-2910Spectrophotometer (Tokyo, Japan). UV-vis spectroscopic analysis was performed via continuous scanning at the range of280–760 nm. The size was calculated by using the following Scherrer formula was used: D ¼ 0.94 k/b Cos h. FTIR analysis was carried out by using FTIR 8400S spectrometer in attenuated total reflection mode and spectral range of 4000–400 cm_1 with a resolution of4 cm_1. Finally, the morphology of the synthesized AgNPs was determined via AFM analysis using AA 3000 Scanning Probe Microscope. The XRD pattern of AgNPs produced from the leaf extract was further illustrated and confirmed by the characteristic peaks observed in the XRD spectra. Four Bragg's reflection patterns at 2h, namely, 32.00, 38.50, 44.40,64.50, and in the entire spectrum of value ranging within10–90, were interpreted from XRD. These patterns corresponding to (122), (111), (200), and (220), respectively. Thus, the UV-vis results indicated that *C. roseus* leaf extract can possibly reduce Ag to AgNPs, and such finding was

further inspected to confirm the synthesis of AgNPs from C. roseus. The XRD pattern clearly revealed that AgNPs are crystalline in nature. The sharp peaks clearly indicate the cubic crystalline nature of the synthesized AgNPs which is in nanoscale. Hence, C. roseus leaf extract is promising target in the development of AgNPs.

Hamouda *et al*., (2019) used UV-Visible absorption spectrum, Fourier transforms infrared (FT-IR), transmission electron microscopy (TEM), and scanning electron microscope (SEM) for biological characterization of silver nanoparticles from the cyanobacterium Oscillatoria limnetica. The quasi-spherical Ag-NPs form was found using TEM images with a size range of 3.30–17.97nm. FT-IR analysis revealed the existence of free amino groups as well as sulfur-containing amino acid derivatives that operate as stabilisers, as well as the presence of sulphur or phosphorus functional groups that may attach silver. In UV-Visible absorption spectrum, silver ions forming spherical AgNPs ranging from 100–200nm in size.

2.4 Drug delivery aspects of silver nanoparticles:

Sahana *et al*., (2014) formulated antibacterial cold cream using biosynthesized silver nanoparticle from flower extract of Cassia auriculata as a reducing agent. The cold cream containing the flower extract alone showed a minimum inhibitory effect on the pathogens whereas the bactericidal cold cream containing the NPs synthesized from the flower extract showed an excellent antibacterial activity.

Veerakumar *et al*., (2014) synthesized AgNP using *Heliotropium indicum* plant leaves and displayed larvicidal effects on A. stephensi, A. aegypti and C. quinquefasciatus vector mosquito species. The (lethal concentration) LC50 and LC90 values of *H. indicum* aqueous leaf extract appeared to be effective against A. stephensi followed by A. aegypti and C. quinquefasciatus further ensuring the potential to be used as an ideal ecofriendly approach for the control of mosquito vector species.

Appadurai and Rathinasamy, (2015) developed formulation with improved antimitotic, apoptotic and antiproliferative activity of plumbagin (PBL) by interacting AgNPs with the anticancer agent PLB, which will further enhance the internalization of PLB. Hence, AgNP could be a promising and potent drug delivery system for enhanced activity of PLB in cancer treatment.

Firdhouse and Lalitha, (2015) described the synthesis of silver nanoparticles using the aqueous extract of Alternanthera sessilis as a reducing agent by sonication, espousing green chemistry principles. The cytotoxic effect of biosynthesized silver nanoparticles was studied by MTT ([(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay against breast cancer cells (MCF-7 scell line) and the NPs exhibited significant cytotoxic activity with IC50 (Half maximal inhibitory concentration) value 3.04 lg/mL compared to that of standard cisplatin. The data obtained in the study revealed the potent therapeutic value of biogenic silver nanoparticles and the scope for further development of anticancer drugs.

Kravets *et al*., (2015) demonstrated the application of luminescent silver nanoparticles as imaging agents for neural stem and rat basophilic leukaemia cells and further studied the experimental size dependence of the extinction and emission spectra for silver nanoparticles. The nanoparticles were functionalized with fluorescent glycine dimers. Spectral position of the resonance extinction and photoluminescence emission for particles with average diameters ranging from 9 to 32 nm was inspected. The nanoparticles were able to penetrate cell membranes of rat basophilic leukaemia and neural stem cells fixed with paraformaldehyde. Additionally, toxicity studies were performed and it was found that towards rat basophilic leukaemia cells, luminescent silver nanoparticles had a toxic effect in the silver atom concentration range of 10–100 lM.

Borrego *et al*., (2016) tested the antiviral activity of AgNP formulated as Argovit against Rift valley fever virus, which represents an important zoonotic pathogen and potential biological weapon. Silver nanoparticles combined with the virus will help to plan a more effective application of Argovit against viral infections both prophylactically and therapeutically.

Casanas *et al*., (2016) reported the synthesis of AgNPs (3.89 ± 0.90 nm) through the polyol method, the generation of AgNP nanocarriers and the bio-conjugation protocol of the nanocarrier with soybean agglutinin (SBA). The free AgNPs, the AgNP nanocarriers, and the SBA-bioconjugatedAgNP nanocarriers were tested for cytotoxicity in breast cancerous (MDA-MB-231and MCF7) and non-cancerous (MCF 10A) cells, using the MTT assay. AgNPs demonstrated cytotoxic activity in vitro, the non-cancerous cells (MCF 10A) being more sensible than the cancerous cells (MDA-MB-231 and MCF7) showing LD50 values of 128, 205 and 319 lM Ag, respectively; the nanoencapsulation diminished the cytotoxic effect of AgNPs in non-cancerous cells, alleviating the effect on the cancer-derived cells, whereas the SBA-bioconjugation allowed AgNP cytotoxic activity with a similar behaviour to the nanocarriers.

Kajani *et al*., (2016) synthesized AgNP using extract of *T. baccata* plant, which showed better anticancer activity than the previously reported ones due to the synergistic role of Taxus compound in the nanoparticle cytotoxicity. It was concluded that the biogenic synthesis of AgNPs in combination with targeted therapy of tumours may give rise an alternative approach for efficient treatment of cancers with fewer side effects.

Khan *et al*., (2016) described the method of synthesis of nanoparticle using *Heliotropium crispum* plant extract. The antibacterial action of AgNPs was found to be species independent and strictly strain dependent as both gram-negative P. aeruginosa (PA) and A. baumannii (AB) and gram-positive Multiple Drug Resistant S. aureus (MRSA) exhibited differential inhibition zones and decrease in bacterial viability.

Mc Laughlin *et al*., (2016) prepared sprayable formulation of AgNPs by exchanging citrate capping agents with LL37- SH peptide thereby forming antimicrobial composite that was acting as an anti-infective and anti-biofilm barrier against P. aeruginosa infection without having any toxic or anti-proliferative effects on human skin fibroblasts and when used as in vivo wound model, the composite remained in the affected area without infiltrating other tissue and organ.

Perez-Diaz *et al*., (2016) prepared chitosan gel loaded with AgNPs to check the anti-biofilm capacity and application in chronic wounds with respect to standard silver sulphadiazine. The results showed that the developed formulation could be used for prevention and treatment of infections in chronic wounds as it completely inhibits the formation of biofilm and kill bacteria in established biofilm.

Rath *et al*., (2016) explored the wound healing capacity of collagen nanofiber mats containing silver nanoparticle. In in vivo study, the wound healing rate of composite nanofiber mats was found to be higher owing to their intrinsic antibacterial, anti-inflammatory, controlled drug release profile and haemostatic properties compared with plain collagen nanofiber.

Tutaj *et al*., (2016) synthesized AgNP using antifungal agent amphotericin B acting both as reducing as well as capping agent. An excellent anti-fungal activity of AmB AgNPs was ascribed to the synergistic effect of antifungal activity of amphotericin-B and antimicrobial properties of silver.

Wildt *et al*., (2016) introduced a novel technique, nanoparticle associated cytotoxicity microscopy analysis (NACMA), which integrates fluorescence microscopy detection using ethidium homodimer-1, a cell permeability marker that binds to DNA after a cell membrane is compromised (a classical dead-cell indicator dye), with live cell time-lapse microscopy and image analysis to concomitantly enquire silver nanoparticle accumulation and cytotoxicity in L-929 fibroblast cells. Studies conducted on 10, 50, 100 and 200 nm silver nanoparticles disclosed size dependent cytotoxicity with particularly high cytotoxicity from 10 nm particles. In addition, NACMA results, when combined with transmission electron microscopy imaging, reveal direct affirmation of intracellular silver ion dissolution and possible nanoparticle reformation within cells for all silver nanoparticle sizes.

Azizi *et al*., (2017) developed a novel nano-composite with the aim of making specific targeting of silver nano particles as a drug for tumour cells and developing new anticancer agents. Albumin coated silver nanoparticles (ASNPs)

were synthesized, and their anti-cancerous effects were evaluated against MDA-MB 231, a human breast cancer cell line. The morphological changes of the cells were observed by inverted, florescent microscopy and also by DNA ladder pattern on gel electrophoresis revealing that the cell death process occurred through the apoptosis mechanism. It was found that ASNPs with a size of 90 nm and negatively charged with a zeta-potential of about 20 mV could be specifically taken up by tumour cells. The LD50 of ASNPs against MDA-MB 231 (5 lM) was found to be 30 times higher than that for white normal blood cells (152 lM) suggesting ASNPs as a good candidate as chemotherapeutic drug.

Bilal *et al*., (2017) synthesized AgNPs loaded chitosan-alginate construct from methanolic extract of E. helioscopia and antibacterial activities against six clinically pathogenic strains including S. aureus, P. aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Morganella morganii and Haemophilus influenza were investigated. All construct exhibited excellent biocompatibility for normal cell line, i.e., L929 and anti-cancer efficacy against HeLa cells. Thus, the newly engineered construct could be a useful candidate for biomedical applications.

Castangia *et al*., (2017) synthesized grape-silver nanoparticles stabilized by phospholipids vesicles, which inhibited proliferation of S. aureus and P. aeruginosa providing safeguard of keratinocytes and fibroblast against oxidative stress that could be used as topical formulation for skin damages.

Goyal *et al*., (2017) synthesized AgNP using b-glucan replacing conventional reducing agents with biocompatible and structural compatible sugar molecules. An efficient nanoemulsions delivery method for AgNP was developed wherein DOX was encapsulated in nanoemulsions containing AgNP and displayed antibacterial effects together with antitumor efficacy, which is quite useful in treatment of cancer cells, while preventing microbial infection. The effective loading of drug was about 15–30%.

Jha *et al.,* (2017) synthesized AgNPs from Ocimum tenuiflorum extract followed by study of AgNP loaded multi-walled carbon nanotubes (MWCNT)

with mammalian sperm to evaluate the increased targeting potential for the development of portable diagnostic tool for the infertility management. Advanced frequency modulation (AFM) demonstrated the loading of AgNP inside MWCNT as surface height of MWCNT increased from 22 to 32 nm, which in turn assured the encapsulation of 10nm size of AgNP inside the tube.

Kumar *et al*., (2017) reported green synthesis of AgNP by *Jatropha curcas* and *Lannea grandis*, which further demonstrated low MIC (Minimum inhibitory concentration) and low minimum biofilm eradication concentration against C. albicans biofilm. The formulation developed was stable and cytotoxic against goat blood RBC and it could be further used for treatment of C. albicans associated infection.

Manukumar *et al*., (2017) developed thymol-loaded chitosan AgNPs by novel route using chitosan as reducing agent and thymol as capping agent, which is highly efficacious against multiple food-borne pathogens and is potent in controlling the human diseases induced by both gram- negative and gram-positive bacteria.

Mugade *et al*., (2017) synthesized mannan sulphate capped silver nanoparticles, which appear a promising topical agent with increased wound healing properties due to faster uptake of mannose receptor and increase site specific delivery. These stable MS-AgNPs exhibited enhanced cytocompatibility, targeting potential and cellular uptake in murine macrophages, human skin fibroblasts and human keratinocytes.

Patil, (2017) synthesized silver nanoparticle via green synthesis using extract of *Lantana camara* L. leaves and found these NPs to exhibit dose dependent antioxidant potential comparable to that of standard ascorbic acid. AgNPs also showed significant antimicrobial activity against gram-positive staphylococcus aureus than gram negative pseudomonas aeruginosa and E. coli comparable with standard, ciprofloxacin.

Sadat *et al*., (2017) developed high drug-loaded Imatinib-loaded silver nanoparticle for potent bioavailability and decreasing dose frequency which is

important in antitumor drug delivery to breast cancer cells, which was incited by apoptosis rather than necrosis. They concluded that green synthesized silver nanoparticles are promising sustained release system to the Imatinib and will be potentially useful for controlled drug delivery.

Yamada *et al*., (2017) immobilized silver nanoparticles on the surface of yttria stabilized zirconia (YSZ) and tested for antibacterial activity against S. aureus, S. mutans, E. coli and A. actinomycet emcomitans, which were found to be concentration dependent on AgNPs whereas excellent antimicrobial activity against E. coli was observed and no cytotoxic effects on L929 cells were detected at coating concentrations below 2.5 mM. Further, AgNP-coated YSZ can be potentially used to control dental caries and periodontal disease.

Alves *et al*., (2018) formulated antimicrobial thermo-responsive gel by interaction of AgNP with PVP (Polyvinylpyrrolidone) and PVA (polyvinyl alcohol) polymers and thereafter incorporated in CUR/P407 (1:2) solid dispersion into a polymer dispersion of 20% P407 an displayed good antioxidant activity. The Minimum Inhibitory Concentration (MIC) values were efficacious for Gram negative bacteria than for Gram positive ones and antibacterial activity of curcumin was alleviated in presence of AgNP for use as hydro gel.

2.5 Applications of Silver nanoparticles:

Asharani *et al*., (2009) conducted research on anti-proliferative activity of silver nanoparticles. In vitro, different dosages of Ag-nps were given to normal human lung fibroblasts (IMR-90) and human glioblastoma cells (U251). Endocytosis (clathrin-mediated process and 22 icropinocytosis) was the major mode of Ag-nps uptake, which was accompanied by a time-dependent rise in exocytosis rate. In both the cytoplasm and the nucleus, electron micrographs indicated a consistent intracellular distribution of Ag-np. Human cells treated with Ag-np showed chromosomal instability and mitotic arrest. Normal human fibroblasts recovered quickly from their arrest, whereas cancer cells failed to proliferate. Their findings show that cancer cells are vulnerable to harm from Ag-np-induced stress due to a lack of recovery. Ag-np is reported to function either

directly or indirectly through intracellular calcium transients and chromosomal abnormalities, as well as catabolic enzyme activation. The signalling cascades are thought to play a role in cytoskeleton deformations and, as a result, cell proliferation inhibition.

Lara *et al*., (2012) reported that variety of diverse in vitro assays are used to analyse silver nanoparticles to determine antiviral action against HIV-1. Silver nanoparticles exert anti-HIV activity at an early stage of viral replication, most likely as a virucidal agent or as an inhibitor of viral entry. Silver nanoparticles bind to gp120 and block CD4-dependent virion binding, fusion, and infectivity, performing as a virucidal agent against cell free virus (laboratory strains, clinical isolates, T and M tropic strains, and resistant strains) and cell-associated virus. Furthermore, silver nanoparticles stop HIV-1 from progressing to the next stage of its life cycle. They concluded that these characteristics make them a broad-spectrum, non-resistant agent that could be utilised to prevent the spread of HIV-1 strains**.**

Gurunathan *et al.,* (2015) conducted research on antiangiogenic properties of silver nanoparticles in5- to 6-week-old C57BL/6 mice. He found that Ag-NPs, like PEDF, could suppress vascular endothelial growth factor (VEGF)-induced cell proliferation, migration, and development of capillary-like tubes in bovine retinal endothelial cells. Ag-NPs may have the ability to suppress angiogenesis, a critical stage in tumor growth, invasiveness, and metastasis, according to research. In the mouse Matrigel plug assay, Ag-NPs also significantly prevented the creation of new blood micro vessels caused by VEGF. He noted that the Ag-NPs can inhibit angiogenesis by inhibiting the activation of the PI3K/Akt signaling pathways.

Abbasi *et al*., (2016) reviewed that the *Tephrosia tinctoria* stem extracts mediated AgNP synthesis was used for control of blood sugar levels. AgNPs scavenged free radicals, reduced the levels of enzymes that bring about the hydrolysis of complex carbohydrates (a-glucosidase and a-amylase), and as a result of which there is an increase in consumption rate of glucose.

Chung *et al*., (2016) reviewed that the pharmaceutical industry seems to be one of the largest beneficiaries of AgNPs; employing these nano materials as antimicrobial and anti-fungal preparations. Silver has been used anciently for burns, wounds and bacterial infections; the utility of AgNP in these treatments is better appreciated in modern context due to growing overwhelming antibiotic resistance in bacteria. AgNPs have been shown to improve efficiency of cancer treatments by increasing effectiveness of drug delivery and producing anti-tumorogenic effects, which display great capability in cancer therapeutics. Studies concentrating on the therapeutic applications of AgNPs in the gastrointestinal tract have displayed that gastric cell can be sensitized to radiation by the use of AgNP and they may bypass the stomach and instead release the drug in small intestine.

Cox *et al*., (2016) studied that the silver nanoparticles as antimicrobial agents have been used largely in the health industry, food storage, textile coatings and a number of environmental applications. *Indigofera aspalathoides* extract mediated silver nanoparticles were assessed in wound-healing applications following excision in animal model. Chrysanthemum morifolium extract mediated AgNPs were added to clinical ultrasound gels, which are used with an ultrasound probe, and were found to possess bactericidal activity, aiding to the sterility of the instrument.

Wang *et al*., (2016) study was undertaken to evaluate green synthesis of silver nanoparticles by Bacillus methylotrophicus, and their antimicrobial activity. He tested antimicrobial activity of AgNPs against a variety of pathogenic microorganisms, including Candida albicans, Salmonella enterica, E. coli, and Vibrio parahaemolyticus. The strains were completely resistant to these antibiotics, but sensitive to AgNPs, according to the findings. Result was found that AgNPs have more antimicrobial activity, compared to standard antibiotics.

Singh *et al*., (2017) reviewed that the silver nano-particles (AgNPs) are one of the most extensively utilised nano-materials in personal care products, dressings as treatments for external wounds, ointments, and surgical tools, because of its strong antibacterial action. Antibacterial activity of AgNPs is broad,

affecting both gram-negative and gram-positive bacteria, including antibiotic-resistant species. Antiviral properties of AgNPs (diameter 5-20 nm, average diameter 10 nm) have been demonstrated against HIV-1, hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, and monkey pox virus. The anti-inflammatory capabilities of AgNPs, as well as nano silver-derived solutions and products, have been demonstrated.

Abdelghany *et al*., (2017) reported that the unique properties of AgNPs, which have a wide range of applications, including antibacterial, antifungal, antivirus, and anticancer drugs, larvicidal excellent catalytic natural action toward dye degradation, excellent antioxidants, treatment of diabetes-related complications, and wound healing.Combining antibiotics with AgNPs to manage microbial infections is a contemporary method for enhancing antibiotic efficacy, as evidenced by AgNPs' damage to microbial deoxyribonucleic acid.

Burdusel *et al.,* (2018) reviewed research on biomedical applications of silver nanoparticles. Drug delivery, wound dressing, tissue scaffolding, and protective coating applications can all benefit from AgNP-based nanosystems and nanomaterials. Anti-inflammatory, anti-oxidant, antibacterial, and anticancer biosubstances were tested as carriers using AgNP-based nanosystems.The intrinsic antibacterial properties of AgNPs were linked to physicochemical characteristics such as size, shape, concentration, surface charge, and colloidal state.

De matteis *et al.,* (2018) conducted research on silver nanoparticles and noted their synthetic routes, in vitro toxicity and theranostic Applications for Cancer Disease.The bactericidal effect of silver nanoparticles produced by a unique electrochemical technique On E. coli, Staphylococcus aureus, Aspergillus niger, and Penicillium phoeniceum cultures. The studies revealed that manufactured silver nanoparticles have a strong antibacterial/antifungal effect when added to water paints or cotton fabrics. Smaller silver nanoparticles have been proven to have stronger antibacterial/antifungal activity.

Nakkala *et al*., (2018) examined the anticancer efficacy of silver nanoparticles (AgNPs) made from aqueous rhizome extract of Acorus calamus (ACRE) was tested in vitro. The MTT experiment revealed that all of the cancer cell lines treated with ACAgNPs (Acorus calamus silver nanoparticles) had lower cell viability. For Hep2, COLO 205, and SH-SY5Y cells, the IC50 values of ACAgNPs were determined to be 29.5 g/mL, 99.4 g/mL, and 149.4 g/mL for 24 hours, and 16.4 g/mL, 73 g/mL, and 97.2 g/mL for 48 hours, respectively (Supplementary data S4). Based on these findings, more susceptible Hep2 cells were chosen for additional experiments to determine the method of action of ACAgNPs due to their low IC50 values. Apoptotic bodies in Hep2 cells treated with ACAgNPs were orange in colour, indicating late apoptosis. The majority of anticancer medicines cause apoptotic cell death in cancer cells by affecting oxidant/antioxidant balance. As a result, DCFH-DA (dichlorofluorescin diacetate) staining was used to measure ROS levels in Hep2 cells treated with ACAgNPs. Green fluorescence intensity was found to be higher in ACAgNPs-treated Hep2 cells than in untreated cells, implying higher reactive oxygen species levels in treated cells.

Dong *et al*., (2019) carried out research on silver nanoparticles to evaluate antibacterial activity of silver nanoparticles of different particle size against Vibrio Natriegens.The antibacterial activity of the NP-treated bacterial culture was further confirmed by inoculating it on Muller-Hinton agar (MHA) plates. UV–visible spectrophotometry and comet assays were used to evaluate the bacteriostatic effects. The smaller the particle size of Ag-NPs, the lower the (minimum inhibitory concentration) MIC and (minimum bactericidal concentrations (MBC) MBC, indicating stronger antibacterial activity.The results conclude that different particle sizes and concentrations of Ag-NPs were efficient, and the smaller the particle size of Ag-NPs, the stronger the antibacterial activity.

Xu *et al*., (2020) reviewed research on silver nanoparticles and noted that the AgNPs have been extensively used for their antibacterial and anticancer effects. AgNPs have been demonstrated to exhibit broad-spectrum antibacterial effects against bacteria, fungi, and viruses. Furthermore, AgNPs can efficiently

harm or kill nematodes and worms. AgNPs' antibacterial properties are influenced by a number of parameters, including their size, shape, dosage, and stabiliser. AgNPs may have differing antibacterial actions against Gram-positive and Gram-negative bacteria, which is interesting. AgNPs have anticancer effects that are extensive in scope. Although the exact mechanisms of AgNPs' antibacterial and anticancer effects are still unknown, several studies have been conducted to test theories. By triggering membranene breakdown, ROS generation, DNA damage, enzyme inactivation, and protein denaturation, AgNPs can inhibit or kill bacteria. AgNPs can be used in a variety of medical applications, including bone restoration and wound healing, in addition to antibacterial and anticancer characteristics. AgNPs can be used as a dental material addition or as a vaccine adjuvant.

2.6 Toxicity of Silver Nanoparticles:

Shavlovski *et al.,* (1995) investigated, embryotoxicity in female rats that had been orally administered 50 mg of silver chloride/animal (corresponding to 190 mg of silver/kg of b.wt./day) during days 1–20 of the term. Post-implantation lethality was found to be increased, and the incidence of visceral damage in the offspring was considerably higher compared to the control group. Moreover, all of the newborns died within 24 h of birth.

Zmeili *et al.,* (1998) investigated the acute and sub-chronic toxicity of a new antismoking (A.S.) mouthwash (0.5% silver nitrate as the active ingredient) in male and female rats and rabbits. The LD50 values for ip administration in male and female rats were 35.7 and 37.2 mg/kg body weight, respectively. The corresponding values in male and female rabbits were 113 and 128 mg/kg body weight, respectively. The oral LD50 values of the mouthwash in male and female rats were 428 and 433 mg/kg body weight, respectively. The corresponding values in male and female rabbits were 1261 and 1320 mg/kg body weight, respectively. Postmortem and histopathological examination revealed congestion, edema, hemorrhage, and mucosal necrosis with brown pigment deposition in the upper gastrointestinal and respiratory tracts for the orally treated animals and ascitis, peritoneal fat necrosis, and pigment deposition in ip administered animals.

Subchronic toxicity involved administration of low (1.5 mg/kg), intermediate (15 mg/kg), and high (150 mg/kg) doses of A.S. mouthwash by swabbing the oral cavity daily for 30 consecutive days. Body weight, hematologic observations, and histopathological examination showed no significant differences between control and treated animals, except for dark coloration in teeth and increased platelet counts in treated rats.

Kim *et al.,* (2008) studied the oral toxicity of silver nanoparticles (60 nm) over a period of 28 days in Sprague-Dawley rats following OECD test guideline 407. Eight-week-old rats, weighing about 283 gm for the males and 192 gm for the females, were divided into four 4 groups (10 rats in each group): vehicle control, low-dose group (30 mg/kg), middle-dose group (300 mg/kg), and high-dose group (1000 mg/kg). After 28 days of exposure, the blood biochemistry and hematology were investigated, along with a histopathological examination and silver distribution study. The male and female rats did not show any significant changes in body weight relative to the doses of silver nanoparticles during the 28-day experiment. However, some significant dose-dependent changes were found in the alkaline phsophatase and cholesterol values in either the male or female rats, seeming to indicate that exposure to over more than 300 mg of silver nanoparticles may result in slight liver damage. There were no statistically significant differences in the micronucleated polychromatic erythrocytes (MN PCEs) or ratio of polychromatic erythrocytes among the total erythrocytes after silver nanoparticle exposure.

Panyala *et al.,* (2008) The unique physical and chemical properties of silver nanoparticles make them excellent candidates for a number of day-to-day activities, and also the antimicrobial and anti-inflammatory properties make them excellent candidates for many purposes in the medical field. However, there are studies and reports that suggest that nanosilver can allegedly cause adverse effects on humans as well as the environment. It is estimated that tonnes of silver are released into the environment from industrial wastes, and it is believed that the toxicity of silver in the environment is majorly due to free silver ions in the aqueous phase. The adverse effects of these free silver ions on humans and all

living beings include permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory, and intestinal tract irritations; and untoward changes in blood cells.

Kim *et al*., (2010) examined the oral toxicity of silver nanoparticles (56 nm) in F344 rats using OECD test guideline 408. The rats were divided into 4 groups (10 rats in each group): vehicle control (0.5% carboxymethylcellulose, CMC), low-dose group (30 mg/kg/day), middle-dose group (125 mg/kg/day), and high-dose group (500 mg/kg/day). At the age of five weeks, they were exposed to silver nanoparticles by gavage for 13 weeks of repeated oral administration (dosing volumes were 10 ml/kg). Although there were no significant changes in food or water consumption during the study period, male rats' body weight decreased significantly after 4 weeks of treatment. The male and female rats showed significant dose-dependent alterations in alkaline phosphatase and cholesterol, indicating that exposure to more than 125 mg/kg of silver nanoparticles may cause minor liver damage. In treated animals, histopathological analysis revealed a greater rate of bile-duct hyperplasia, with or without necrosis, fibrosis, and/or pigmentation. Silver accumulated in all tissues studied in a dose-dependent manner. In the kidneys, there was a gender-related differential in silver accumulation, with female kidneys accumulating twice of silver in male kidneys. From research it was concluded that the target organ for the silver nanoparticles in both male and female rats was liver. The current investigation suggested a NOAEL (no observable adverse effect level) of 30 mg/kg and a LOAEL (lowest observed adverse effect level) of 125 mg/kg.

Hadrup *et al*., (2012) investigated subacute oral toxicity of nanoparticulate and ionic silver in rats. In the 28-day oral toxicity study, 4-week-old 44 female in four groups and 12 four-week-old males were in two groups were sellected. Animals received orally by gavage either 11.5 mg/mL PVP (vehicle control) (10 females and 6 males administered twice a day) or polyvinyl pyrrolidone stabilised Ag-NPs in doses of 2.25 mg Ag/kg bw/day (eight females), 4.5 mg Ag/kg bw/day (8 females) or 9.0 mg Ag/ kg bw/day (10 females and 6 males administered twice

a day with 4.5 mg/kg bw) or 14 mg Ag-acetate/kg bw/day (equal to 9 mg silver/kg bw/day) in 11 mg/mL PVP (eight females administered twice a day). On day 18, animals were placed individually in metabolic cages, with free access to water but not to feed. In female rats administered Ag-NP, haematocrit was increased in the high-dose group, MCH was lower in all groups, MCHC was lower for the medium and high doses and P\0.01, respectively vs. controls). In the male Ag-NP group, lower MCHC was the only haematological parameter significantly different from the vehicle controls. Differential counts revealed no differences in distribution of WBC. In all Ag-NP groups, plasma and urine clinical biochemical parameters were unaffected. In the Ag-acetate group, plasma ALP (Alkaline phosphatase) activity was higher (P\0.05) and plasma urea concentration was lower compared with vehicle controls. Histological examination of the liver, kidneys, ileum and myocardium did not exhibit any differences compared with the vehicle control groups of Ag-NP and Ag-acetate respectively.

Van der Zande *et al*., (2012) evaluated immunotoxicity of silver nanoparticles. In this experiment rats were exposed daily for 28-days by oral gavage to 90 mg/kg body weight (bw) Ag < 20 or Ag < 15-PVP particles, to 9 mg/kg bw AgNO3, or to their respective vehicle solutions only. The 10 times lower dose of AgNO3 was intentionally chosen in the range of the soluble silver content in the Ag < 20 suspension. Two different negative control groups were included since the vehicle solution of the Ag <20 particles contained additional AgNP stabilizing agents. At day 29 the animals were euthanized and organs were collected. Wash-out groups, identically exposed for 28-days to Ag < 20, Ag < 15-PVP, or AgNO3, were euthanized at day 36 and 84. The determination of IgM and IgG levels in plasma indicated that exposure to silver did not affect total serum IgG and IgM levels. Additionally, proliferation of mitogenically stimulated T-or B-cells, isolated from the spleen and mesentric lymph node (MLN) was not significantly altered in the exposure groups. Furthermore, cytokine levels in the supernatants of these stimulated T- and B-cells were unaffected in the exposure groups. Finally, the activity of natural killer (NK) cells, isolated from the spleen, was unaffected by the silver exposure. Taken together, these results indicate that oral AgNP exposure does not result in nonspecific immune responses *in vivo.*

Adeyemi and Adewumi, (2014) research was undertaken to evaluated biochemical evaluation of silver nanoparticles in wistar rats. Forty male rats were divided into eight experimental groups of five animals each. Groups B to H were given daily doses of 100, 1000, and 5000 mg/kg of silver nanoparticles (AgNPs) for 7, 14, and 21 days, respectively. The rats were slaughtered once the treatments were stopped, and the blood and other critical organs were collected. Rat exposure to AgNPs resulted in significant changes in serum and tissue AST, ALT, and ALP levels. Rat serum and tissue AST and ALT levels were considerably reduced and elevation in total protein levels in rat liver after exposure to 100 mg/kg AgNPs. AgNPs treatment increased ALP levels in rat serum and tissues.

Ema *et al.,* (2017) reviewed, reproductive and developmental toxicity of silver nanoparticle in laboratory animal. Following oral administration of AgNPs (15nm in size) for 2–4 weeks, at 30 or 300 mg/kg/d caused changes in histopathology including inflammation, apoptosis and degenerated follicles in the ovaries of female rats. Male and female mice fertility was checked by intravenously injecting with AgNPs and it was concluded that that their gene expressions were down regulated resulting in debilitated development of spermatocytes and oocytes. Oral AgNPs (15 nm) exhibit ovarian toxic potential at 30 mg/kg/d. Intravenous AgNPs (20 nm) lowers the number of follicles and augment embryonic deaths at 0.5 mg/kg.

Nosrati *et al*., (2021) conducted research on silver nanoparticles to evaluate its toxicity. The male wistar rats were given silver nanoparticles solution in doses of 30, 125, 300, and 700 mg/kg. The effects were more pronounced in the 30 and 125 mg/kg groups. In both the cortex and the medulla, collagen intensity was raised in 30 treated groups. The middle dose groups (125 and 300 mg/kg) showed much more apoptosis. Reverse transcription- polymerase chain reaction (RT-PCR) results showed that Bcl-2 and Bax mRNAs were elevated in treated groups, and epidermal growth factor (EGF), tumor necrosis factor and Transforming growth factor-1 (TNF and TGF-1) data showed that AgNPs caused larger gene expression changes in the 30 and 700 mg/kg groups than in the control

group. From result it was concluded that the AgNPs played a critical role in their *in vivo* renal toxicity.

2.7 *Jatropha curcas*:

Jatropha curcas L. is a multipurpose plant that belongs to the Euphorbeaceae family, includes over 175 species of succulents, shrubs, and trees. The Portuguese traders spread *Jatropha* as a prized hedge plant throughout Africa and India (Thomas, *et al.,* 2008). *Jatropha curcas* is a drought-resistant shrub or tree native to Central and South America, Africa, India, and Southeast Asia. In "Species Plantarum," it was the first to name the physic nut *Jatropha* L., which is still valid today. *Jatropha* derives its name from the Greek words jatr'os (doctor) and troph'e (food), implying medical properties. In Malabar, India, the physic nut is known by the name *Curcus*. By definition, a physic nut is a small tree or large shrub that grows to a height of three to five metres, but can grow to a height of 8 or 10 meters under ideal conditions. The tree has a straight trunk and grey or reddish bark with huge white patches that cover it. It has green leaves that are 6 to 15 cm long and wide, with 5 to 7 shallow lobes. The leaves are arranged in a zigzag pattern.The seeds of ripe *Jatropha* fruits (7–11) are black and average 18 mm length (11–30) and 10 mm broad. The *Jatropha curcas* plant has a lifespan of more than 50 years (Kamal, *et al*., 2011).

The constituents of *Jatropha curcas* include curcin, with hemagglutinating activity, phorbol esters (Lin, *et al*., 2010), lathyranlactone (Devappa, *et al*., 2013),, jatrocurcasenones A-E and jatrophodiones B-E with cytotoxic activity (Liu, *et al*., 2015),, curcusones A-E, jatrogrossidione and 2-epi-jatrogrossidione, reported for their cytotoxity (Liu, *et al*., 2013), isoamericanol A, which has an inhibitory effect on cell proliferation of MCF-7 (Katagi, *et al*., 2016), curcacycline A, which displays a moderate inhibition of proliferation of human T-cells (Van den Berg, *et al*., 1995) , and curcacycline B, known to enhance the rotamase activity of human cyclophilin B (Auvin, *et al*., 1997). Phytol (47.42%) in the leaf, 1- butoxyperethyl-homotetrasilsesquioxane (36.11%) in the petiole, 7-benzyl-8-(methylthio) theophyllin (12.53%) in the stem, as well as γ-gurjunene

(13.99 %), β-elemol (13.45%) and guaiene (13.13%) in the root, were major components present in the essential oil (Mahalaxmi *et al*., 2016). *Jatropha* oil contains about 14% free fatty acid (FFA), which is far beyond the limit of 1% FFA level that can be converted into biodiesel by transesterification using an alkaline catalyst (Tiwari *et al*., 2007). Palmitic acid (11.3%), stearic acid (17%), arachidic acid (4.7%), oleic acid (12.8%), and linoleic acid (47.3%) are the fatty acids found in *Jatropha curcas* oil (Adebowale and Adedire, 2006). *Jatropha* oil contains approximately 24.60% of crude protein, 47.25% of crude fat, and 5.54% of moisture contents (Kamal, *et al*., 2011). The seed which is black and oval in shape is rich in fixed oil. The oil content of the seed is high (66.4%) (Laxane, *et al*., (2013). The oil is composed of 97.6% neutral lipids, 0.95% glycolipids and 1.45% phospholipids (Rao, *et al*., 2009).

2.7.1 Medicinal uses of *Jatropha curcas*:

Osoniyi and Onajobi, (2003) conducted research to evaluate the coagulant activity of *Jatropha curcas* latex, and found that, at low concentrations the ethyl acetate fraction (*Fe*) significantly ($P < 0.05$) decreased in blood clotting time while the butanol *(Fb)* fraction significantly increased ($P < 0.05$) the clotting time in a concentration-dependent manner. He further concluded that the latex fractions *F*e, *F*b and *F*a, the results of the plasma prothrombin time (PT) and activated partial thromboplastin time (APTT) tests failed to show the presence of a procoagulant in any of the fractions, yet the whole blood clotting time test indicates that the coagulant activity of *Jatropha curcas* latex is associated with the ethyl acetate fraction (*F*e).

Esimone *et al*., (2008) carried out research on cutaneous wound healing activity of herbal ointment containing leaf extract of *Jatropha curcas* L on albino rats. A methanol leaf extract ointment was prepared with the concentrations of 0.5, 1.0 and 1.5 g of *Jatropha curcas* extract per 10 g of the ointment base with control and standard treatments with blank ointment base and Gentamycin ointment (1%). Topical administration methanol leaf extract of *J. curcas* on a rats excision wound resulted in a significantly (P0.05) wound healing at 1.5g concentration ointment.

Kannappan *et al*., (2008) examined the action of a methanolic extract of *Jatropha curcas* Linn. leaves on pylorus ligation and Aspirin-induced stomach ulcers, in wistar rats. 30 albino wistar rats of both sexes placed into five groups of six rats each. Pylorus ligation (PL) combined with aspirin (200mg/Kg, PO) causes gastric lesions. The aspirin and pylorus ligation (PL) rats were given treatment with *Jatropha curcas* extract (100 mg/kg body weight, 200 mg/kg body weight) and ranitidine (50 mg/kg body weight) for 6 days to absorb the acid parameters. After treatment with 100 mg and 200 mg *Jatropha curcas* extracts in Pylorus ligation plus aspirin caused ulcers, a significant dose-dependent reduction (P 0.05) in acid parameters. The results showed that *Jatropha curcas* extract has antiulcer effect against stomach lesions. The antiulcer effect is demonstrated by a decrease in acid-secretory parameters (total and free acid), stomach volume, and ulcer score, implying that acid inhibition speeds and ulcer healing. Further he also confirms the LD50 as370 mg/kg of body weight by using OECD-423.

Uche and Aprioku, (2008) recorded the analgesic and anti-inflammatory effects of the methanolic extract of the leaves of *Jatropha curcas* in mice and rats. The analgesic efficacy was tested in mice using an acetic acid-induced writhing test whereas anti-inflammatory activity was determined by Egg albumin-induced oedema of the rat paw. In anti-inflammatory activity test, *Jatropha curcas* extract (10-80mg/kg) inhibited inflammation caused by egg albumin in the rats' paw significantly. In an acetic acid-induced writhing test in mice, *Jatropha curcas* (10-80mg/kg) also reduced the mean number of writhes elicited by acetic acid significantly.

Balaji *et al*., (2009) studied the anti-metastatic effect of the methanolic fraction of *Jatropha curcas* in C57BL/6 mice using B16F10 melanoma cells. Administration of methanolic fraction at 100 and 200 mg/kg, p.o. substantially (p 0.01) inhibited the proliferative colony formation of melanoma in the lungs by 47.54 and 69.52%, respectively, with a rise in the survival rate of metastatic tumour bearing animals when compared to untreated control animals. The hydroxyproline content of lung collagen in control mice was extremely high, which was significantly reduced in the methanolic fraction administered groups.

Control group have higher levels of lung hexosamines and uronic acid compared to treatment group. The methanolic fraction treated mice had lower levels of serum sialic acids and -glutamyl transpeptidase (both of which are indicators of neoplastic proliferation) compared to control group. On the basis of these he concluded that the methanolic fraction of *Jatropha curcas* effectively suppress the metastasis of B16F10 melanoma cells and possessed anti-metastatic and antiproliferative activity at the dose levels evaluated.

Mishra *et al*., (2010) undertaken the research to evaluated antihyperglycemic activity of 50 % ethanolic extract of *jatropha curcas* leaves (JCE), in alloxan-induced diabetic mice. The five group of rats comprising of six in each group. Diabetic rats were administered ethanolic extract of *jatropha curcas* at 250 and 500 mg/kg body weight and glibenclamide 600µg/kg body weight as standard drug. Oral administration of *Jatropha curcas* extract at 250 and 500 mg kg revealed strong antihyperglycemic action. The reduction in blood glucose level was 219.5 - 116.5, in 250 mg/kg whereas 237.0-98.83 at 500 mg/kg dose, it confirms the antihyperglycemic potency of *Jatropha curcas* leaves in alloxan induced diabetes. Further the LD50 value was determined as 2500 mg/kg by employing OECD-423 guideline.

Olukunle *et al*., (2011) studied the anti-inflammatory and analgesic properties of the aqueous extract of *Jatropha curcas* leaves (150 mg/kg oral). Indomethacin had the highest percentage inhibition of paw volume 83.9%, aspirin had a 64.3% inhibition, and the extract of *Jatropha carcus* had a 60.7% inhibition of paw volume was found in anti-inflammatory study. The result was revealed that the aqueous extract of *Jatropha curcas* leaves could be used as a supplemental therapy or as an alternative anti-inflammatory therapy in the management of inflammatory disorders. Even the aqueous extract of *J. curcas* leaves at a dose of 150 mg/kg also exhibited significant analgesic activity in mice. The mean number of writhing was significantly lower ($P < 0.05$) in mice given aqueous extract of *Jatropha curcas* leaves compared to the control but higher than that of the standard analgesic, paracetamol in analgesic activity.

Juliet, *et al*., (2012) investigated the effects of an ethanolic extract of *Jatropha curcas* leaves as a first step in developing a safe and environmentally friendly therapeutic treatment to combat tick and tick-borne disease concerns. The adult immersion test was used to test the ethanolic extract of *Jatropha curcas* at various dilutions such as 50, 60, 70, 80, 90, and 100 mg / ml against ticks. When compared to control, the extract significantly reduced the hatchability of eggs at all concentrations. The extract of *Jatropha curcas*, on the other hand, did not cause adult engorged ticks to die. The extract had no effect on the number of eggs laid by the ticks that were treated. The result finding indicated that the *Jatropha curcas* extract's eclosion-blocking activity is a promising trait of the plant that could be used to manage tick populations.

Abdelgadir and Van Staden, (2013) reviews on *Jatropha curcas* activity as anti-inflammatory, antioxidant, antimicrobial, anticancer, antiviral, antidiabetic, analgesic, hepatoprotective, wound healing, anticoagulant and antifertility activitity**.** For decades, *Jatropha curcas* has been a popular source of medicine in many cultures. According to review he concluded that *Jatropha curcas* is a valuable source of medicinally relevant compounds, as well as providing compelling evidence for its prospective usage in modern medicine.

Dahake *et al*., (2013) studied effect of Potential Anti-HIV Activity of *Jatropha curcas* Linn. Leaf Extracts. He assessed the anti-viral activity of *Jatropha curcas* Linn. leaf extracts. the *in vitro* micro co-culture methods were used to isolate HIV, and drug susceptibility assays were used to determine resistance to Zidovudine (AZT), Lamivudine (3TC), and Stavudine (d4T). The compounds from the leaves of *Jatropha curcas* Linn were extracted using the Soxhlet equipment, and methanolic and aqueous extracts were selected anti-viral activity. High-Performance Thin Layer Chromatography was used to detect secondary metabolites, and the MTT assay was used to determine in vitro cytotoxicity. He obtained seven HIV isolates (isolation rate: 23.33%) with drug IC50 values ranging from 0.001418- 82.73 μM AZT, 2.645-15.35 μM 3TC and 18.55-66.23 μM d4T. Tannins, Flavonoids, Saponins were detected in aqueous extract and Flavonoids, Saponins from methanolic extract. The anti-viral activity

was evaluated by inhibition of HIV replication as determined by HIV p24 antigen ELISA. Post-infection (4 isolates) interaction studies showed IC50 values ranging from 0.0255-0.4137 mg/mL and 0.00073-0.1278 mg/mL for aqueous and methanolic extracts respectively. The preinfection (1 isolate) interaction studies showed 100% inhibition by methanolic and 97.19% inhibition by aqueous extract at 25 mg/mL each. He concluded that obtained HIV isolates were potentially resistant to AZT/3TC/d4T, and *Jatropha curcas* Linn. leaf extracts demonstrated effective antiviral and likely entrance inhibitory action against potentially drug-resistant HIV. The result showed that *Jatropha curcas* Linn. could be a promising candidate for anti-HIV therapy.

Laxane *et al*., (2013) reviewed that the *Jatropha curcas* herb has been utilised for medical purposes. The anti-inflammatory, antimetastatic, anticancer, coagulant and anti-coagulant (dose dependent), disinfecting, antiparasitic, wound healing, insecticidal, pregnancy termination, and antidiarrhoeal properties are all present in the plant. Curcin, curcusone-B, curcain, and other plant elements are the most important in *Jatropha curcas* herb.

Baroroh *et al*., (2014) conducted research to evaluate the antiarthritic effects of ethanolic extract of *Jatropha curcas* leaves. Freund's adjuvant induced arthritis model was used to assess the anti-arthritic activity. Forty-eight wistar rats were divided into six groups (n=8). I group as normal group, injected with 0.9% NaCl, (II) negative control group, induced by CFA (complete Freund's adjuvant) (III) positive control group, given sodium diclofenac at a dose of 6.75 mg/kg, and (IV-VI) treatment groups, given ethanol extracts *J. curcas* leaves at doses of 150 mg/kg, 300 mg/kg and 600 mg/kg. The treatment extracts and sodium diclofenac were administered orally on days 14-28. The extract at a dose of 150 mg/kg significantly decreased the arthritis scores after the 20th day and there were significant differences in arthritis scores compared with sodium diclofenac on the 20thday. Rats treated with *J. curcas* extract at doses of 300 and 600 mg/kg showed significant decreases in arthritis scores from the 20th to the 28th day compared to arthritic control rats. The *Jatropha curcas* extract reduced edoema and cartilage breakdown in arthritic joints, according to histopathology

investigations. So, it was concluded that the anti-arthritic effects of the *Jatropha curcas* leaf extract was seen in the reduction of arthritis and mobility scores.

Anigbogu *et al*., (2015) reported that the effects of ethanol extract of *Jatropha curcas* on lipid profile of anemic rats. Wister Albino rats weighing 102-200kg were used in this study. The rats were divided into five groups of five rats each at random. The rats in groups 2, 3, 4, and 5 were given cyclophosphamide to produce anaemia, whereas group one acted as a control group. Group 2. 3 anaemic rats received 100mg and 200mg ethanol extract of *Jatropha curcas.* Group 4 rats with anaemia served as positive control whereas Group 5, was on standard medication Chemiron. The ethanol extract of *Jatropha curcas* significantly increased the concentrations of HDL-cholesterol of groups 2, 3 and 5 rats. The findings revealed that an ethanol extract of *Jatropha curcas* could raise (High density lipoprotein) HDL cholesterol levels while lowering LDL (Low density lipoprotein) cholesterol levels. This demonstrates that traditional herbalists were correct when they asserted that the ethanol extract of *Jatropha curcas* might be used to treat cardiovascular problems.

Kumar *et al*., (2016) conducted an experiment to evaluate the anti-diabetic effect of 50% hydroethanolic extract of *Jatropha curcas* leaves. It was tested on 25 rats divided into five groups of five each. After a single injection of alloxan drug @ 100 mg/kg b.wt., i.p., followed by treatment with metformin (300 mg/kg) in group 3rd, hydroethanolic extract of *Jatropha curcas* leaves (JCE) (200 mg/kg) in group 4th, and *Jatropha curcas* extract (400 mg/kg) in group 5th. In comparison to the alloxan-induced diabetic group, the metformin and *Jatropha curcas* extract treated groups had significantly lower blood glucose levels (3 -5 groups). Serum creatinine, urea, cholesterol, and enzymes all increased significantly in group 2nd group, but decreased dramatically in groups 3rd and 5th. From research it was observed that hydroethanolic extract of *Jatropha curcas* leaves having anti-diabetic properties.

Oyama *et al*., (2016) studied the antibacterial activity of *Jatropha curcas* using aqueous, methanol and ethanolic extracts against *Escherichia coli, staphylococcus aureus, Proteus* spp., *Klebsiella pneumonia* and *Pseudomonas*

aeruginosa. The antimicrobial activity of the plant was evaluated using the agar well diffusion method. 100 µl of the plant extracts at 1mgml-1 were used. Inhibition zones were measured and recorded. All the extracts demonstrated significant inhibition against the microorganisms However, ethanolic extract shown greatest inhibition when compare with other solvent extracts.

Adeosun *et al*., (2017) carried out research on the chemical constituents and anti-inflammatory activity of the essential oil of *Jatropha curcas* leaf in wistar rats. The inflammation was studied using egg albumin as a phlogistic agent. The anti-inflammatory activity of the essential oil reveals a minimal to significant activity.

Salim *et al*., (2018) assessed the *Jatropha* sap cream's activity on day 5th of the healing process of skin wounds of mice infected with Staphylococcus aureus. They used twenty-seven male mice (Mus musculus) weighing 30- 40 g. All mice were infected with 0.2 ml suspension of S. aureus ATCC 25923 PK/5 after a 20 mm incision in the dorsal area (back). The treatment of exposed wounds took place twice a day. Cream base was applied to group P1 (negative control); 0.1% sulfadiazine cream was applied to group P2 (positive control); and 10 % *Jatropha* sap cream was applied to group P3. On day 5, the pathological anatomy of the skin wounds revealed hyperaemia (redness), swelling (oedema) of the wound edges, and wetness due to exudate. The result was found that the impact of 10% *Jatropha* cream and sulfadiazine cream was clearly superior than the basis cream based on the length of the incision on 5th day. 10% *Jatropha* cream can be utilised as a topical medication for infected wounds which had antibacterial activity, anti-inflammatory, and neovascular stimulator in mice skin wound infected with S. aureus.

Elimian and Eze, (2019) the study was done to determine the wound healing effects of *Jatropha curcas* latex on intact and wounded skin in wistar rats. A total of 24 wistar rats were used, with each group consisting of six wistar rats (labelled A, B, C, and D). The wistar rats in group A got a 3cm long incision on their backs and were treated with *Jatropha curcas* latex. This was compared to group B, received 0.9 % normal saline treatment. On undamaged skin, group C

received *Jatropha curcas* crude sap. Nothing was applied to the skin in group D, and no wound was present. After 28 days, the animals were slaughtered, and the normal and injured skin histology was studied. Unlike those treated with regular saline, the *J. curcas* developed blood clots within 5 seconds after administration. In Wistar rats, topical application of latex to intact skin resulted in stratum corneum exfoliation. The result was found that latex of *Jatropha curcas* has been proven to have haemostatic characteristics.

Ahmed *et al*., (2020) carried out research to evaluated anti-cancer Activity of Curcin and Latex Isolated from *Jatropha* Plant (*Jatropha Curcas* L.) He observed that different extracts of *Jatropha curcas* were tested in vitro against MCF-7, HepG2, and HCT-116 cancer cell lines, as well as the in vivo antitumor effectiveness of the most efficient extract on a chemically produced hepatocellular carcinoma (HCC) rat model. The result showed that the methanolic extract of Latex *Jatropha curcas* had the maximum in vitro cytotoxic activity against the HepG2 cell line, with 19.11 g/ml.

Airaodion and Ogbuagu, (2020) evaluated the abortifacient properties of *Jatropha curcas* leaf extract in female wistar rats. The females were dosed with diethylstilbestrol dissolved in paraffin oil at a concentration of 1 mg/kg body weight to synchronise their estrus. Then, a male rat was placed in each cage for mating. The pregnant rats were divided into four groups. Group A was treated with normal saline. Groups B, C, and D dosed @ 1 g/kg body weight of *Jatropha curcas* leaf extract for 24, 48, and 72 hours respectively. 60 % of animals treated with *Jatropha curcas* leaf extract for 24 hours heaved litter, indicating that miscarriage occurred in 40 %. The result was revealed that *in vitro* and *in vivo* doses of *Jatropha curcas* leaf extract caused numerous contractions in pregnant rats.

2.8 Toxicity of *Jatropha curcas:*

Li *et al*., (2010) assessed the acute toxicity of phorbol esters given by intragastric administration to determine the LD50 for swiss hauschka mice. Six dosages 36, 32.40, 29.16, 26.24, 23.62, and 21.26 mg/kg were selected with each

group consisting of ten mice. The LD50 and 95% confidence limits for male mice were 27.34 mg/kg and 24.90–29.89 mg/kg and the LD5 and LD95 were 18.87 and 39.62 mg/kg, respectively were observed. The histopathological studies in dead mice showed, no significant abnormal changes in the organs at the lowest dose (21.26 mg/kg), prominent lesions mainly found in lung and kidney, with diffused haemorrhages in lung, and glomerular sclerosis and atrophy in kidney at 32.40 mg/kg doses and multiple abruptions of cardiac muscle fibres and anachromasis of cortical neurons at the highest dose of 36.00 mg/kg.

Igbinosa *et al*., (2013) evaluated the LD50 of methanol extract of *Jatropha curcas* leaves in rats and its potential toxicity. 40 rats for LD50 estimation and 40 for toxicity testing. 4 dosages 86, 58, 46, and 34 kg were selected based on the pretest that was done to determine a range of toxicity. The rats were divided into four groups, each containing ten rats. Rats in groups 1, 2, 3, and 4 were given 0, 500, 1000, and 2000 mg/kg body weight of *Jatropha curcas* extract, respectively, by oral intubation for 21 days. Thereafter, clinical signs, change in body weight, toxicity symptoms, and biochemical parameters were obtained. The LD50 at 95% confidence limits for rats was 46.0mg/kg body weight (44.95–52.69mg/kg body mass). There were no clinical and biochemical signs of toxicity when the extract was administered at 500, 1000, and 2000mg/kg body weight, respectively ($P > 0.05$). Results obtained from this study suggest that liver, kidney, and haematological system of rats tolerated methanolic leave extract of *Jatropha curcas* at a certain concentration.

Vipin Kumar *et al*., (2014) assessed the sub-acute toxicity of *Jatropha curcas* seed and seed oil in rats. 30 healthy rats weighing 150-200gm, randomly divided into five groups of six rats each. The daily oral dosing of *Jatropha curcas* seed @ 4seed/rat and seed oil @ 1ml/rat followed by the treatment with the therapeutic module comprising of sodium thiosulphate (33.75mg/ml) and glutathione (22.5mg/ml) @1ml /rat in group III and V for 28 days. Whereas group II and IV dosed with *Jatropha curcas* seed (4 seeds/rat) and seed oil (1 ml/rat) respectively. The clinical signs of toxicity envisages the letharginess, dullness, disinclined to move and blood stained diarrhoea appeared on fourth week of the

experimental trial in group II, III, IV and V. There was only single mortality recorded in group II (16.67%), III (16.67%) and V (16.67%) on 26th, 27th and 25th day, respectively whereas two mortalities occurred in group IV (33.33%) on day 24th and 25th of trial. Non- significant changes were observed in the body weight of the treated groups i.e., II, III, IV and V. On the basis of results, he concluded that therapeutic module comprising of sodium thiosulphate and glutathione in sub-acute study produced the ameliorating effect by improving the hematobiochemical values in the treatment groups

Azubike *et al*., (2015) examined the acute toxicity and histopathological damage caused by crude aqueous leaf extract of *J. curcas*, in mice. Thirty-six mice of both sexes, weighing 25-35 g, were used. The experiment was done in two stages. In stage 1, exploratory trials were performed in five groups of 3 mice each of both sexes. Group I (control) received no treatment while the rest of the groups [II-V] were given graded doses [10, 100, 1000 and 2000 mg kg b.wt., respectively] of the extract (i.p.). The obtained doses were 100 and 1000 mg/ kg b.wt., respectively. Hence within these limit, graded doses of 200, 400, 600 and 800 mg kg b.wt. were then derived for the Stage 2 experiment using 4 groups [VI-IX] of 6 mice each. Each animal was given a single intraperitoneal dose of the crude aqueous extract after a 24 h fast in the respective groups. After the drug administration, clinical observations were monitored hourly for 24 h and mortality and/or clinical signs of toxicity were recorded. The intraperitoneal [i.p.] doses of the crude extract of *J. curcas* produced dose dependent organ toxicities including the heart, liver, kidney, lungs and spleen. The LD50 obtained being 141.4 mg kgG1 b.wt. (i.p.) suggests that the substance would be considered harmful and its use may be linked to tissue structural damage in important organs.

See *et al.,* (2016) evaluated the acute and chronic toxicity testing of *Jatropha curcas* by decoction and ethanol leaf extraction methods in rats. In acute toxicity, no mortality was recorded in first two hours to 24-hour period following the administration of decoction and ethanol leaf extracts of *Jatropha curcas* @ 200mg/kg, 500mg/kg, and 1000mg/kg. However, in chronic test, total of twenty-one rats died during the course of the 45 days period after administration both the

leaf extracts of *Jatropha curcas* at 200mg/kg, 500mg/kg, and 1000mg/kg. Even the remaining rats shown drastic decrease or change in body weight. The significant change in weight indicates chronic pain in rats. Results showed that extracts were not acutely toxic with 0% mortality but lethal to the rats when taken for longer period of time marked by behavioral changes that signifies chronic toxicity in rats.

Mahe *et al.,* (2017) conducted research to evaluate *in vitro* and *in vivo* toxicity of crude methanol *J. curcas* leaf extract on albino rats. *J. curcas* leaf extract at 10, 100, and 1000 mg/kg fed orally in three rats of each group. Five rats were fed with the crude extract at doses of 1400, 1800, 2200, 2600 and 3000 mg/kg orally. Both phases were monitored 24 hrs for mortality and general behaviour. There was no death recorded in the first phase, while in the second phase death was recorded in animals administered 2600 mg/kg and 3000 mg/kg body weight of the extract. The estimated oral LD50 of *Jatropha curcas* extract was 2,391.65 mg/kg whereas the LC50 values for the crude leaf extract, fraction 1, and fraction 2 were 109.07 g/ml, 100 g/ml, and 883.26 g/ml, respectively. The crude methanol leaf extract was found to have a minor hazardous effect according to various toxicity indices.

Sawadogo *et al*., (2018) evaluated an acute and subacute toxicity of aqueous extract *Jatropha curcas* leaves. Dose limits of 2000 and 5000 mg/kg were used for acute toxicity. Following oral administration at dose of 5000 mg/kg body weight, acute toxicity data showed no signs of toxicity. Hence, he concluded that the LD50 value of aqueous leaves extract *Jatropha curacs* is greater than 5000 mg/kg of body weight.

2.9 *Jatropha curcas* coated silver nanoparticles:

Sadgire *et al*., (2014) evaluated the effect of *Jatropha curcas* coated silver nanoparticles *in vitro* and *in vivo* on breast cancer in mice by using MTT assay against C1271 breast cancer cell lines for 50 days. The cell cytotoxicity and percent inhibition were evaluated using the MTT assay. At a dose of 50g/ml of JCSNP, the greatest percent inhibition of the Vero cell line was observed.

According to the tetrazolium reaction, 54.74 % of cells were killed when using silver nanoparticle alone at 100 g/ml concentration, 54.41 % of cells were dead, and 61.49 % of cells were dead when utilising *Jatropha curcas* at 200 g/ml concentration. On that basis he concluded that JCSNP treatment prevented tumour growth, as evidenced by histological tests, and had a restorative impact in the mammary gland, as evidenced by microscopic observation. It also restored haematological parameters, bringing haemoglobin content and RBC count back to more or less normal levels. This suggests that JCSNP has a protective effect on the hemopoietic system, demonstrating the validity of the parameters used to assess the efficacy of anticancer drugs.

Dhaije *et al*., (2018) reported that the Green synthesised SNPs and coating with JcLE (*Jatropha curcas* leaf extract) were evaluated for sub-acute dermal toxicity. 28-day dermal application of a combination of 1mg/kg SNPs and 150 mg/kg JcLE (Low dose), 2 mg/kg SNPs and 300 mg/kg JcLE (Medium dose), and 4 mg/kg SNPs and 600 mg/kg JcLE (High dose) in three group respectively. The final group was designated as a satellite group, receiving 8 mg/kg SNPs with 1200 mg/kg JcLE and being followed for a further 14 days to assess the post-withdrawal effect. No abnormal behavioural symptoms or skin changes during the experimental period except for a rise in neutrophil count and a compensatory increase in TEC count on the 28th day of all treatment groups were observed. The haematological parameters exhibited no significant alterations as a marker of toxicity, and this was not dosage dependent. In the JcLE coated SNPs groups, serum biochemical results showed an increase in ALT and AST. However, the increase in BUN readings in the trial was not dose dependent, and there was no significant increase in creatinine dose on the 28th day. In the trial, there was no significant increase in total protein on the 14th day. In the liver and kidney treated with the maximum dose JcLE coated SNPs, grossly minor haemorrhages, congestion, and architectural changes were detected. Skin tissues, on the other hand, showed no histoarchitectural changes.

Materials and Methods

MATERIALS AND METHODS

Present research describes the materials and methods used to evaluate the sub-acute oral toxicity of *Jatropha curcas* Linn. leaf extract coated silver nanoparticles in wistar rats (OECD-407). The study included the evaluation of repeated dose exposure of *Jatropha curcas* extract coated silver nanoparticles for 28 days and its post exposure (15 days) effects on rats. The toxicity was evaluated through Haematological, Biochemical and Histopathological changes in kidney, liver, and heart tissues.

3.1 Location:

The present investigation was carried out in small animal laboratory house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra.

3.2 Experimental animals:

The Institutional Animals Ethical Committee (IAEC) approved the experimental protocol vide Resolution no. IAEC/80/21 dated 13/10/2021. The present study was conducted on 50 wistar rats of 8-10 weeks and 160-220 gm body weight of either sex. Rats were selected in accordance with their health monitoring report and behavioural examination. The selected rats were randomized according to their body weight and the range was within ±20 percent of the mean body weight. The wistar rats were maintained as per the standard laboratory conditions by implementing the CPCSEA guidelines. The rats were provided with *ad-libitum* food and water and were kept in the experimental room of laboratory animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani. The experimental animals were kept quarantine for a week before commencement of the experiment. All necessary managemental procedures were adopted to keep the animals free from stress.

3.2.1 Husbandry

Housing	**:**	Cage system (Individual animal in a cage)
Temperature	**:**	22 ± 3^0C
Relative Humidity	**:**	30- 70%
Photoperiod	**:**	12 hours light and 12 hours dark period
Light intensity	**:**	130-400 Lux
Food	**:**	Pelleted diet
Water	**:**	Clean water purified by aqua guard water system

3.2.2 Animal housing

Animals were housed in small animal laboratory animal house, at room no.3 of College of Veterinary and Animal Sciences, Parbhani, Maharashtra. Corncob was used as bedding material and cages were changed thrice a week.

3.2.3 Environmental conditions

Wistar rats were kept in an environmentally controlled room with 22 ± 3^0 C temperature and 30-70% relative humidity with 12 hours light and dark period.

3.2.4 Drinking water and diet

Purified drinking water and conventional standard pelleted laboratory animal feed (VRK pellet feed) was provided *ad-libitum* throughout the experimental period.

3.2.5 Acclimatization and grouping

Acclimatization period of a week was followed. Wistar rats were acclimatized to experimental room condition for a week. During acclimatization period daily health and behavioural monitoring were followed. After the acclimatization, 50 wistar rats were randomly divided into five groups, each comprising of 10 rats of either sex. All the animals were maintained for the experimental period of 28 days except the satellite group, which was maintained for 15 more days, for reversibility, persistence, or delayed occurrence of toxic effects or to study the post withdrawal effect with highest dose given.

3.2.6 Animal numbering and identification

Rats were identified with the unique numbers allocated and four rats in a cage were kept in accordance with their groups. Each cage was identified with a label, cage number, group details and treatment given respectively.

3.3 Drugs and chemicals:

All the drugs and chemicals used in the investigation are as follows

3.3.1 Silver Nitrate

Silver Nitrate was used to produce silver nanoparticles (SNPs) by biological reduction method by using *Azadirachta indica* (Neem) leaf extract.

Compound name	**:**	Silver Nitrate
Chemical name	**:**	Nitric acid silver (I) salt
Mol. Wt.	**:**	169.87
Product code	**:**	0574200010
Molecular formula	**:**	AgNO3
CAS No.	**:**	7761-88-8
UN No.	**:**	1493
Batch No.	**:**	B212131610
Company	**:**	Loba Chemie Pvt. Ltd, Mumbai
Physical appearance	**:**	White colored powder
Storage condition	**:**	0 – 40 C
Vehicle	**:**	Deionized water

3.4 Plant material

3.4.1 *Jatropha curcas*

Botanical name	**:**	*Jatropha curcas*
Family	**:**	*Euphobiaceae*
Common name	**:**	*Jatropha*
English	**:**	*Jatropha*
Hindi	**:**	Bagbherenda, Jangaliarandi, Safed arand
Sanskrit	**:**	Kananaeranda, Parvataranda
Marathi	**:**	Mogalierenda, Ranayerandi
Gujarati	**:**	Jamalgota, Ratanjota
Telugu	**:**	Nepalamu, Pedanepalamu

3.4.1.a Botanical description:

Jatropha curcas Linn. has thick glorious branchlets. The tree has a straight trunk and grey or reddish bark masked by large white patches. The branches contain whitish latex, which causes brown stains, which are very difficult to remove. Normally, five roots are formed from seeds, one tap root and 4 lateral roots. Plants from cuttings develop only lateral roots. Inflorescences are formed terminally on branches. The plant is monoecieous and flowers are unisexual. Pollination is by insects. After pollination, a tri-lobular ellipsoidal fruit is formed. The exocarp remains fleshy until the seeds are mature. (Kamal, *et al*., 2011). The leaves are of the paracytic (Rubiaceous) type, with five to seven lobed, hypostomatic, and stomata. The colours range from bright green to pale green (Gupta, 1985). Fruits are 3–4 cm long ovoid capsules that are somewhat trilobite and break into three cells. There are three seeds per fruit, each of which is huge, rectangular, and 2 cm long, with a sweet flavour (Kochhar *et al*., 2008; Neuwinger, 1996). The seeds are fully ripe when the capsule turns yellow after 3–4 months of flowering. The seeds are black, with a seed weight per 1000 of roughly 727 g and an average of 1375 seeds per kg. With 2n = 22 chromosomes, the physic nut is a diploid species (Li *et al*., 2011)**.** Even on gravelly, sandy, and saline soils, *Jatropha* grows almost anyplace except wet places. It can thrive in a

wide range of rainfall conditions, from 250 to over 1200 mm per year. It is now grown in most every tropical and subtropical country (Singh, 1970).

3.4.1.b Active principle:

Aerial part of the plant contains organic acids (o and p-coumaric acid, p-OH-benzoic acid, protocatechuic acid, resorsilic acid), saponins and tannins. Stem bark contains amyrinsitosterol and taraxerol 30.

Leaves contains cyclic triterpenes stigmasterol, stigmasterol-5-en-3,7diol, cholesterol 5-en-3,7diol, campesterol, sitosterol, 7-keto-sitosterol, 7-keto-sitosterol as well as the d-glucoside of sitosterol. Flavonoids apigenin, vitexin, isovitexin. Leaves also contain the dimer of the triterpene terpene alcohol (C63H11709) and flavonoidal glucoside 30,31,32. Latex contain curcacyline A, a cyclic octapeptide curcain (a protease) 33. Seeds contain curcin, a lectin Curcin, a lectin Phorbolesters Esterases (JEA) and Lipase (JEB). Kernal and press cake contains phytate saponin and a trypsine inhibitor 34. Roots contain sitosterol and its d-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusone A-D, diterpenoids jatrophol and jatropholone A and B, the coumarin tomentin, the coumarin –lignan jatrophin as well as taraxerol 35,36 (Akintayo, 2004, Augustus *et al*., 2002, Khafagy *et al*., 1977, Hufford and BO (1978)., Mishra *et al*., 2010, Nath and Dutta.,1992).

Deffated meal of *Jatropha* contain protein (58-66) and phytate) (9-11%). It has 66% phytate and 22% crudeprotein (Kumar *et al*., 2011)**.** *Jatropha* meals/seeds contain very high quantity of trypsin and phytate inhibitors (Aderibigbe *et al*., 1997). Crude *Jatropha* oil contains 78-80% of phorbol esters (Devappa *et al*.,2013).

3.4.1.c *Jatropha curcus linn.* Leaf extract:

Aqueous extract of *Jatropha curcas* leaf was prepared by cold extraction method. *Jatropha curcas* leaves (figure 3.1) were procured from the surrounding area of VNMKV, Parbhani. The collected leaves were authenticated from the department of Veterinary Pharmacology and Toxicology. The collected leaves

were then washed with the tap water and allowed for shed dry at department laboratory. The dried leaves were then crushed and ground to powder with the help of an electric grinder shown in figure 3.2. 200 gm powders of leaves were dissolved in distilled water and the final volume was made to 1 liter. The flask was kept in refrigerator for 48 hrs for complete maceration. During 48 hrs intermediate shaking was done for thoroughly mixing of powder and distilled water with an electrically operated flask shaker. The resulting solutions after 48 hrs was first filtered by using muslin cloth and then by whatmans No.42 filter paper. The filtrate obtained was then poured in the evaporating dishes and allowed for shed dry at room temperature.

After complete drying the obtained extracts was weighed and stored in screw cap vial and used as and when required shown in figure 3.3.

3.4.2 *Azadirachta indica*

English	:	Indian lilac
Hindi	:	Neem
Sanakrit	:	Nimba
Tamil	:	Vembu
Malayam	:	Aryaveppu
Marathi	:	Kadu-Limba

3.4.2.a Botanical description

Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), rarely to 35–40 meters (115–130 ft). It is evergreen but in severe drought it may shed most or nearly all of its leaves.

3.4.2.2 Active principle

Three bitter compounds were extracted from neem oil, which were named nimbin, nimbinin, and nimbidin. The seeds contain a complex secondary metabolite azadirachtin.

(A)

(B)

Figure 3.1 A. *Jatropha curcas* whole Plant

***B. Jatropha curcas* leaves**

Figure 3.2 *Jatropha curcas* leaf Powder

Figure 3.3 *Jatropha curcas* leaf extract

3.5 Synthesis of Silver nanoparticles (SNPs)

3.5.1 Collection and extract preparation:

The plant material was collected and allowed to dry at room temperature. The *Azadirachta indica/neem* leaves (5% aqueous extract) were used for the synthesis of silver nanoparticles and the aqueous leaf extract of *Jatropha curcas* was used for coating of silver nanoparticles. The plant material stored at 4^{o}C was used whenever required.

3.5.2. Synthesis of Silver nanoparticle:

The 5 gm dried leaves of *Azadirachta indica* were cut into small pieces and putted in test tube. 100 ml of sterile distilled water was dispensed in the tube and kept in water bath for one hour at 80ºC. Then the leaf extract was collected in separate conical flask and filtered through whatmans No.42 filter paper.

10^{-3} M Silver nitrate ($AgNo_3$) solution was prepared and store in brown bottles separately. 5ml of leaf extract (*Azadirachta indica*) was taken in BOD bottle and 95 ml of 10^{-3} M $AgNo_3$ solution was added. Then the BOD bottle was incubated at room temperature for 28 hours. The colour changes of the solution from pale green to dark brown were checked periodically. The colour change to brown indicates synthesis of AgNPs from the leaves (*Azadirachta indica*). Dhaije *et al*., (2018).

3.5.3 Coating of silver nanoparticles with *Jatropha curcas* extract:

For coating of synthesised silver nanoparticles (SNPs) with *Jatropha curcas* leaf extract, the synthesised silver nanoparticles solution was (0.17 mg/kg b.wt.) mixed with the different doses of aqueous leaf extract of *Jatropha curcas* (100, 200, 400 and 800 mg). The resulting solution was maintained at room temperature and stirred for 1 hour to covalently modify the surface of SNPs with the phytochemicals present in *Jatropha curcas* leaf extract.

3.5.4 Characterization of silver nanoparticles:

Characterization of the synthesized and purified AgNPs were carried out with the help of Transmission Electron Microscopy (TEM) according to method of Banerjee *et al.* (2014).

Floatation method for sample preparation for -TEM Model-JEM-1400(HR).

A drop of sample was placed on a piece of parafilm and placed the carbon coated copper grid on top of the sample drop and wait for 10 minutes. Absorb the excess with the help of piece of filter paper. Wash with distilled water and stained with 2% aqueous Uranyl Acetate and remove the excess water with the help of piece of filter paper and air dried alter observed under transmission electron microscope (Camera-C camera emesis) at various magnification. As par the standard staining procedures carried out at shri Sai histopathology centre, Shivaram pally, Rajendranagar, Hyderabad India. The visualization of images was taken from the National Institute of Animal Biotechnology, Hyderabad.

3.6 Technical programme:

The present investigation was planned to study the sub-acute oral toxicity of aqueous leaf extract *Jatropha curcas* coated silver nanoparticles in wistar rats. as per the OECD-407 guideline.

The silver nanoparticles were prepared and coated to the *Jatropha curcas* leaf extract. Both silver nanoparticles and their coated *Jatropha curcas* extract were used in the present investigation.

The pilot study was carried out in 10 wistar rats to determine the doses to be used in the final experiment.

3.6.1 Preparation of animals for administration of drug:

Based on OECD Guideline 407, the test extracts were administered daily for 28 days orally as per the mentioned groups. The rats were observed closely during the entire period of the experiment for any alternation or sign of toxicity.

The satellite group, was dosed for 15 more days, for reversibility, persistence, or delayed occurrence of toxic effects.

3.7 Experimental design:

The 50 rats were divided in 5 groups, each comprising of 10 rats (5 male and 5 female). Group – I serve as healthy control; no any treatment was posed in this group.

Group – II, III and IV were treatment groups.

Group –V was satellite group

The silver nanoparticles at dose @ 0.17 mg/kg b.wt. was used for different concentrations. The dose details of the above groups were mentioned in the following table no.

Table 3.1: Experimental design:

Groups	Name of Group	Treatment	Dose	Route
I	Healthy control	Distilled water	1 ml	Oral
II	*Jatropha curcas* leaf extract coated SNP Dose – I	Silver nanoparticles	0.17 mg/kg	Oral
		Jatropha curcas leaf extract	100 mg/kg	
III	*Jatropha curcas* leaf extract coated SNP Dose – II	Silver nanoparticles	0.17 mg/kg	Oral
		Jatropha curcas leaf extract	200 mg/kg	
IV	*Jatropha curcas* leaf extract coated SNP Dose – III	Silver nanoparticles	0.17 mg/kg	Oral
		Jatropha curcas leaf extract	400 mg/kg.	
V	Satellite Group	Silver nanoparticles	0.17 mg/kg Bwt	Oral
		Jatropha curcas leaf extract	800 mg/kg Bwt.	

3.7.1 Animal grouping

The experimental animals will be divided into 5 groups, each comprising of 10 rats of either sex as detailed below.

a) Group I: (Control)

Control group was treated orally with vehicle for 28 days (distilled water). The conventional feed and supply of pure drinking water was offered during entire research.

b) Group II: (Leaf extract of *J. curcas* coated AgNPs - Dose - I)

Rats were treated orally with leaf extract of *Jatropha curcas* coated AgNPs at 100 mg/kg body weight (Dose-I) for 28 days.

c) Group III: (Leaf extract of *J. curcas* coated AgNPs - Dose - II)

Rats were treated orally with leaf extract of *Jatropha curcas* coated AgNPs at 200 mg/kg body weight (Dose-II) for 28 days.

d) Group IV: (Leaf extract of *J. curcas* coated AgNPs - Dose - III)

Rats were treated orally with leaf extract of *Jatropha curcas* coated AgNPs at 400 mg/kg body weight (Dose-III) for 28 days.

e) Group V: (Satellite study)

Rats were treated with leaf extract of *Jatropha curcas* coated AgNPs at 800 mg/kg body weight (Dose-III). The dosing will be continued for 15more days of experimental period.

The volume of different doses was made up to 1ml/rat/day in control as well as treatment groups. The volume of each dose was not exceeded to 1ml/rat/day (orally) (Figure 3.4).

3.7.2 Collection of Sample

Blood samples were collected on the 0^{th} day, 7^{th} day, 14^{th} day and on the day of animal sacrifice (28^{th} day) from each group. The blood sample was

Figure 3.4 - Dosing of rats by oral gavage.

collected from retro-orbital plexus with the help of capillary tube. The collected blood was analyzed for estimations of haematological and biochemical parameters.

Animals were sacrificed by using excess dose of inhalation anesthesia (Carbon monoxide) at the end of experiment. Post mortem examination was carried out in the sacrificed animals. Liver, kidney, and heart were collected in 10 % formalin for histopathological examination.

3.8 Parameters studied:

General observations (behavioral changes, food and water intake, body weight), haematological, serum biochemical estimations, and pathological observations (gross pathology, organ weights and histopathology) were studied for the evaluation of sub-acute oral toxicity of *Jatropha curcas* leaf extract coated silver nano particles in wistar rats.

3.9 General observations:

3.9.1 Behavioral changes

All the grouped animals were daily examined for any abnormal behavioral changes, changes in water and feed intake if any.

3.9.2 Body weight

The body weights were taken on day 0^{th}, 7^{th}, 14^{th} and day 28^{th} i.e., on the termination day of the experiment.

3.10 Haematological parameter estimations:

Blood samples were collected at $0^{th,}$ 7^{th}, 14^{th} and 28^{th} days were analyzed for following haematological parameters: -

Haemoglobin (Hb)

Total Erythrocyte Count (TEC)

Total Leucocyte Count (TLC)

Blood clotting time

Differential Leucocyte Count (DLC)

Fully automatic blood cell counter (ERMA PCE- 210N) was used for estimation of haematological parameters.

3.11 Serum biochemical parameter estimations:

Blood samples were collected from rats by puncture of lateral canthus retro-orbital plexus by using micro-capillaries at 0^{th}, 7^{th}, 14^{th} and 28^{th} days. Serum was separated from blood samples using clot activator tubes and stored at 4^0C for further biochemical estimations. The following biochemical estimations were carried out:-

AST / SGOT	:	(UV kinetic method)
ALT / SGPT	:	(UV kinetic method)
BUN	:	(Berthelot method)
Creatinine	:	(Alkaline picrate method)
Total Protein	:	(Biuret method)

3.11.1 Liver function enzymes:

3.11.1.a Aspartate aminotransferase (AST/SGOT) (UV Kinetic method)

1000 µl of working reagent was added with 100 µl of serum sample. The mixed sample was incubated for 1 minute at 37^0C and the absorbance was measured at 340 nm with a factor of 1745. The values were expressed in IU/L.

3.11.1.b Alanine aminotransferase (ALT/SGPT) (UV Kinetic method)

1000 µl working reagent was added in 100 µl of serum. The mixed sample was incubated for 1 minute at 37^0C and the absorbance was measured at 340 nm with a factor of 1745. The values were expressed in IU/L.

3.11.1.c Total protein (Biuret method)

20 µl of serum was added in 1000 µl of working reagent and incubated for 10 minutes at 37^0C. After incubation period the absorbance was measured at 546 nm and the values were expressed in g/dl. Prior to analysis, analyzer was calibrated with 1000 µl of working reagent + 20 µl distilled water as blank and 1000 µl of working reagent + 20 µl protein standard as standard.

3.11.2 Kidney function test:

3.11.2.a Blood urea nitrogen (Berthelot Method)

10 µl of serum was added in 1000 µl of working reagent and incubated for 30 second at room temperature. After incubation period, two absorbances were measured at 340 nm with a factor of 23.4 and the values were expressed in mg/dl. Prior to analysis, analyzer was calibrated with 1000 µl of working reagent + 10 µl standard same as above.

3.11.2.b Creatinine (Alkaline Picrate method)

1000 µl of working reagent was added with 100 µl of serum sample. The mixed sample was incubated for 1 minute at room temperature and two absorbances were measured within 60 second interval at 505 nm. The values were expressed in mg/dl. Prior to analysis, analyzer was calibrated with 1000 µl of working reagent + 100 µl standard same as above.

3.12 Pathological observations:

Animals were sacrificed by using excess dose of inhalation anesthesia (Carbon monoxide) at the end of experiment. Post mortem examination was carried out in the sacrificed animals. Organs were subjected to the gross, microscopic and histopathological examinations. Liver, kidney, and heart were collected in 10 % formalin for histopathological examination.

3.12.1 Gross pathology:

All the organs in experimental sacrificed wistar rats were observed for the presence of any abnormal changes.

3.12.2 Organ weight:

The absolute organ weights of liver, kidney and heart were noted in control and post treatment sacrificed wistar rats.

3.12.3 Histopathological changes:

Liver, kidney, and heart were collected in 10 % buffered formalin and were dehydrated in ascending grade of alcohol, cleared in xylene and embedded in paraffin wax. Four-to-five-micron thick sections were stained with routine Haematoxylin and Eosin-staining method (Culling, 1974) and examined.

3.13 Statistical analysis:

The data obtained from various parameters from all the groups was analyzed as per method suggested by Snedecor and Cochran (1994) using factorial randomized block design (FRBD) and completely randomized block design (CRD) using WASP (Anonym, 2018 WASP version 2.0 http://www.ccari.res.in/wasp2.0/index.php).

Results and Discussion

RESULTS AND DISCUSSION

The present experiment was designed to assess the repeated dose oral toxicity of *Jatropha curcas* Linn. Leaf extract coated silver nano particles in wistar rats. The results were shown from table 4.1 to 4.18 and figure from 4.1-4.33 interpreted in the present topic.

4.1 *Jatropha curcas* leaf extracts preparation:

For the aqueous extraction of *Jatropha curcas,* the collected leaves were weight and the collected leaves were 21.5 kg. Through these collected leaves, 3 kg of leaves powder was obtained after the drying and grinding of these leaves. In the cold aqueous extract preparation, from the 3kg of powder average 140 gm of aqueous extract was obtained. Percent extractability was calculated and it was found 4.66% for 100 gm of powder of *Jatropha curcas* leaves. However, Jadhav *et. al.,* (2021), received percent extractability of 11.10% in the whole plant of *Jatropha curcas*. Similarly, Shivani *et al.,* (2011), reported the influence of extraction methods on yield percentage of extract. By soxhlet extraction, the recorded yield of *Jatropha curcas* L. seed oil extract with hexane, isopropanol and petroleum ether solvents was 78.66%, 59.66% and 53.66% respectively. Whereas, through filtration method, it was 20 %, 15.66% and 10.3% and through centrifugation extraction, 52.66%, 53.33% and 48.3% for hexane, isopropanol and petroleum ether respectively. In another study El-Baz *et al*., (2014), evaluated the extractability yield of leaf extract of *Jatropha curcas* in different solvents. The reported yield for different solvent systems i.e., crude methanol, successive methanol, ethyl acetate and petroleum ether were 5.97%, 3.69%, 1.32% and 1.36% respectively.

4.2 Synthesis, coating and characterization SNPs:

The process of synthesis of silver naopartical was carried out as per the method suggested in 3.5.2 of matrial and methods. As per the interpetation, the colour was changed from pale green to dark brown. The changed colour from pale green to dark brown indicated synthesis of AgNPs from the leaves. The synthesis of silver nanoparticles was confirmed by change in colour and it was shown in

figure 4.1. Abhinna *et al*., (2014), also reported similar finding in gold nanoparticles synthesis, after 2 hrs of tube intubation the colour intensified and turned to reddish- black colour. To confirm this colour change is due to the formation of gold nanoparticles. Similar result was observed by Namratha and Monica, (2013) there was observed colour change from silver nitrate solution to reduced silver nanoparticles is indicated synthesis of silver nanoparticles. Dhaije *et al*., (2018) also reported same finding changed colour from pale green to dark brown indicated synthesis of AgNPs from the neem leaves. Characterization of the synthesized AgNPs was carried out with the help of Transmission Electron Microscopy (TEM) according to method of Banerjee *et al.,* (2014).

The synthesis of silver nanoparticles of various shapes, primarily spherical, was demonstrated by transmission electron microscopy (TEM) pictures as shown in figure 4.2. Average particles size of silver nanoparticles was found to be 22.47 nm, by measuring the diameter of these nanoparticles. Similar observation was reported by Okafor *et al*, (2013) reported spherical shape surrounded by biological molecules, which prevent AgNPs from aggregation in synthesis of silver nanoparticles. The average size of AgNPs was in the 3–9 nm range. Zargar *et al*., (2011), also reported same result the TEM study showed the formation of silver nanoparticles in the 10–30 nm range and average 18.2 nm in size.

Coating of silver nanoparticles was confirmed by TEM images. The TEM images *Jatropha curcas* leaf extract shown their presence specifically around the silver nanoparticles. The extract did not find visible in the pace where silver nanoparticles were not present. Even, change in average diameter of silver nanoparticles was observed after coating with *Jatropha curcas* leaf extract. The average diameter of coated silver nanoparticles was found 31.92 nm. Hence, it was confirmed that *Jatropha curcas* leaf extract has affinity for silver nanoparticles and capable of coating with them and was shown in figure 4.3. Accordingly, the aqueous leaf extract of *J. curcas* coated silver nanopartical was confirmed. Similar observation was reported by Sadgire *et al*., (2014), the TEM image of Ag, colloid particles were observed as spherical and average particle was in the range of 8-52 nm. Common average particle size was found 27.169nm.

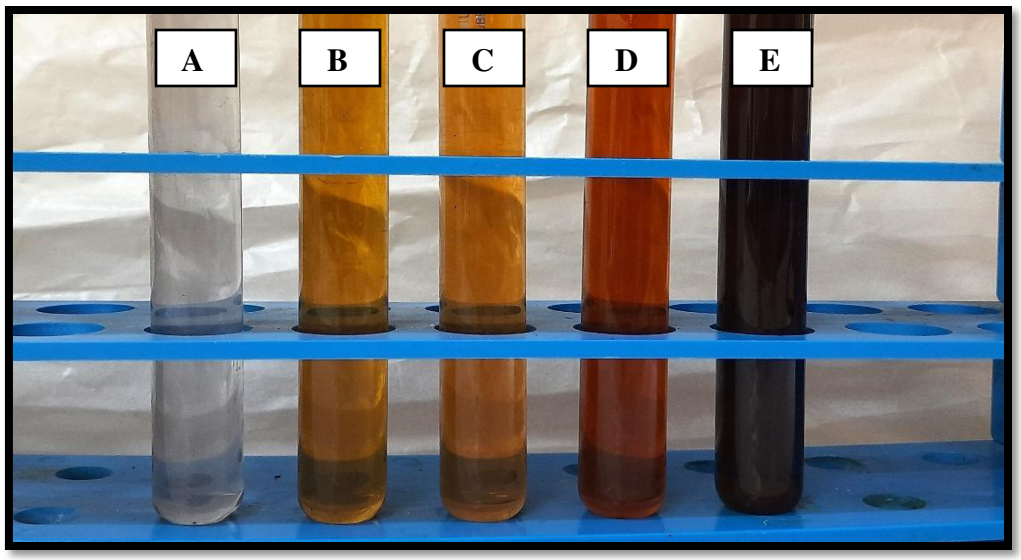
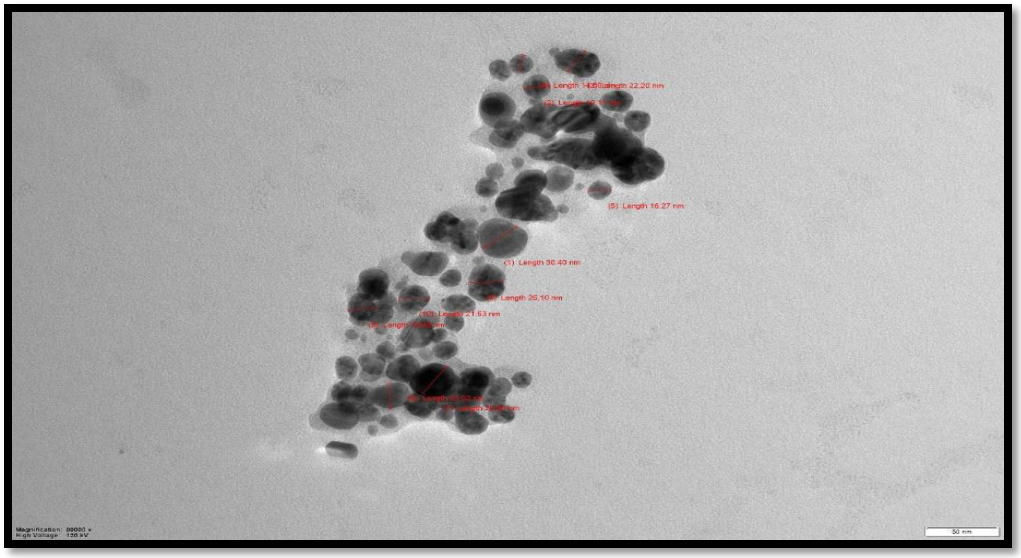
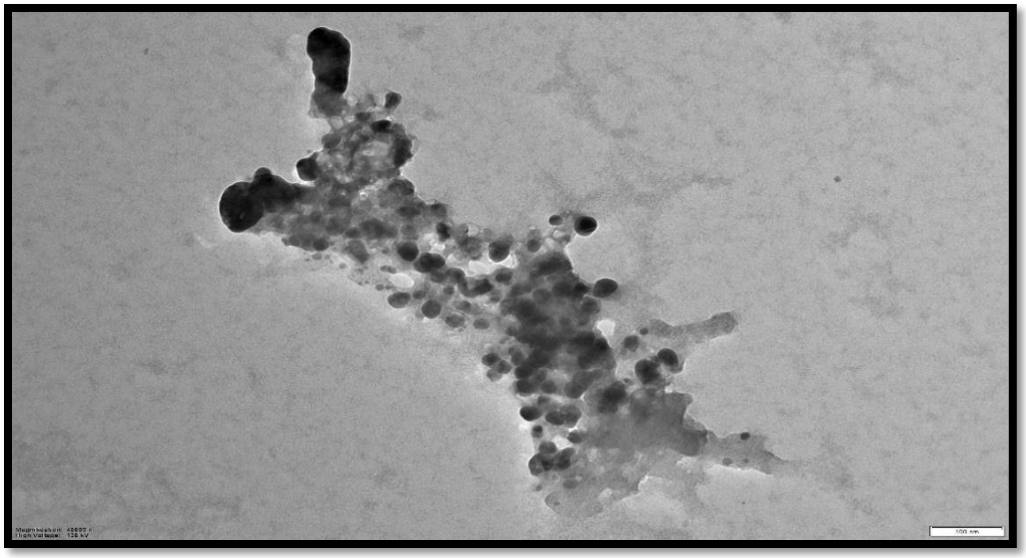


Figure 4.1- Confirmation of silver nanoparticles formed by observing change in colour from pale green to dark brown.

A. Natural colour of 1mM solution of silver nitrate.
B. Natural colour of 5% aqueous leaf extract of *Azadirachta indica* (Neem).
C. Pale green colour formed after addition of 1mM solution of silver nitrate and 5% aqueous leaf extract of *Azadirachta indica* (Neem) plant.
D. Colour change from pale green to dark brown after addition of 1mM solution of silver nitrate and 5% aqueous leaf extract of *Azadirachta indica* (Neem) plant after 2 hrs of incubation.
E. Reddish black colour observed after mixing AgNPs with *Jatropha curcas* leaf extract.



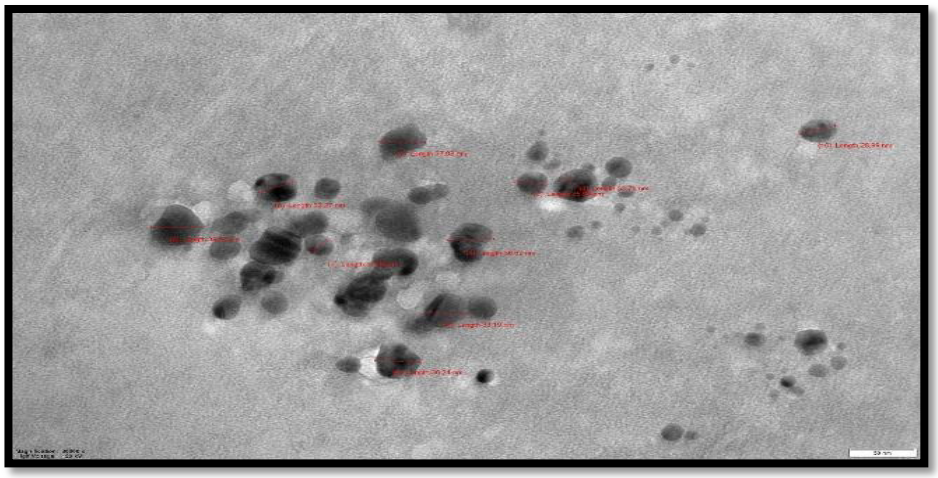
(A)



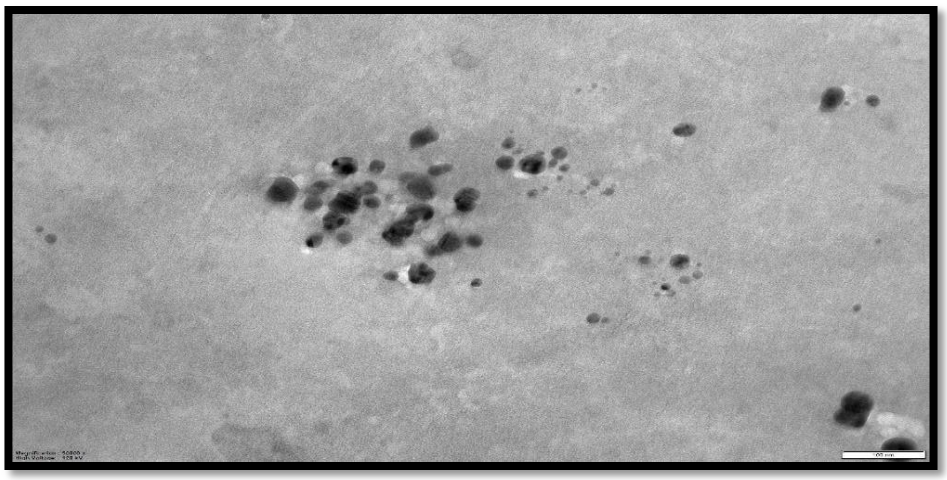
(B)

Figure 4.2 TEM image of SNPs

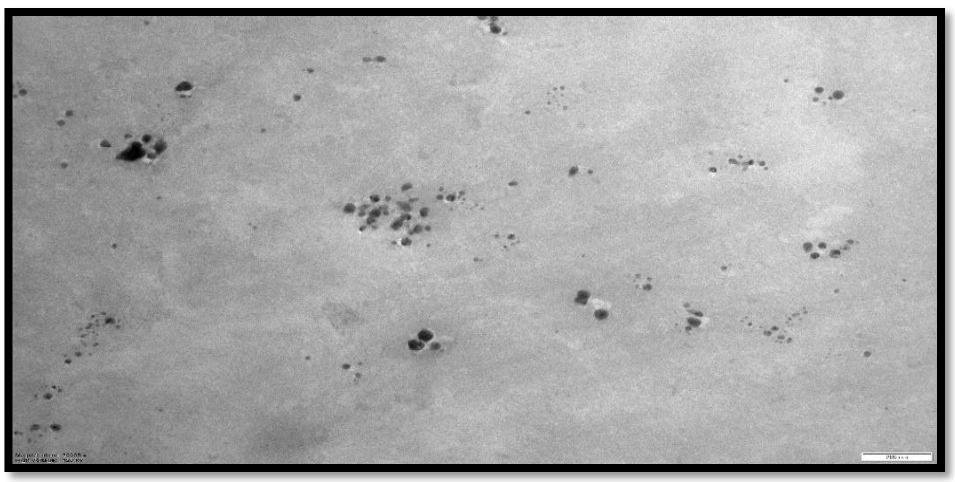
A) Magnification 80000X
B) Magnification 40000X



(A)



(B)



(C)

Figure 4.3 TEM images of JCLE coated SNPs

•Magnification- 80000X;
•Magnification- 50000X
•Magnification- 20000X

Debi *et al*., (2016) also reported similar finding the images revealed the formation of gold nanoparticles of different shaped but mostly observed spherical. By measuring the diameter of these nanoparticles average particles size was observed 40.54nm. Change in average diameter of gold nanoparticles was noticed after coating with *Morinda citrifolia* fruit extract coated GNPs found 51.37nm.

4.3 General Observations:

During the period of acclimatization of five days and throughout the entire experimental period all the experimental rats were appeared alert, active and healthy without any untoward manifestations suggesting proper managemental condition in the animal house. No any deviation in the general observation were observed in rat either in control as well as treatment group as shown in table 4.1.

The animals from control group remained active and alive throughout the experimental period, however, two mortality was recorded in experimental group. Two mortality was observed on 14^{th} day of experiments in group III and IV. Though the single mortality was there in two groups, none of the rest animals were shown the sign of toxicity or mortality upto 28 days of experimental period. Moreover, no delayed mortality was recorded in the satellite group (V) where the rats were maintained for 15 more days. Mahe *et al*., (2017) reported similar result in albino rats treated with crude methanol extract of *J. curcas* leaf and he noted that no death was observed acute toxicity of first phase, however, in the second phase death was recorded in animals administered with 2600 mg/kg and 3000 mg/kg body weight methanol extract of *J. curcas* leaf. Igbinosa *et al*., (2013), reported death of rats administered with *J. curcas* methanol extract occurred at a dose-dependent manner with starting dose of 34 mg/kg and at highest dose of 86.0 mg/kg, majority of rats exhibited signs of depression, closing of eyes, languishment, loss of body mass, and black excreta. Rats began to die on day 2 after administering *J. curcas* extract at 86.00, 58.00, 46.00, and 34.0 kg/body weight in dose range study. See *et al*., (2016) reported that decoction and ethanol leaf extract of *Jatropha curcas* acutely nontoxic with 0% mortality but lethal to the rats when taken for longer period of time marked by behavioral changes that signifies chronic toxicity such as weight loss, anorexia, reluctance to move and restlessness. Whereas, Azubike *et al*., (2015) observed percentage mortality

(number of deaths) of 100 % in first phase at 1000 and 2000 mg/kg dose and 50% mortality observed at 200 mg and 100 % mortality at 400, 600, and 800 mg/kg dose in the mice treated with crude aqueous extract of *Jatropha curcas* leaves in acute toxicity study (intraperitonally). There were tremors, vomiting, anorexia, weakness, diarrhea and death also observed during clinical examination.

Table 4.1. Behavioral Changes of rats in treatment and control groups for 28 days.

Symptoms	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
Convulsion	-	-	-	-	-
Tremors	-	-	-	-	-
Hyperactivity	-	-	-	-	-
Restlessness	-	-	-	-	-
Grooming	-	-	-	-	-
Decreased activity	-	-	-	-	-
Sleep	-	-	-	-	-
Gait	-	-	-	-	-
Piloerection	-	-	-	-	-
Aggressiveness	-	-	-	-	-
Salivation	-	-	-	-	-
Vomiting	-	-	-	-	-
Urination	-	-	-	-	-
Diarrhoea	-	-	-	-	-
Respiration	-	-	-	-	-
Appetite	-	-	-	-	-
Thirst	-	-	-	-	-

Scoring: - : No effect, + = mild effect, ++ = moderate effect, +++ = severe effect

4.3.1. Feed consumption:

There were also no significant differences in food consumption and water intake was observed in the experimental rats in both treated and control group rats throughout the experimental period. The feed consumption values were incorporated in table 4.2. In control group animals on day 7^{th}, 14^{th} ,21^{th} and 28^{th}

were 671.8± 8.47, 670.25 ± 5.13, 674.64 ± 8.51, 673.31 ± 5.02 respectively. In group II feed consumption values were 671.06 ± 7.63, 670.11 ± 6.24, 672.30 ±7.68, 666.74 ± 7.01 respectively. In group III and IV feed consumption values were 672.20 ± 5.51, 671. 89 ± 3.81, 671.78 ± 3.05, 669.84 ± 8.31 and 671.78 ± 7.10, 674.25 ± 5.55, 668.95 ± 4.06, 667.30 ± 6.75 respectively. Throughout the experimental period feed consumption values observed increased on 21th day in group I and values observed decreased on day 28th in group II.

Table 4.2 Weekly feed intake of rats in the treated and control groups for 28 days.

Group	Treatment	7thday (Mean± S.E.)	14th day (Mean± S.E.)	21th day (Mean ±S.E)	28th day (Mean± S.E.)	Stat	CD
I	Healthy control	671.85 ±8.47	670.25 ±5.13	674.64 ±8.51	673.31 ±5.02	NS	At 5% 16. 304 At 1% 21. 423
II	*Jatropha curcas* leaf extract coated SNP's dose-I	671.06 ± 7.63	670.11 ±6.24	672.30 ±7.68	666.74 ±7.01	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	672.20 ±5.51	671.98 ±3.81	671.78 ±3.05	669.84 ±8.31	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	671.78 ± 7.10	674.25 ±5.55	668.95 ±4.06	667.30 ±6.75	NS	
V	Satellite group	671.59 ± 3.83	668.22 ±3.40	670.60 ±3.01	667.40 ±4.38	NS	
Stat		NS	NS	NS	NS	-	
CD	At 5% = 16.304; At 1%= 21.423						

4.3.2. Body Weight

The mean body weight of rats in treatment and control groups were recorded and presented in table 4.3; figure 4.4. In control group animals on day 0th, 7th, 14th and 28th were 193.12 ±3.88, 192.50 ±8.18, 199.37 ±7.09, and 195.37 ±5.24 respectively. In II group animals the body weight were 190.62±5.03, 188.75±8.49, 193.62±6.24, and 190.62± 3.71respectively. In III groups the animal body weight was 190.62±6.15, 191.25±5.49, 193.75±5.65, and 191.75±6.82 respectively. In group IV and V, animals the body weight were 191.87±6.12, 183.75±7.54, 198.75±6.95, 183.87±6.89 and 191.87±4.21, 190±8.66, 198.12±10.43, and 196.25 ± 5.57 respectively.

In group I, II, III, IV and V there was statistically non-significant difference was observed in body weight on day 0^{th},7^{th},14^{th}, and 28^{th} day. All values observed at par when compared with each other.

In group II there was non-significant increase in body weight observed on day 14^{th} as compared with its respective day values. However, decrease in body weight observed on day 7^{th}. Body weight values observed at par when compared with each other. In group III there was non-significant increase in body weight observed on day 14^{th} as compared to 7^{th}, and 28^{th} day weight. In group IV there was increase in body weight observed on day 14^{th} as compared to other day and decrease in body weight observed on day 7^{th}. All values observed at par when compared to each other.

On day 0^{th}, 7^{th}, 14^{th} and 28^{th} there was also statistically non-significant difference was observed in body weight in group I, II, III, IV and V. On day 7^{th} non-significant decreased in body weight was observed in treatment group IV as compared to group I, II, III and V.

On day 14^{th} statistically non-significant decrease in body weight observed in group II and III as compared to other groups. Whereas increase in body weight was found in group IV and V compared to group II and III. Body weight decreases in all groups when compared to group I.

On 28^{th} day there was decrease in body weight observed in group IV as compared to other groups. However, increase in body weight was found in group V as compared to other groups II, III and IV.

Similar result was observed by Adeyemi and Adewumi, (2014) in rats treated with silver nanoparticles (AgNPs) at 100, 1000, and 5000 mg/kg daily for 7, 14, and 21 days alternately. Rats did not produce significant loss in body weight. Qin *et al*., (2017) also reported non- significant effects in body weight in rats treated with silver nanoparticles at doses of 0.5 and 1 mg/ kg body weight daily for 28 days. Igbinaduwa, *et al*., (2011) also revealed the same result in rats treated with *Jatropha tanjorensis* leaf extract at 500 and 2000 mg/ kg for 28 days. Rats did not cause any changes in body weight. Kim *et al*., (2010) also reported similar result in fisher- 344 rats, treated with 30, 125 and 500mg/kg/day silver nanoparticle for 13 weeks oral dosing. There were no significant dose-related

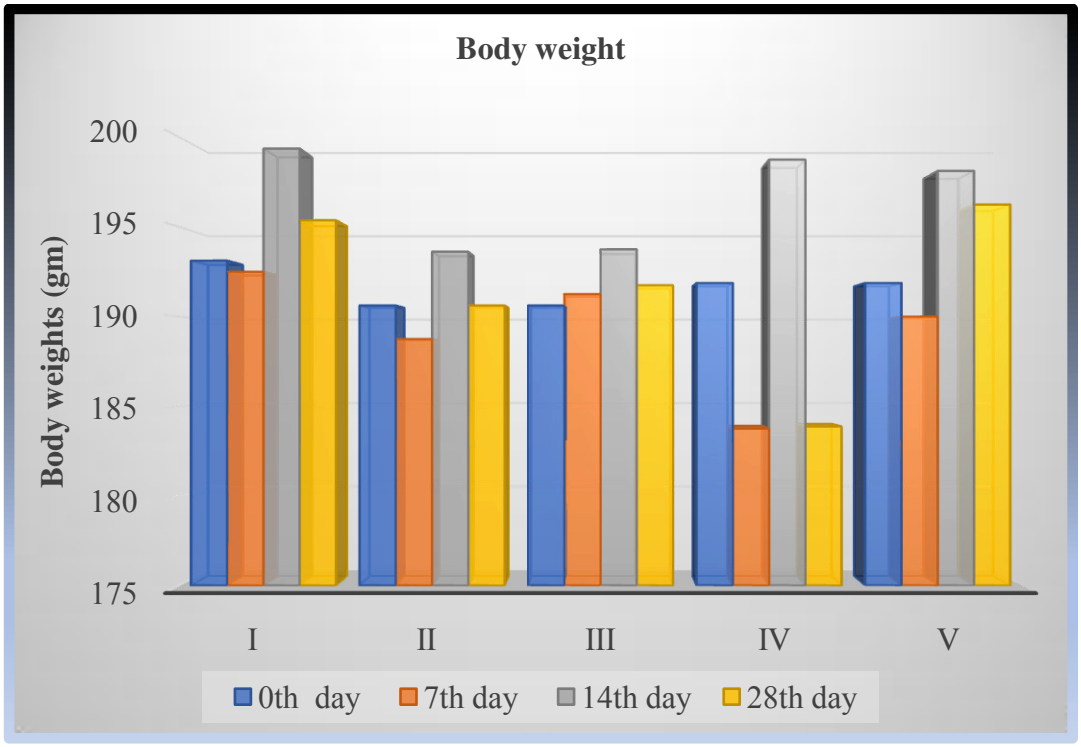


Figure 4.4- Mean Body weight values (gm) of experimental groups at different intervals.

changes in the body weight of female rats. On the basis of present research and literature it was confirmed that neither silver nanoparticles or *J. curcas* shown any significance difference on body weight gain at any of the concentration and duration of exposure. Hence it is concluded that aqueous leaf extract of *J. curcas* coated silver nanoparticles did not possess any toxic effect on the body weight of exposed rats.

were observed in this experiment.

Table 4.3 Mean Body weight (Mean ± S.E., gram) on different days in experimental rats of different groups. Overall, in general observation, feed consumption and body weight gain of rats either in control group or treatment group, no significant difference was observed in any of time duration. Hence the present research revealed that, the aqueous leaf extract of *J. curcas* did not shown any observed toxicity in the rats treated for 28 days, moreover no sign of delayed toxicity on rats

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean±S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	193.12am ±3.88	192.50am ± 8.18	199.37am ±7.09	195.37am ±5.24	NS	At 5% 15.931 At 1% 20.946
II	*Jatropha curcas* leaf extract coated SNP's dose-I	190.62am ± 5.03	188.75am ± 8.49	193.62am ±6.24	190.62am ± 3.71	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	190.62am ±6.15	191.25am ± 5.49	193.75am ±5.65	191.75am ± 6.82	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	191.87am ± 6.12	183.75am ± 7.54	198.75bm ±6.95	183.87am ±6.89	NS	
V	Satellite group	191.87am ±4.21	190.00am ± 8.66	198.12am ±10.43	196.25am ± 5.57	NS	
Stat		NS	NS	NS	NS	-	
CD	At 5% = 15.931; At 1%= 20.946						

4.4 Haematological parameters:

The various haematological parameters viz. haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) values were analyzed from blood collected at 0^{th}, 7^{th} ,14^{th} and 28^{th} day of the experimental period. The data was recorded in table and figure from 4.4 to 4.13.

4.4.1 Haemoglobin (Hb):

The mean haemoglobin values were recorded and presented in table 4.4 and figure 4.5. It was observed that the haemoglobin values in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 13.88 $\pm$0.19, 13.97$\pm$0.11, 14.13$\pm$0.09 and 14$\pm$0.18 respectively. In II groups the haemoglobin values were 13.75 $\pm$ 0.12, 14.04$\pm$ 0.20, 13.66 $\pm$0.34, 13.70 $\pm$ 0.16 and in the III, IV and V the haemoglobin values were 14.06 $\pm$ 0.19, 13.98$\pm$0.15, 14.02 $\pm$ 0.20, 14.58$\pm$ 0.19; 13.89$\pm$0.31, 14.11$\pm$0.17, 14.18$\pm$0.23, 13.76 $\pm$ 0.26; 13.97$\pm$0.15, 14.78 $\pm$0.26, 13.81 $\pm$ 0.10, 14.22$\pm$0.25 respectively.

Non-significant difference in haemoglobin was observed in group II on day 0^{th}, 7^{th} 14^{th} and 28^{th}. There was statistically significant difference in haemoglobin was observed on day 7^{th} and 28^{th} in group III of treatment group. On 7^{th} day the hemoglobin values were decrease as compared to 0^{th} day of experiment. However, from 14^{th} day onwards the increased in haemoglobin values were observed. Though the statistically significant difference was observed on day 7^{th}, 14^{th} and 28^{th} all the haemoglobin values on different days were within the normal range. Non-significant difference was also observed in group IV. However, in group V there was statistically significant difference in haemoglobin was observed on day 0^{th}, 7^{th}, 14^{th} and 28^{th}. On 14^{th} day the haemoglobin value was found decrease as compared to 7^{th} day but on 28^{th} day haemoglobin value was found increased. The haemoglobin value in all the days were found within the normal range only.

On day 0^{th} no significant difference in haemoglobin value was observed in groups I, II, III, IV and V. However, on day 7^{th} statistically significant difference was observed in I, II, IV and V groups. The haemoglobin values in groups II, IV

and V were increased as compared to group I and III, but in all the treatment groups the haemoglobin values were within the normal range.

On 14th day statistically significant difference was observed in groups II and IV groups. The haemoglobin values in groups I, II and V were observed at par when compared with each other. Though the haemoglobin values were increased in group III and IV on day 14th. The haemoglobin values in groups II and V were found decrease as compared with control groups. However, the haemoglobin values in all the groups were observed within the normal range.

On 28th day statistically significant difference was observed in I, II, III and IV groups. The haemoglobin values in groups II and III observed decrease than other groups. But the haemoglobin values in all the groups were within the normal range.

Throughout the experimental period there was highly increase in haemoglobin values was observed on 7th day in group V (satellite group), as compared to other groups.

Similar result was observed in fisher- 344 rats, by Kim *et al*., (2010) treated with 30, 125 and 500mg/kg/day silver nanoparticle for 13 weeks oral dosing. The haemoglobin values were 16.76, 16.47 and 16.93 respectively. No significant difference was observed in haemoglobin values at any of the dose. Sawadogo *et al*., (2018) also concluded, non-significant effect on the haemoglobin values in mice treated with aqueous extract of *J. curcus* at 200, 400 and 800 mg/kg body weight in subacute toxicity. The haemoglobin values were within the normal range in any of the dose. Poon *et al*., (2011) also reported same finding in rats treated with *Jatropha* oil at 0.5, 5, 50 and 500 mg/kg body weight per day for 28 consecutive days. Haemoglobin values were 14.18, 14.55, 14.52, 13.92 respectively. Non-significant effect was observed in haemoglobin. Haemoglobin values observed decreases than control, but observed within normal range.

Hence on this basis it was concluded that no effect of silver nanoparticles as well as *J. curus* was observed in the haemoglobin values. In this research, though the binding of silver nanoparticles with *J. curcas* was confirmed but they

could not be shown any deviation in haemoglobin values at any of the dose of the experiment.

Table 4.4 Mean Hb Values (Mean±S.E., g/dl) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S .E.)	Stat	CD
I	Healthy control	13.88^{am} ±0.19	13.97^{am} ± 0.11	14.13^{amn} ±0.09	14.00^{amn} ±0.18	NS	At 5% 0.581 At 1% 0.760
II	*Jatropha curcas* leaf extract coated SNP's dose-I	13.76^{am} ± 0.12	14.04^{amn} ± 0.20	13.66^{am} ± 0.34	13.70^{am} ± 0.16	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	14.06^{abm} ±0.19	13.98^{am} ±0.15	14.02^{abmn} ±0.20	14.58^{bmn} ± 0.19	S	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	13.89^{am} ±0.31	14.11^{amn} ±0.17	14.18^{an} ±0.23	13.76^{am} ±0.26	NS	
V	Satellite group	13.97^{am} ±0.15	14.78^{bn} ±0.26	13.81^{am} ±0.10	14.22^{bmn} ±0.25	HS	
Stat		NS	HS	S	HS	-	
CD	At 5% = 0.581; At 1%= 0.760						

Superscript a, b shows the significant difference within the row (between different day in a specific group). (P<0.05).

Superscript m, n, o shows significant difference in within the column (between different group on specific day). (P<0.05).

4.4.2 Total Erythrocyte Count (TEC):

The mean total erythrocyte count (TEC) values in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 7.72±0.17,7.60 ±0.13, 7.70 ±0.20 and 7.67±0.14 respectively. In II groups the total erythrocyte count (TEC) values were 7.80± 0.25, 7.58± 0.10, 7.67±0.18, 7.89± 0.16 respectively. In III group the total erythrocyte count (TEC) values were 7.73± 0.20, 7.55 ±0.13, 8.18± 0.32, 7.78± 0.12 respectively. In groups IV and V, the total erythrocyte count (TEC) values were 7.70±0.19, 8.07±0.23, 7.68±0.10, 7.98± 0.13 and 7.68±0.24, 7.72±0.29, 7.65± 0.22, 7.89 ±0.15 respectively. The data was represented in table 4.5 and figure 4.6.

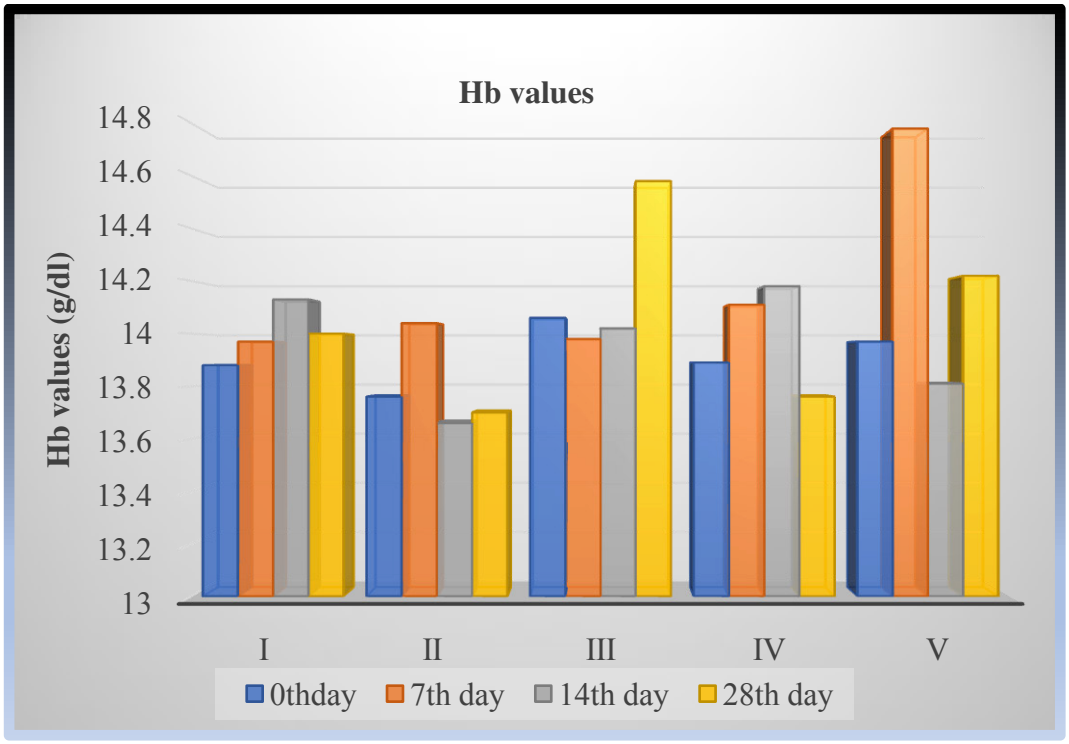


Figure 4.5- Mean Hb values (gm/dl) of experimental groups at different intervals

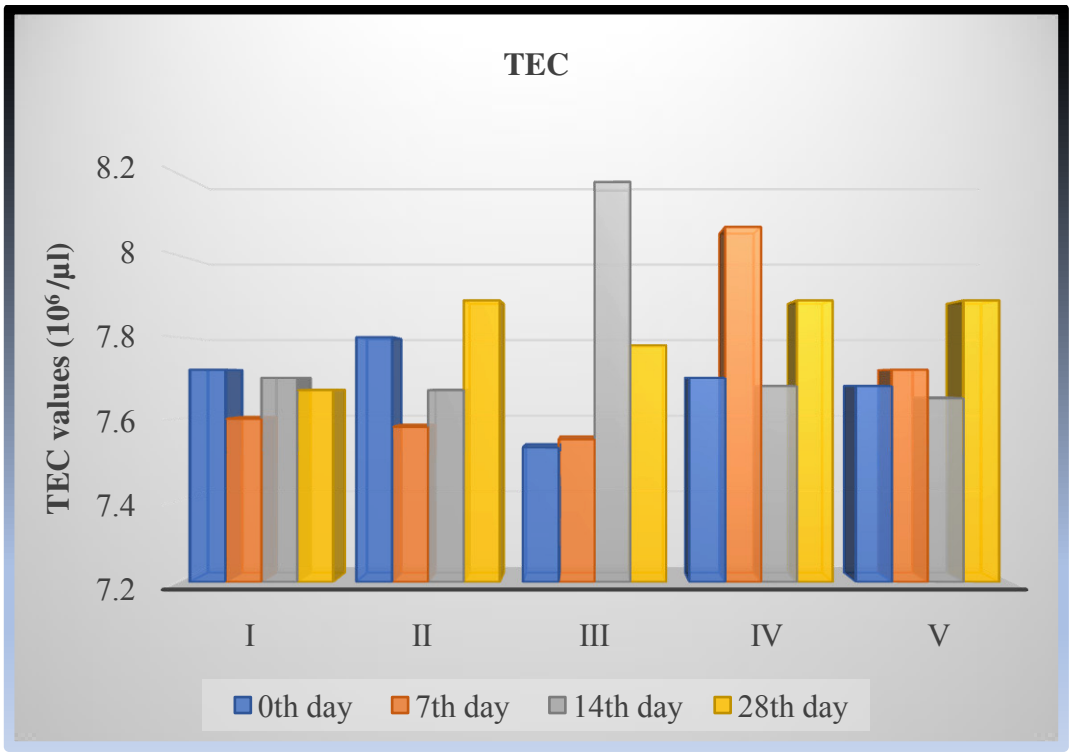


Figure 4.6- Mean TEC values (10^6 /µl) of experimental groups at different intervals.

Table 4.5 Mean TEC Values (Mean ± SE, X 10^6 / µl) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	7.72^{am} ± 0.178	7.60^{am} ± 0.131	7.70^{amn} ± 0.202	7.67^{am} ± 0.146	NS	At 5% 0.558 At 1% 0.723
II	*Jatropha curcas* leaf extract coated SNP's dose-I	7.80^{am} ± 0.252	7.58^{amn} ± 0.102	7.67^{amn} ± 0.182	7.89^{am} ± 0.168	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	7.73^{abm} ± 0.201	7.55^{an} ± 0.136	8.18^{bn} ± 0.327	7.78^{abm} ± 0.128	S	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	7.70^{am} ± 0.194	8.07^{ao} ± 0.235	7.68^{amn} ± 0.109	7.89^{am} ± 0.134	NS	
V	Satellite group	7.68^{am} ±0.243	7.72^{amn} ± 0.290	7.65^{am} ± 0.224	7.89^{am} ± 0.159	NS	
Stat		NS	S	S	NS	-	
CD	At 5% = 0.558; At 1%= 0.723						

Superscript a, b shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o shows significant difference in within the column (between different group on specific day). ($P<0.05$).

In treatment groups I, II, IV and V statistically non-significant difference was observed in total erythrocyte count on 0^{th}, 7^{th}, 14^{th} and 28^{th} day of experiment. Total erythrocyte count (TEC) values were observed at par when compared with each other on 0^{th}, 7^{th}, 14^{th} and 28^{th} day of experiment. In group II total erythrocyte count (TEC) was increase on 28^{th} day however, decreased on day 7^{th} of experiment. In treatment groups III statistically, significant difference was observed in total erythrocyte count on 7^{th} and 14^{th} day of experiment. There was increase in total erythrocyte count was observed on day14th and decreased on day 7^{th} as compared to other days values. In treatment group IV and V there was increase in total erythrocyte count (TEC) value on 28^{th} day of experiment and decrease on day 14^{th} as compared to other groups.

Statistically significant difference in total erythrocyte count (TEC) were observed on day 7^{th} in group III, IV and on day 14^{th} in III and V group. On 7^{th} day

the total erythrocyte count (TEC) value in group III was decreased and on 14th day it was increased as compared with other treatment groups but all the values in the treatment groups were within the normal range.

The increase in total erythrocyte count (TEC) values in group IV was observed and decreased in II and III group as compared to other treatment groups on day 7th of experiment. On day 14th there was increase in total erythrocyte count (TEC) was observed in group III, and decreased in II, IV and V group was observed compared to control group. But all the total erythrocyte count (TEC) values in all the groups were observed within normal range.

Though the total erythrocyte count was found decreased on 7th day of the experiment in group III, all the total erythrocyte count was observed to be non-significant in all treatment groups. On day 28th there was statistical, non-significant difference was observed in group I, II, III, IV and V.

Throughout the experimental period there was lowered in total erythrocyte count (TEC) value was observed on day 7th in group III as compared to other treatment groups, but this value was observed within normal range.

Similar result was found by Igbinosa *et al*., (2013) in rats treated with methanolic extract of *J. curcus.*Total erythrocyte count values were 9.84, 8.70 and 8.45 at 500,1000 and 2000mg/kg body weight respectively for 21days study. Non-significant difference was observed in total erythrocyte count. Qin *et al*., (2017) also concluded, non-significant effect in the total erythrocyte count values in sprague–dawley rats treated with silver nanoparticle at 0.5 and 1 mg/kg in subacute toxicity. Total erythrocyte count values were 8.09, 7.40 respectively. The total erythrocyte count values were within the normal range in any of the dose.

Hence on this basis of literature it was concluded that no effect of silver nanopartical as well as *J. curus* was observed in the total erythrocyte count values. However, in this research, *J. curcas* coated silver nanoparticles also not shown any sign of toxicity on total erythrocyte count at any of the concentrations used for 28 days.

4.4.3 Total Leucocyte Count (TLC):

The mean total leucocyte count (TLC)values were recorded and shown in table 4.6 and figure 4.7.

Table 4.6 Mean TLC values (Mean ± SE, 10^3/µl) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	10.19^{am} ±0.32	11.12^{am} ± 0.73	10.22^{am} ±0.44	11.04^{am} ±0.60	NS	At 5% 1.816 At 1% 2.389
II	*Jatropha curcas* leaf extract coated SNP's dose-I	10.12^{am} ± 0.40	13.15^{cn} ± 0.80	11.13^{abm} ± 0.58	12.16^{bm} ± 0.58	HS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	10.01^{am} ± 0.39	11.56^{abm} ± 0.58	13.09^{co} ± 0.90	12.13^{bm} ± 0.86	HS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	10.62^{am} ± 0.41	11.20^{abm} ± 0.59	14.54^{cp} ± 0.85	13.23^{bn} ± 0.73	HS	
V	Satellite group	10.15^{am} ±0.32	12.85^{bo} ± 0.65	12.46^{bcno} ± 1.01	14.20^{co} ± 0.65	HS	
Stat		NS	S	HS	HS	-	
CD	At 5% = 1.816; At 1%= 2.389						

Superscript a, b, c, d shows the significant difference within the row (between different day in a specific group). (P<0.05).

Superscript m, n, o, p shows significant difference in within the column (between different group on specific day). (P<0.05).

In control group animals, total leucocyte values, on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 10.19 ±0.32, 11.12 ±0.73, 10.22 ±0.44and 11.04 ±0.60 respectively. In II and III groups the total leucocyte count (TLC) values were 10.12 ± 0.40, 13.15± 0.80, 11.13 ±0.58, 12.16± 0.58 and 10.01± 0.39, 11.56 ±0.58, 13.09± 0.90, 12.13± 0.86 respectively. In groups IV and V, the total leucocyte count (TLC) values were 10.62 ±0.41, 11.20 ±0.59, 14.54 ±0.85, 13.23± 0.73 and 10.15 ±0.32, 12.85±0.65, 12.46±1.01, 14.20±0.65 respectively, observed in all the experimental animals exposed for 28 days.

In group I there was non-significant difference observed in total leucocyte count on 0^{th},7^{th}, 14^{th} and 28^{th} day of experiment. However, statistically significant difference in total leucocyte count (TLC) was observed in group II, III, IV and V.

Statistical, significant difference was observed on day 0^{th} ,$7^{th}14^{th}$ and 28^{th} in group II, the total leucocyte count (TLC) was increased when compared to 0^{th} day value. Though there was increased in total leucocyte count (TLC) on day 7^{th}, all the total leucocyte count (TLC) in other days were within normal range.

In group III, statistically significant difference in total leucocyte count (TLC) was observed on day 0^{th},7^{th} and 28^{th}. There was increased in total leucocyte count (TLC) found on 14^{th} day and again decreased on day 28^{th} but the total leucocyte count in all the experimental days were observed within normal physiological limit. In group IV there was statistically significant difference in total leucocyte count (TLC) were observed on day 0^{th} ,7^{th}, $14^{th}$and 28^{th}. Highly increase in total leucocyte count (TLC) values were observed on day $14^{th}$of group IV compared to 0^{th}, 7^{th} and 28^{th} days.

Statistical, significant difference in total leucocyte count (TLC) values was observed on day 0^{th} ,7^{th}, $14^{th}$and 28^{th}in group V. There was increased in total leucocyte count (TLC) observed on day 28^{th} but total leucocyte count (TLC) values were found within normal range in all the experimental days.

On day 7^{th} statistically significant difference was observed in groups I, II, IV and V. Increased in total leucocyte count (TLC) was observed in group II. However, decrease in total leucocyte count (TLC) value found in group IV. There was increased in total leucocyte count (TLC) values was observed in all treatment groups as compared to group I. But all total leucocyte count (TLC) values were found within normal range. Total leucocyte count values in groups I, III and IV were observed statistically similar but these values observed numerically different.

On day 14^{th} there was statistically significant difference was observed in group I, II, III, IV and V. Increased in total leucocyte count (TLC)was found on all groups as compare to group I. Highly increased in total leucocyte count (TLC) was observed in treatment group IV However, decrease in total leucocyte count (TLC) was observed in treatment group II compared with III, IV and V groups.

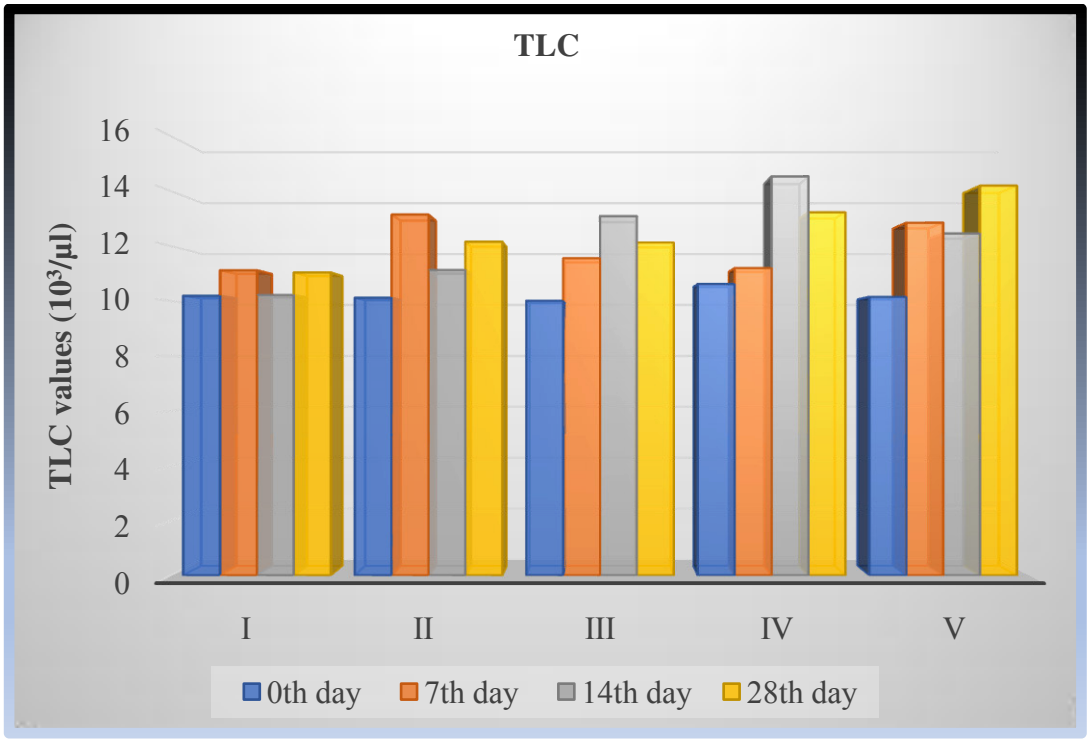


Figure 4.7- Mean TLC values (10^3/µl) of experimental groups at different intervals.

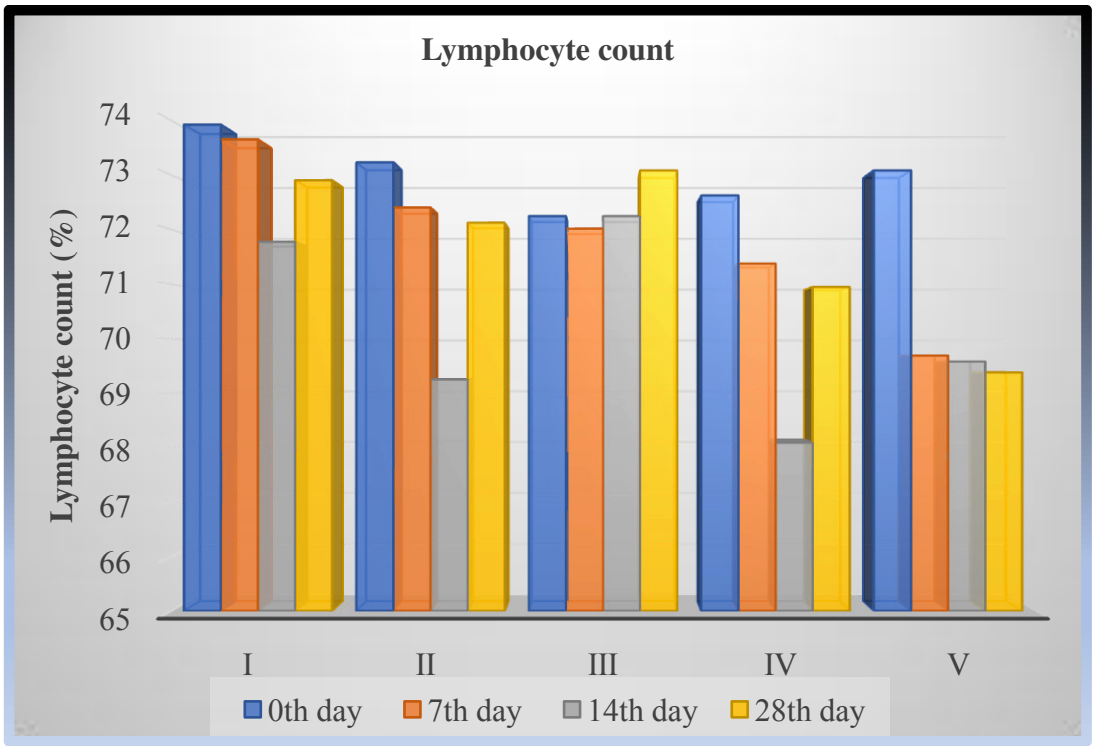


Figure 4.8- Mean Lymphocyte values (%) of experimental groups at different intervals.

On day 28th, statistically significant difference was observed in total leucocyte count (TLC) in group I, II, III, IV and V. Increase in total leucocyte count (TLC) was observed in group V and decreased in group III compared with group II, IV and V.

Throughout the experimental period there was highly increase in total leucocyte count (TLC) was observed in group V on 28th day of experiment.

Qin *et al*., (2017) also reported the similar finding in rats treated with silver nanoparticles at 0.5 and 1 mg/kg in males and female. Total leucocyte count values were in male 7.31, 7.80 and in female 7.41, 8.03 respectively. Increase in total leucocyte counts observed as compared to control but the values were within the normal range. Kim *et al*., (2008) also observed similar finding in sprague-dawley male rats treated with silver nanoparticles at 30, 300, 1000 mg/kg body weight. Total leucocyte counts values were 12.45, 12.68,11.20 respectively. Nonsignificant effect was observed. Igbinosa *et al*., (2013) also concluded, nonsignificant effect on total leucocyte count in rats treated with methanolic extract of *J. curcus* at 500,1000 and 2000mg/kg body weight for 21days study. Total leucocyte count values were 7.50, 9.02,8.9 respectively. The total leucocyte count values were within the normal range in any of the dose. In the present experiment there also nonsignificant difference was observed in all the groups, though there was deviation in the values in all the groups at different days no sign of toxicity with respect to increase or decrease in value was observed. No toxicity sign was observed in aqueous extract *J. curus* leaves coated silver nanoparticles when dosed for 28 days of experiment.

4.4.4 Differential Leucocyte Count (DLC):

4.4.4.a Lymphocyte Count (%)

Table 4.7 and figure 4.8. shown the mean lymphocyte counts values, in control group animals on day 0th, 7th, 14th and 28th were 73.93±0.78, 73.66± 1.06, 71.78± 2.52and 72.91± 1.90 respectively. In II, III, IV, V and VI groups, the lymphocyte counts were 73.24±1.19, 72.41± 0.92, 69.25±1.27, 72.13±2.18; 72.25±0.57, 72.02± 1.10, 72.25±1.11, 73.09 ±1.48; 72.63±0.91, 71.38±1.73, 68.09±1.30, 70.95±0.87; 73.09±1.42, 69.69±1.85, 69.58± 1.54, 69.38±2 respectively.

In group I, II, III and V there was non-significant difference in lymphocyte counts was observed on day 0^{th}, 7^{th}, 14^{th} and 28^{th}. All lymphocyte counts values observed at par when compare with each other. In group II decrease in lymphocyte counts was observed on 14^{th} day as compared with its respective day values.

In group III there was increase in lymphocyte counts and in group V there was decrease in lymphocyte counts on 28^{th} day of experiment. However, the lymphocyte counts values were observed with in normal physiological limit in all the experimental days

In group IV there was statistical, significant difference in lymphocyte counts were observed on 0^{th} and 14^{th} day of experiment. There was decrease in lymphocyte counts on day $14^{th}$and increase on 7^{th} day of experiment was observed.

On day 0^{th},7^{th} and 28^{th} statistically non-significant difference in lymphocyte counts was observed in all groups. On day 7^{th} there was non-significantly decrease in lymphocyte counts found in group V and increase in lymphocyte counts in group II was observe when compared with rest of the groups. On day 28^{th} non-significantly increased in lymphocyte counts observed in group III as compared to groups I, II, IV and V. Whereas decrease in lymphocyte counts observed in V group. But the all-lymphocyte counts were observed within normal range in all the treatment groups.

On day 14^{th} statistically significant difference in lymphocyte counts was observed in group III and IV. There was increased in lymphocyte counts group III and decrease in group IV was observed. In group I, II and V lymphocyte count values were statistically similar but numerically different, however, all values were observed within normal range.

Kim *et al*., (2010), observed similar result in male and female rats after 90-day oral administration of silver nanoparticles at 30, 125 and 500 mg /kg. Lymphocyte values were 68.21, 68.93, 69.34 and 68.32, 69.59, 71.22 respectively. No significant effect was observed in lymphocyte count, all the values observed within normal range. Igbinaduwa, *et al*., (2011) also reported

non-significant effect in rats treated with *Jatropha tanjorensis* leaf extract at 500 and 2000 mg/kg. Lymphocyte values were observed within normal rang.

Sawadogo *et al*., (2018) also concluded, no significant effect on lymphocyte count in mice treated with aqueous extract of *jatropha curcas* leaves at 0, 200, 400, 800 mg/kg. The lymphocyte values were 71.4, 71.2, 70.2, 67.4. No sign of toxicity was observed in any of the concentrations. Hence on this basis it was concluded that no effect of silver nanoparticle as well as *J. curus* was observed in the lymphocyte count. Though there was coating of silver nanoparticles to the aqueous extract of *J. curcas* no any sign of toxicity in terms of increase or decrease of lymphocyte count was observed at any of the dose of the experiment.

Table 4.7 Mean Lymphocyte values (Mean ± SE, %) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S .E.)	Stat	CD
I	Healthy control	73.93^{am} ±0.78	73.66^{am} ± 1.06	71.78^{amn} ±2.52	72.91^{am} ±1.90	NS	At 5% 4.347 At 1% 5.717
II	*Jatropha curcas* leaf extract coated SNP's dose-I	73.24^{am} ± 1.19	72.41^{am} ± 0.92	69.25^{amn} ± 1.27	72.13^{am} ± 2.18	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	72.25^{am} ± 0.57	72.02^{am} ± 1.10	72.25^{an} ± 1.11	73.09^{am} ± 1.48	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	72.63^{am} ± 0.91	71.38^{am} ± 1.73	68.09^{bm} ± 1.30	70.95^{abm} ± 0.87	S	
V	Satellite group	73.09^{am} ±1.42	69.69^{am} ± 1.85	69.58^{amn} ± 1.54	69.38^{am} ± 2.92	NS	
Stat		NS	NS	S	NS	-	
CD	At 5% = 4.347; At 1%= 5.717						

Superscript a, b shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n shows significant difference in within the column (between different group on specific day). ($P<0.05$).

4.4.4.b Monocyte Count (%):

The mean monocyte count values were represented in table 4.8 and figure 4.9. In control group animals, mean monocyte count on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 5.62±0.49, 5.83±0.48, 0.69±0.38and 5.97±0.48 respectively. In II and III groups the monocyte counts were 5.95± 0.36, 7.02± 0.18, 6.93 ±0.20, 6.91 ± 0.12 and 5.97± 0.38, 6.81 ± 0.24, 7.55 ± 0.22, 7.92±0.26 respectively. In groups IV and V, the monocyte counts were 5.90±0.39, 7.06±0.27, 7.68±0.28, 7.60±0.25 and 5.86±0.43, 7.01 ±0.13, 7.57±0.20, 7.90 ±0.28 respectively.

In group I there was statistically non-significant difference in monocyte counts was observed on 0^{th}, 7^{th}, 14^{th} and 28^{th} day of experiment.

In group II there was statistically significant difference in monocyte counts observed on 0^{th}, 7^{th}, 14^{th} and 28^{th} days of experiment. There was decreased in monocyte counts was observed on 14^{th} and 28^{th} day and increased in monocyte counts was observed on 7^{th}day of experiment.

In group III statistically, significant difference was observed in monocyte counts on 0^{th}, 7^{th},14^{th} and 28^{th} days of experiment. Statistically increase in monocyte counts was observed on day14^{th} and 28^{th} as compared to 0^{th} and 7^{th} day values. Monocyte values were observed increases from 7^{th} day onwards.

In group IV there was also statistically significant difference in monocyte count observed on 0^{th}, 7^{th},14^{th} and 28^{th} days of experiment. The monocyte count was increased on day 14^{th} of experiment and decrease on day 7^{th}day of experiment.

Statistically, significant difference was observed in monocyte counts on 0^{th},7^{th},$14^{th}$and 28^{th} day in group V. There was statistical increase in monocyte counts was observed on 28^{th} day of experiment. Monocyte counts values observed increases from 7^{th} day onwards.

On day 0^{th} there was non-significant difference was observed in monocyte counts in groups I, II, III, IV and V groups when compared with each other. On day 7^{th} there was statistical, significant difference in monocyte counts was observed in group I, II, III, IV and V. In group III decrease in monocyte counts was observed as compared to group II, IV and V. In group II, IV, and V the count were statistically similar but numerically different was observed.

On day 14th there was statistical, significant difference was observed in monocyte counts in group I, II, III, IV and V. Increase in monocyte counts was observed in group IV compared to groups I, II, III and V, whereas in group II decrease in monocyte counts was observed as compared to groups III, IV and V. In groups II, III and V values were statistically similar but observed numerically different.

On day 28th statistical, significant difference in monocyte counts was observed in group I, II, III, IV and V. Increase in monocyte counts was observed in group III and decrease in monocyte counts was observed in group II as compared to III, IV and V group. Throughout the experimental period there was highly increase in monocyte counts was observed on 28th day of experiment in treatment group III.

Table 4. 8 Mean Monocyte values (Mean ± SE, %) on different days in experimental rats of different groups.

Group	Treatment	0th day (Mean± S.E.)	7th day (Mean± S.E.)	14th day (Mean ±S.E)	28th day (Mean±S.E.)	Stat	CD
I	Healthy control	5.62^{am} ±0.49	5.83^{am} ± 0.48	5.69^{am} ±0.38	5.97^{am} ±0.48	NS	At 5% 0.899 At 1% 1.179
II	*Jatropha curcas* leaf extract coated SNP's dose-I	5.95^{am} ± 0.36	7.02^{bn} ± 0.18	6.93^{abn} ± 0.20	6.91^{abo} ± 0.12	HS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	5.97^{am} ±0.38	6.81^{bm} ± 0.24	7.53^{cn} ± 0.22	7.92^{cp} ± 0.25	HS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	5.90^{am} ± 0.39	7.06^{abn} ± 0.27	7.68^{bo} ± 0.28	7.60^{bn} ±0.25	HS	
V	Satellite group	5.86^{a} ±0.43	7.01^{bn} ± 0.13	7.57^{cn} ± 0.20	7.90^{cp} ± 0.28	HS	
Stat		NS	HS	HS	HS	-	
CD	At 5% = 0.899; At 1%= 1.179						

Superscript a, b, c shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o, p shows significant difference in within the column (between different group on specific day). ($P<0.05$).

In the present study there was significant increase in monocyte count was observed than the normal range from II to V group of treatment. Kim *et al*.,

(2010) received the increase in monocyte values as 3.85, 3.65, 4.54 in female rats after 90-day oral administration of silver nanoparticles only at 30, 125 and 500 mg /kg but the values were within the normal range. However, Azzaz *et al*., (2011), concluded that there was significant decrease in monocytes count in exposed groups fed with *Jatropha* seed (as meal 200gm/kg) daily for one month as compared to treatment group. Poon *et al.,* (2011), also confirmed that there was decreased in monocyte count in the rats treated with *Jatropha* oil diluted in corn oil at 0.5, 5, 50 and 500 mg kg body weight (b.wt.) for 28 consecutive days.

Sawadogo *et al*., (2018) also reported that aqueous extract of *Jatropha curcas* shown non-significant difference in monocyte count in mice after 28 days of treatment with doses of 200, 400 and 800 mg/kg body weight.

In present study the monocyte count was gradual increases from group II to group V. It was also observed that the monocyte count was also significantly increase in rats treated with silver nanoparticles. So based on the results and literature it was concluded that though plant not shown the toxicity but the silver nanoparticles coating itself shown the toxicity by increasing in the monocyte values.

4.4.4.c Neutrophil count (%):

The mean neutrophil counts were observed in control group animals on day 0th, 7^{th}, 14^{th} and 28^{th} were 17.95±1.25, 18.45±0.64, 20.32 ±2.23 and 18.65±2.95 respectively. In II and III group the neutrophil counts were17.77 ±1.25, 18.42 ±1.05, 21.40±1.12, 18.58±2.13 and 19.41±1.06, 18.91± 1.13, 17.56± 0.84, 16.55±2.17 respectively. In group IV and V the neutrophil counts were 19.18 ±1.21, 19.15 ± 1.86, 21.85 ± 0.76, 18.97± 0.96; 18.93± 1.39, 21.02±1.81, 20.65 ±1.86,20.25 ±2.71 respectively. The recorded mean neutrophil values were shown in table 4.9 and figure 4.10.

In group I, II, III, IV and V there was statistically non-significant difference was observed in neutrophil counts on day 0^{th}, 7^{th} ,14^{th} and 28^{th} day of experiments and all values observed within normal physiological limit.

In group II, and IV there was non-significant increase in neutrophil counts was observed on day 14^{th} of experiment when compared to day 0^{th}, 7^{th} and 28^{th}. In

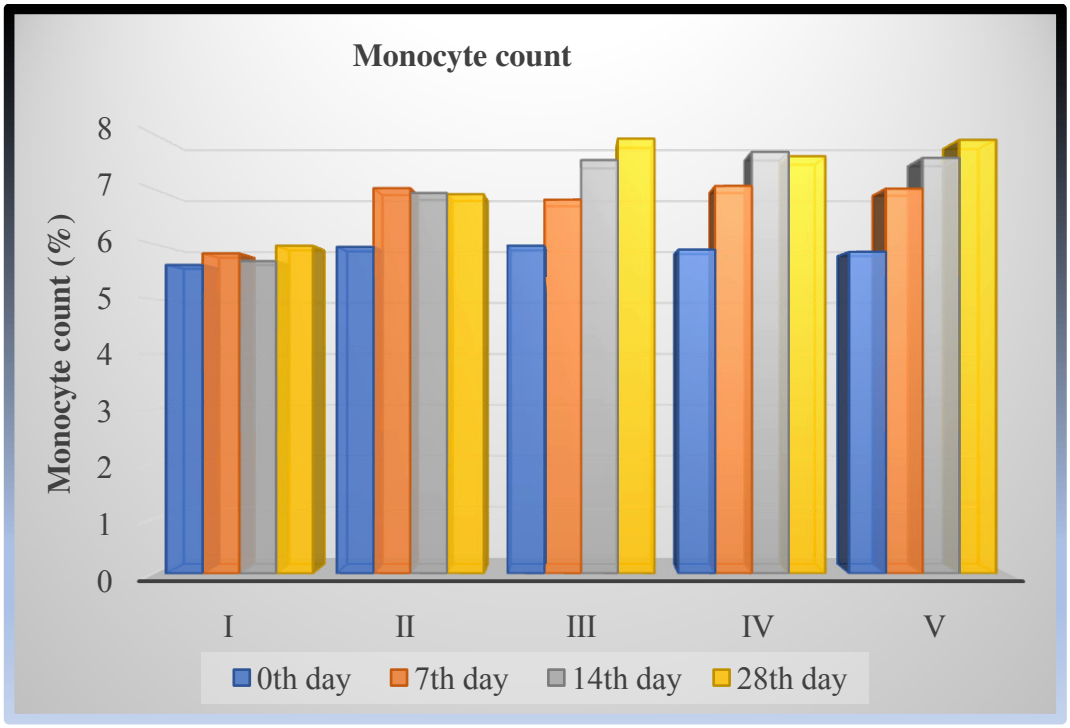


Figure 4.9 Mean Monocyte values (%) of experimental groups at different intervals.

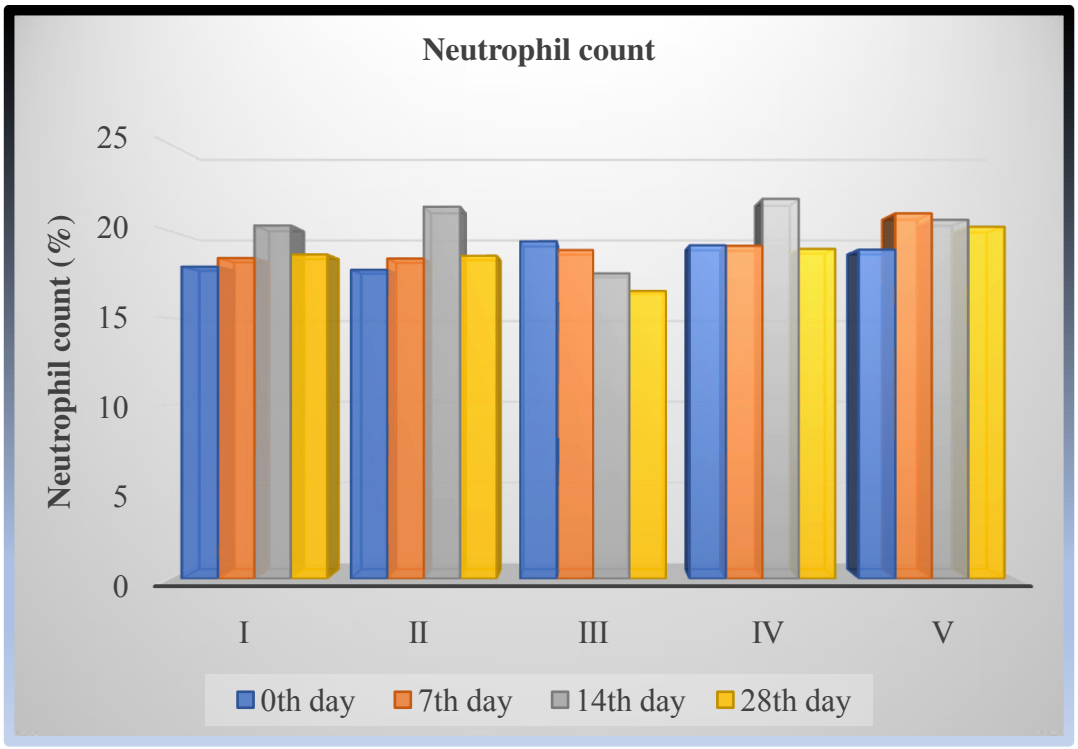


Figure 4.10- Mean Neutrophil values (%) of experimental groups at different intervals.

group III and IV there was non-significant decrease in neutrophil counts observed on 28th day of experiment as compare to 0th, 7th and 14thdays values.

In group V there was statistically non-significant increase in neutrophil counts observed on 7th day and decreased on 28th day of experiment. In groups I, II, III, IV and V all neutrophil counts values were observed at par when compared with each other. However, all values were within normal physiological limit.

On day 0th, 7th 14th and 28ththere was statistical, non-significant difference in neutrophil counts was observed in groups I, II, III, IV and V.

On day 7th there was non-significant increase in neutrophil counts observed in group V as compared to other groups. On day 28th there was nonsignificant, increased in neutrophil counts in group V when compared to another groups I, II, III and IV. However, decrease in neutrophil counts was observed in group III as compared to other groups. On day 0th, 7th, 14th and 28th days of experiments all neutrophil counts values observed at par when compared with each other.

Throughout the experimental period there was increase in neutrophil counts was observed in treatment group IV on day 14th when compared to another treatment groups.

Similar result was found by AL-Baker *et al*., (2020) in rats treated with silver nanoparticles at 0.2 and 0.4 gm/kg for a period of 3 days. Neutrophil counts values were 17, 10 respectively, so the neutrophil count was observed within the normal range. Igbinaduwa *et al*., (2011) also reported the non-significant effect of methanolic extract of *Jatropha tanjorensis* leaf on rats dosed with 500 and 2000 mg/kg for 28 days. Koker *et al*., (2015) reported that the increase in neutrophil values as 35.31, 47.36, 70.30 in rats at 50, 100 and 200 mg/kg dose of ethanolic extract of *Jatropha curcas* seeds intraperitoneally for 3 days. Significantly increased neutrophils values when compared to their respective controls. But the values were observed increases than the normal range. The results of present study confirms that no effect of *J. curcas* coated silver nanoparticles was observed in the neutrophil count in any of rats of the groups.

Table 4.9 Mean Neutrophil values (Mean ± SE, %) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	17.95^{am} ±1.25	18.45^{am} ± 0.640	20.32^{am} ±2.23	18.65^{am} ±2.95	NS	At 5% 4.598 At 1% 6.045
II	*Jatropha curcas* leaf extract coated SNP's dose-I	17.77^{am} ± 1.25	18.42^{am} ± 1.057	21.40^{am} ± 1.12	18.58^{am} ± 2.13	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	19.41^{am} ±1.068	18.91^{am} ± 1.13	17.56^{am} ± 0.84	16.55^{am} ± 2.17	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	19.18^{am} ± 1.21	19.15^{am} ± 1.86	21.85^{am} ± 0.76	$18..97^{am}$ ±0.96	NS	
V	Satellite group	18.93^{am} ±1.39	21.02^{am} ± 1.81	20.65^{am} ± 1.86	20.25^{am} ± 2.71	NS	
Stat		NS	NS	NS	NS	-	
CD	At 5% = 4.598; At 1%= 6.045						

4.4.4.e Eosinophil Count (%):

The eosinophil values of control and treatment groups were shown in table 4.10 and figure 4.11.

On day 0^{th}, 7^{th}, 14^{th} and 28^{th} the mean eosinophil counts in control group animals were 2.37 ±0.27, 1.95±0.32, 2.11±0.17and 2.37 ±0.20 respectively. In II groups the eosinophil counts were 2.87 ± 0.24, 2.00± 0.32, 2.25 ±0.18, 2.12±0.35 respectively. In III group the eosinophil counts were 2.29±0.20, 2.12±0.39, 2.50±0.32, 2.25±0.36 respectively. In group IV and V, the eosinophil counts were 2.17±0.23, 2.25±0.36, 2.25±0.36, 2.25±0.36 and 2.00±0.37, 2.12±0.35, 2.00±0.26, 2.25±0.36 respectively.

The I, III, IV and V groups shown non-significant difference in eosinophil count on day 0^{th}, 7^{th}, 14^{th} and 28^{th}. All eosinophil count values were observed within normal range. However, group II, reveled statistically significant difference in eosinophil counts on 0^{th} and 7^{th} day of experiment. Significant, increase in eosinophil counts was observed on day $14^{th}$and on day 7^{th} there was decrease in eosinophil counts was observed as compared to 0^{th}, 14^{th} and 28^{th} days values.

Table 4.10 Mean Eosinophil values (Mean ± SE, %) on different days in experimental rats of different groups.

Group	Treatment	0th day (Mean ± S.E.)	7th day (Mean± S.E.)	14th day (Mean ±S.E)	28th day (Mean±S.E.)	Stat	CD
I	Healthy control	2.37^{am} ±0.27	1.95^{am} ± 0.32	2.11^{am} ±0.17	2.37^{am} ±0.20	NS	At 5 At 5% 0.861 At 1% 1.143
II	*Jatropha curcas* leaf extract coated SNP's dose-I	2.87^{ao} ± 0.24	2.00^{bm} ± 0.32	2.25^{abm} ± 0.18	2.12^{bm} ± 0.35	S	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	2.29^{amn} ±0.20	2.12^{am} ± 0.39	2.50^{am} ± 0.32	2.25^{am} ± 0.36	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	2.17^{an} ± 0.23	2.25^{am} ± 0.36	2.25^{am} ± 0.36	2.25^{am} ±0.36	NS	
V	Satellite group	2.00^{amn} ±0.37	2.12^{am} ± 0.35	2.00^{am} ± 0.26	2.25^{am} ± 0.36	NS	
Stat		S	NS	NS	NS	-	
CD	At 5% = 0.861; At 1%= 1.143						

Superscript a, b, c shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o shows significant difference in within the column (between different group on specific day). ($P<0.05$).

Non-significant, increase in eosinophil counts observed in group III on day 14th whereas decrease in eosinophil counts was observed on day 7th of experiment. In group V there was non-significant increase in eosinophil counts observed on 28th day. However, value observed decrease on day 0th and 14th.

In groups I, II, III, IV and V values were observed at par when compared with each other. But all eosinophil counts values were observed within normal physiological limit.

Though on day 0 there was significant difference was observed in group II and V, the eosinophil values were within the normal range only. On day 7th, 14th and 28thstatistically non-significant difference was observed in eosinophil counts in all groups. On day 7theosinophil counts observed increase in group IV when compared to other groups and decrease in eosinophil counts observed in group II as compared to groups III, IV and V.

On day 14th there was statistically non-significant increase in eosinophil counts observed in group VI as compared to group I, II, III, IV and V and decrease in eosinophil count values observed in group V compared to groups I II, III and IV.

On day 28th there was statistically non-significant decrease in eosinophil counts observed in group II as compared to I, III, IV and V group. In group III, IV and V values of eosinophil counts observed similar. But all eosinophil count values were observed within normal physiological limit.

Akanmu *et al*., (2020) analyzed the haematological parameters of SA Mutton Merino lambs drenched for 75 days with extracts of *Moringa oleifera*, *Jatropha curcas* and *Aloe vera*. He reported that non-significant difference was observed in eosinophil count in lambs treated with *Jatropha curcas* extract in the dose range of 10 to 50gm/kg dry matter for 75 days. Poon *et al*., (2011), also reported the similar finding with the present study. Non-significant difference was observed in eosinophil count in the rats treated with *Jatropha* oil diluted in corn oil at 0.5, 5, 50 and 500 mg kg body weight (b.wt.) for 28 consecutive days. Lee *et al*., (2018) also observed similar result in male Sprague-Dawley rats treated with silver nanoparticles at 10 and 100 micro g/kg/day for 4 weeks intravenously. Eosinophil values were 0.86, 1.24 respectively. Kim *et al*., (2008) also observed similar finding in sprague-dawley male rats treated with silver nanoparticles at 30, 300, 1000 mg/kg body weight. Eosinophil values were 0.28, 0.27 and 0.14 respectively. Non-significant decreased in eosinophil count was observed but values observed within normal range. No toxicity or pattern of significant difference in eosinophil count at any dose range with different time intervals was observed hence it was confirmed that aqueous extract of *J. curcas* coated silver nanoparticle did not shown the derivation in the eosinophil count in any of the treatment group dosed for 28 days.

4.4.4.d Basophil count (%):

The mean basophil counts value were shown in table 4.11 and figure 4.12. The values in control group animals on day 0th, 7th, 14th and 28th were 0.125±0.125, 0.115 ±0.112, 0.105 ±0.105 and 0.102±0.108 respectively. In II, III and IV groups the basophil counts were 0.122± 0.080, 0.152±0.099, 0.17±0.114,

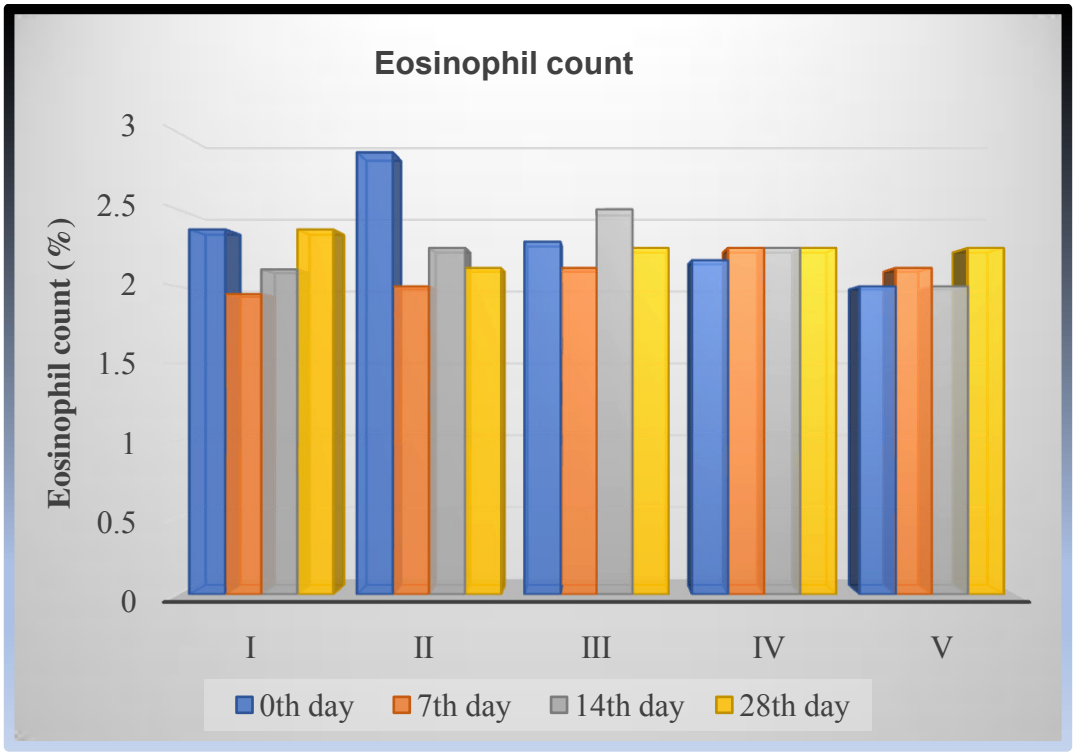


Figure 4.11- Mean Eosinophil values (%) of experimental groups at different intervals.

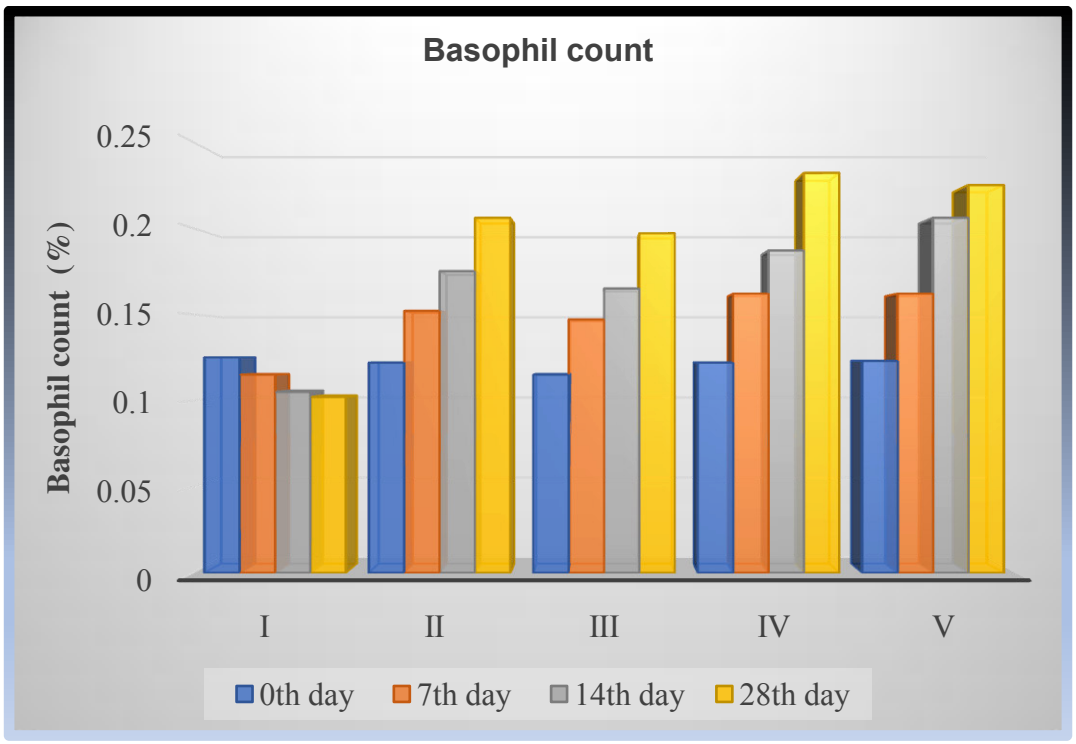


Figure 4.12- Mean Basophil values (%) of experimental groups at different intervals.

0.202± 0.206; 0.115± 0.084, 0.147 ±0.097, 0.165± 0.108, 0.197± 0.132 and 0.122±0.083,0.162± 0.110, 0.187±0.123, 0.232± 0.152respectively. In groups V basophil counts were 0.123±0.081, 0.162±0.112, 0.206± 0.136, 0.225±0.147 respectively.

In groups I, II, III, IV and V statistically non-significant difference in basophil counts were observed on day 0^{th} ,7^{th} 14^{th} and 28^{th} day and all-basophil counts values were observed within normal physiological limit. In treatment group I, II, III, IV and V in basophil counts values were observed at par when compared with each other.

In group II statistically non-significant increase in basophil counts was observed on day 28^{th} and decrease in basophil counts observed on day 7^{th} of experiment. But all values observed within normal range.

In group III there was non-significant decrease in basophil counts was observed on 7^{th} day and increase in basophil count was observed on 28^{th} day of experiment. In group IV nonsignificant increase in basophil count was observed from 7^{th} day onwards and on day 28^{th} day it was found highest. In group V basophil counts was observed non-significantly increase on day 28^{th}as compared to 0^{th} ,7^{th} and 14^{th} day.

On day 0^{th}, 7^{th}, 14^{th} and 28^{th} there was statistical non-significant difference was observed in basophil counts in groups I, II, III, IV and V. All values were observed within normal physiological limit. On day 7^{th}non-significant increase in basophil counts observed in group IV and V and decrease in basophil counts observed in group III as compared to II, IV and V groups. All basophil counts were observed within normal range.

On day 14^{th}there was non-significant increase in basophil counts observed in group V. However, decrease in basophil counts in group III as compared to groups II, IV and V. On day 28^{th} non-significant increase in basophil counts was observed in group IV and decrease in basophil counts was observed in group III as compared to groups II, IV and V.

Throughout the experimental period there was increase in basophil counts observed in treatment group IV on 28^{th} day of experiment as compared to other treatment groups but the value was within the normal range only.

Similar result was reported by Igbinosa *et al*., (2013) in rats treated with methanolic leaves extract *Jatropha curcas* at 500, 1000, 2000 mg/kg body weight dose range. Basophil values 0.55,0.53,0.50 respectively. He concluded that the basoplil values observed within normal range in the experiment. Qin *et al*., (2017) also reported the similar finding in rats treated with silver nanoparticles at 0.5 and 1 mg/kg in males. Basophil count values were 0.18 and 0.17 respectively. Non-significant increase in basophil counts observed as compared to control but the values were within the normal range.

Table 4.11 Mean Basophil values (Mean ± SE, %) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	0.125^{am} ±0.125	0.115^{am} ± 0.112	0.105^{am} ±0.105	0.102^{am} ±0.108	NS	At 5% 0.325 At 1% 0.429
II	*Jatropha curcas* leaf extract coated SNP's dose-I	0.122^{am} ± 0.080	0.152^{am} ± 0.099	0.175^{am} ± 0.114	0.202^{am} ± 0.206	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	0.115^{am} ±0.084	0.147^{am} ± 0.097	0.165^{am} ± 0.108	0.197^{am} ± 0.132	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	0.122^{am} ± 0.083	0.162^{am} ± 0.110	0.187^{am} ± 0.123	0.232^{am} ±0.152	NS	
V	Satellite group	0.123^{am} ±0.081	0.162^{am} ± 0.112	0.206^{am} ± 0.136	0.225^{am} ± 0.147	NS	
Stat		NS	NS	NS	NS	-	
CD	At 5% = 0.325; At 1%= 0.429						

In the present experiment there also, non-significant difference in basophil count was observed in all the groups. The literature also releveled the similar results in *J. curcus* and silver nanoparticles separately. But the present investigations shown non-toxic pattern on basophil count in rats treated with *J. curcas* coated silver nanoparticles for 28 days.

4.4.5 Blood Clotting Time (second):

The mean blood clotting time in control group animals on day 0th, 7th, 14th and 28th were 50.06±0.12, 51.60± 0.15, 50.02± 0.08 and 52.20± 0.10 respectively. In II and III group the blood clotting times were 51.41± 0.09, 53.40±

0.11, 59.63± 0.14, 54.32± 0.13 and 51.64 ± 0.11, 59.63± 0.29, 62.12 ± 0.18, 63.83± 0.12 respectively. In group IV and V, the blood clotting times were 52.24± 0.08, 61.02± 0.16, 62.52± 0.27, 69.61 ± 0.22 and 51.85± 0.14, 65.88 ± 0.14, 77.11 ± 0.20, 85.27± 0.18 respectively. The mean blood clotting time of control and treatment groups were recorded in table 4.12 and figure 4.13.

In group I there was statistically non-significant difference was observed in blood clotting time on day 0^{th}, 7^{th}, $14^{th}$and 28^{th}. However, in groups II, III, IV and V statistically highly significant difference in blood clotting time was observed on day 0^{th}, 7^{th}, $14^{th}$and 28^{th}.

In group II increase in blood clotting time observed on day 14^{th}as compared to day 0^{th},7^{th} and 28^{th} and decrease in blood clotting time was observed on day 7^{th}as compared to 14^{th} and 28^{th} days values. In group III there was increase in blood clotting time was observed when compared to group I and II. However, the highly increased in blood clotting time was observed on 28^{th} day of experiments, and decreased on day 7^{th} as compared to day 14^{th} and 28^{th}.

Increased in blood clotting time was observed in group IV and V on day 28^{th} as compared to 0^{th}, 7^{th}, 14^{th}. However, in group V there was increase in blood clotting time was observed on day 28^{th} as compared with all the other groups.

On day 0^{th}, non-significant difference was observed on all treatment groups I, II, III, IV and V. However, on 7^{th}, 14^{th} and 28^{th} day of experiments there was highly significant difference in blood clotting time was observed in all the groups.

On day 7^{th} increased in blood clotting time was found in group V and decrease in blood clotting time in group II was observed. The increased blood clotting time was observed in group II, III, IV and V as compared to group I of the experiment.

On day 14^{th} there was increased in blood clotting time was observed in group V and decrease in blood clotting time observed in group II compared to other group III, IV and V. On day 28^{th} there was statistically increased in blood clotting time was observed in group V and decrease in blood clotting time was observed in group II of the experiment.

Throughout the experimental period there was increase in blood clotting time was observed in group V on 28th day of experiment when compared to other treatment groups. However, Osoniyi and Onajobi, (2003) reported that the latex of *Jatropha curcas* showed significantly reduced the clotting time in human blood. The blood coagulation varied between 4 and 8 min, with a mean of 5.83±1.25 min. He found that in the presence of 1ml (1.05 g) of whole latex, the mean of clotting time was significantly reduced to 3.83±1.01 min with a range of 2–5 min. He also reported that diluted latex, prolonged the clotting time at high dilutions, the blood did not clot at all. This indicates that *Jatropha curcas* latex possesses both procoagulant and anticoagulant activities. Prothrombin time (PT) and activated partial thromboplastin time (APTT) tests on plasma confirm these observations. Similarly, Oduola *et al*., (2005) also reported that, the clotting time without adding stem latex was 6 minute 25 seconds while it was 5 seconds when stem latex was added. The difference was statistically significant. The reference range for whole blood clotting time is 6 to 9 minutes at 37 ^{0}C (12) Coagulant activity of the stem latex of *Jatropha gossypifolia* was demonstrated based on the findings of this study. The reduced clotting and bleeding times recorded in the experiment with stem latex as compared to those without stem latex was evidence that the stem latex possess coagulating agent thereby providing scientific basis for its use as a haemostatic agent.

In the present study difference in blood clotting time was observed in all the groups with different time factors. The clotting time significantly increase with increasing dose and duration of exposure. Throughout the experimental period there was increase in blood clotting time was observed in group V on 28th day of experiment it confirms that the significant increase in blood coating time was dose and duration of exposure dependent.

Table 4.12 Mean Blood clotting time (Mean ± SE, Seconds) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	50.06^{am} ±0.12	51.60^{am} ± 0.15	50.02^{am} ±0.08	52.20^{am} ±0.10	NS	At 5% 5.267 At 1% 6.922
II	*Jatropha curcas* leaf extract coated SNP's dose-I	51.41^{am} ± 0.09	53.40^{abm} ± 0.11	59.63^{cn} ± 0.14	54.32^{bm} ± 0.13	HS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	51.64^{am} ±0.11	59.63^{bn} ± 0.29	62.12^{bcno} ± 0.18	63.83^{bcn} ± 0.12	HS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	50.24^{am} ± 0.08	61.02^{bo} ± 0.16	62.52^{bcno} ± 0.27	69.61^{dno} ±0.22	HS	
V	Satellite group	51.85^{am} ±0.14	65.88^{bp} ± 0.14	77.11^{cp} ± 0.20	85.27^{dp} ± 0.18	HS	
Stat		NS	HS	HS	HS	-	
CD	At 5% = 5.267; At 1%= 6.922						

Superscript a, b, c, d shows the significant difference within the row (between different day in a specific group). (P<0.05).

Superscript m, n, o, p shows significant difference in within the column (between different group on specific day). (P<0.05).

4.5 Biochemical Parameters:

The biochemical parameter in context of liver function tests and kidney function tests were recorded and represented in table and figure 4.13 to 4. 18.

4.5.1 Liver function test:

4.5.1.a Aspartate aminotransferase (AST/ SGOT)

The mean aspartate aminotransferase values were recorded and shown in table 4.13 and figure 4.14.

The values, in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 130.81 ±2.20, 129.98±1.83, 130.56±1.18 and 131.23± 1.61 respectively. In II groups the aspartate aminotransferase values were 130.36 ± 2.40, 145.10 ± 2.33, 152.01±2.22, 154.70 ± 3.02 respectively. In III group the aspartate aminotransferase values were 129.46 ±1.68, 156.82 ±3.66, 159.93 ± 2.06, 162.81± 3.20 respectively. In groups IV and V, the aspartate aminotransferase

values were 130.35 ±1.52, 163.69 ±2.26, 164.08±1.89, 170.01 ±1.62 and 131.85 ±1.37, 169.13 ±1.23, 169.74 ± 3.19, 171.94 ±1.66 respectively.

Table 4.13 Mean Serum AST / SGOT level (Mean ± SE, IU/L) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean±S.E.)	Stat	CD
I	Healthy control	130.81^{am} ± 2.20	129.98^{am} ± 1.89	130.56^{am} ± 1.98	131.23^{am} ± 1.61	NS	At 5% 6.087 At 1% 7.997
II	*Jatropha curcas* leaf extract coated SNP's dose-I	130.36^{am} ± 2.40	145.10^{bn} ± 2.33	152.01^{cn} ± 2.22	154.70^{cn} ± 3.02	HS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	129.46^{am} ±1.68	156.8^{bo} ± 3.66	159.93^{co} ± 2.06	162.81^{co} ± 3.20	HS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	130.35^{am} ± 1.52	163.69^{bp} ± 2.26	164.08^{bp} ±1.89	170.01^{cp} ± 1.62	HS	
V	Satellite group	131.85^{am} ± 1.37	169.13^{bq} ± 1.23	169.74^{bq} ± 3.19	171.94^{bp} ± 1.66	HS	
Stat		NS	HS	HS	HS	-	
CD	At 5% = 6.087; At 1%= 7.997						

Superscript a, b, c shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o, p shows significant difference in within the column (between different group on specific day). ($P<0.05$).

In group I there was non-significant difference in aspartate aminotransferase values was observed on 0^{th} ,7^{th}, 14^{th} and 28^{th} days of experiments. However, in group II, III, IV and V there was statistical, significant difference in aspartate aminotransferase values was observed on 0^{th},7^{th}, 14^{th} and 28^{th} days of experiments

In group II and III there was significant increase in aspartate aminotransferase values was observed on day 28^{th} as compared to other days values. Aspartate aminotransferase values were increases from 7^{th} day onwards in both II and III group. Similarly in treatment group IV and V there was also significant increase in aspartate aminotransferase values was observed on day 28^{th} as compared to other days values. Also, the aspartate aminotransferase value was

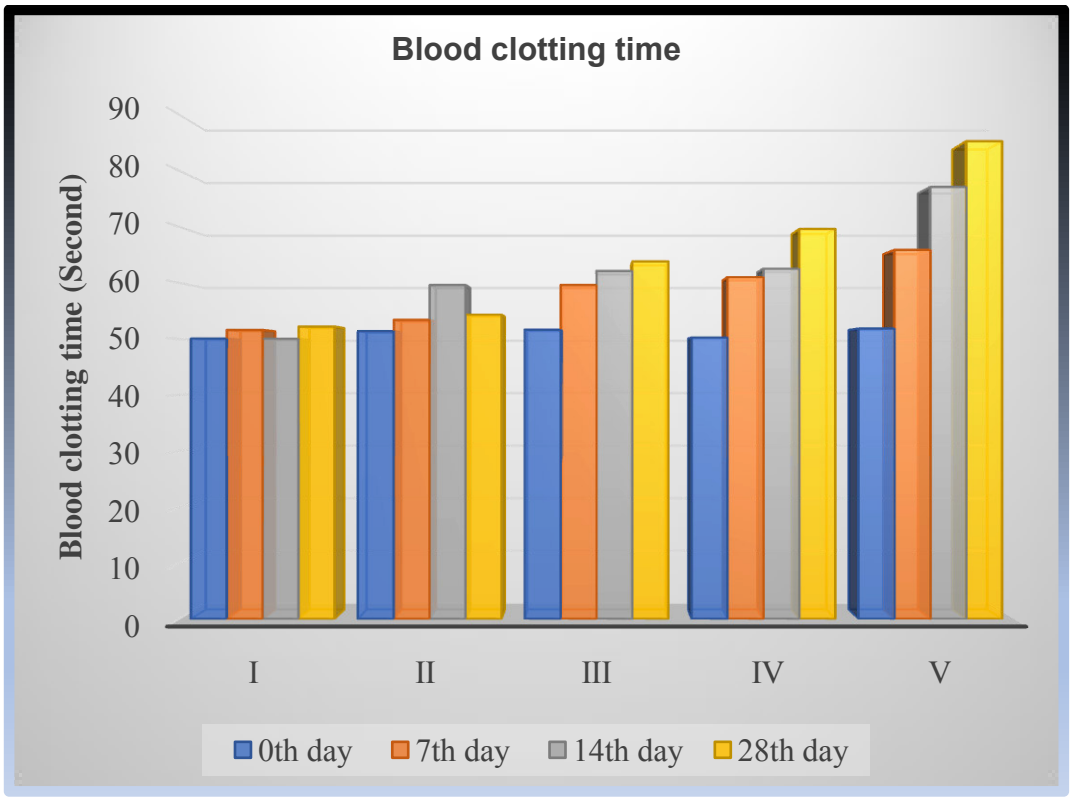


Figure 4.13-Mean Blood clotting time values (second) of experimental groups at different intervals.

started increasing from 7th days onward in IV and V group. All aspartate aminotransferase values were observed increase as compared to 0th day values.

On day 0th there was non-significant difference in aspartate aminotransferase values was observed in group I, II, III, IV and V. On day 7th, 14thand 28th there was significant difference in aspartate aminotransferase values was observed in group I, II, III, IV and V. On day 7thand 14th statistically increase in aspartate aminotransferase values was observed in group V and decreased aspartate aminotransferase values was observed in group II. On day 28th statistically increase in aspartate aminotransferase values was observed in group V as compared to other groups. In groups II, III, IV and V aspartate aminotransferase values were observed increased and the values were beyond the normal physiological limit as compared with control group. Throughout the experimental period there was observed increase in aspartate aminotransferase values in group V on 28th day of experiment.

Igbinosa *et al.,* (2013) reported the similar result in wistar rats treated with methanolic leaf extract of *jatropha curcas* at 500 mg/kg, 1000mg/kg and 2000 mg/kg body weight concentrations for 21 days. The SGOT values were 24.05, 25.47 and 24.93 respectively. As per their results, significant difference was observed in SGOT values in all of the doses used as compared to control. Mahe *et al*., (2017) also recorded similar result in rats treated with leaf extract of *Jatropha curcas* at 100, 200 and 250mg/kg/ body weight, for 28 days oral dosing. The SGOT values were 90.73, 106.81 and 138.96 respectively. There were variations in pattern of AST compared to the control, with a rise in AST activity in group administered with 250 mg/kg though statistically not significant ($p > 0.05$). Similar result was also revealed by Adeyemi and Adewumi, (2014), in rats treatment with 1000 mg/kg and 5000 mg/kg AgNPs led to a significant increase in AST levels for the 14 and 21 day treatment groups. Heydrnejad *et al*., (2015) concluded, same result in BALB/C mice, the serum AST level showed statistical increase in male and female mice treated with 20 and 50 ppm of nanosilver compared to control group. However, in the study conducted by Sulaiman *et al*., (2015) in rats, shown decreased the activities of rat serum AST at 10, 50 and 100

mg/kg body weight of silver nanoparticles. The observed aspartate aminotransferase values were 21.17, 11.17 and 21.50 respectively.

So, the present study concluded that there were gradual increases in aspartate aminotransferase values from group II to group V, which confirms the dose dependent toxicity in aspartate aminotransferase values as compared with control group of experiment.

4.5.1. b Alanine Transaminase (ALT) / SGPT

The alanine transaminase recorded values were presented in table no 4.14 and figure 4.15. The mean alanine transaminase values in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 70.60 $\pm$2.57,70.28$\pm$2.33, 69.22 $\pm$1.97and 70.76$\pm$2.98 respectively. In II groups the alanine transaminase values were 70.98 $\pm$ 2.70, 71.06 $\pm$ 2.10, 70.12 $\pm$0.91, 71.13 $\pm$ 1.58 respectively. In III, IV and V groups the alanine transaminase values were 70.80 $\pm$ 4.37, 71.70 $\pm$2.64, 72.87 $\pm$ 2.91, 73.22 $\pm$ 3.19; 70.45 $\pm$2.06, 73.29$\pm$3.08, 73.10 $\pm$1.13, 73.37 $\pm$2.92 and 71.93 $\pm$2.65, 74.47 $\pm$3.62, 75.52$\pm$0.93, 81.35 $\pm$4.47 respectively.

In group I, II, III and IV there was statistically non-significant difference was observed in alanine transaminase values on day 0^{th} ,7^{th}, 14^{th} and 28^{th} days of experiment. However, in group V and statistically significant difference was observed in alanine transaminase values on day 0^{th}, 28^{th}. In group II and IV there was non-significant increase in alanine transaminase values was observed on 28^{th} day of experiment as compared to 0^{th} ,7^{th} and 14^{th}day's values. However, in group III and V non-significant increase in alanine transaminase value was observed on 28^{th} day and decreased on day 7^{th} of experiment as compared to 14^{th} and 28^{th} day values. Increase in alanine transaminase values observed from 7^{th} day onwards. But all values were observed within normal range.

On day 0^{th}, 7^{th} and 14^{th} there was non-significant difference was observed in alanine transaminase values in groups I, II, III, IV and V. However, there was statistically significant difference observed in alanine transaminase values on 28^{th} day in group I, II, III, IV, V respectively.

On day 7^{th}, 14^{th} and 28^{th} there was non-significant increase in alanine transaminase values was observed in group V as compared to other groups and decrease in alanine transaminase values observed in group II as compared to

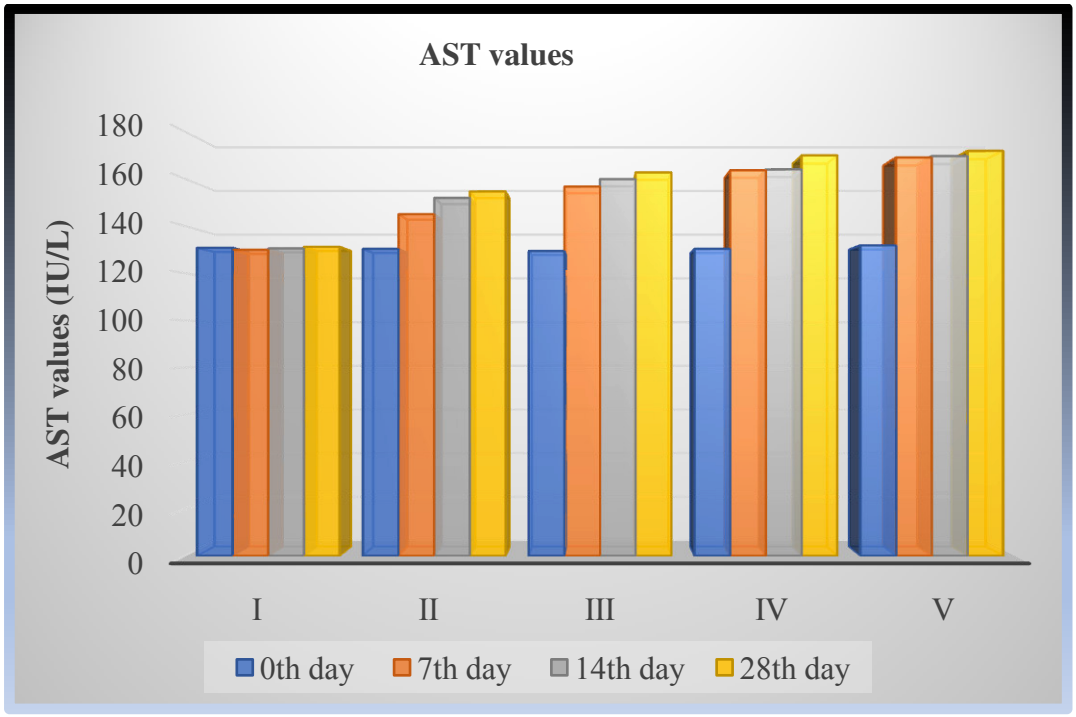


Figure 4.14- Mean AST values (IU/L) of experimental groups at different intervals.

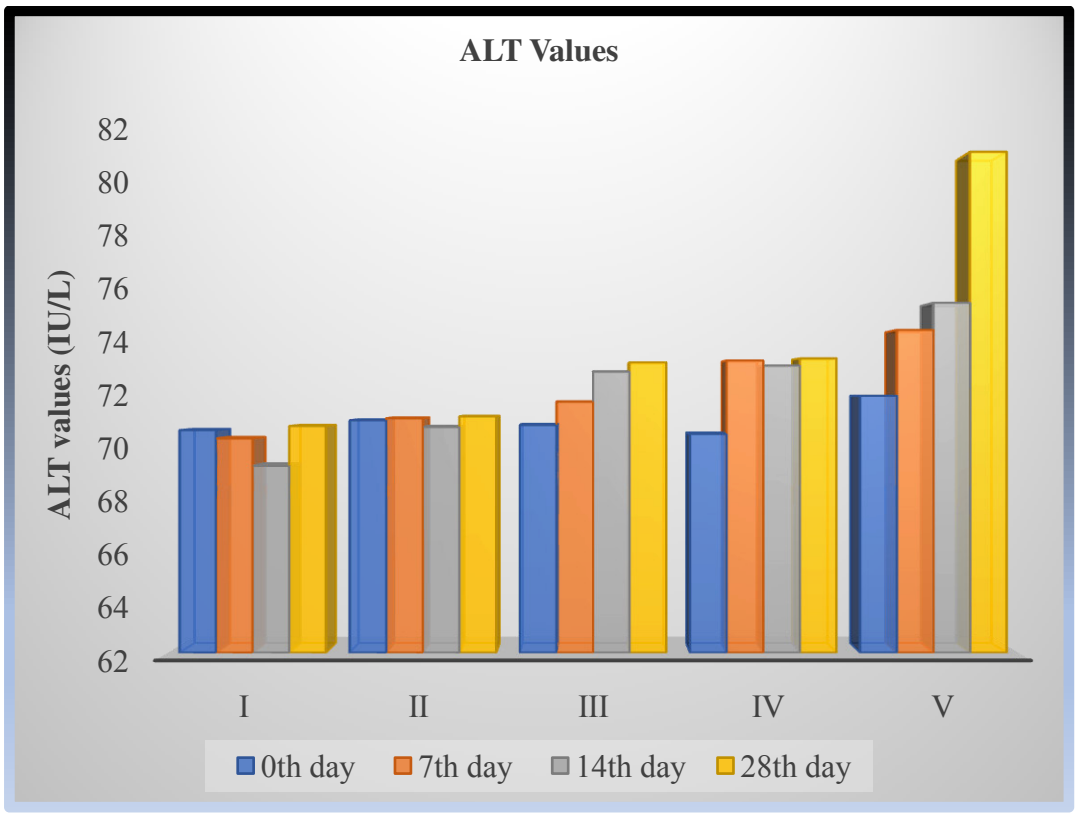


Figure 4.15- Mean ALT values (IU/L) of experimental groups at different intervals.

group III, IV and V. Alanine transaminase values observed at par when compared with each other.

Table 4.14 Mean Serum ALT / SGPT level (Mean ± SE, IU/L) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean± S.E.)	Stat	CD
I	Healthy control	70.60^{am} ± 2.57	70.28^{am} ± 2.33	69.22^{am} ± 1.97	70.76^{am} ± 2.98	NS	At 5% 7.445 At 1% 9.798
II	*Jatropha curcas* leaf extract coated SNP's dose-I	70.98^{am} ± 2.70	71.06^{am} ± 2.10	70.72^{am} ± 0.91	71.13^{amn} ± 1.58	NS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	70.80^{am} ±4.37	71.70^{am} ± 2.64	72.87^{am} ± 2.91	73.22^{an} ± 3.19	NS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	70.45^{am} ± 2.06	73.29^{am} ± 3.08	73.10^{am} ± 1.13	73.37^{an} ± 2.92	NS	
V	Satellite group	71.93^{am} ± 2.65	74.47^{abm} ± 3.62	75.52^{bm} ± 0.93	81.35^{co} ± 4.47	HS	
Stat		NS	NS	NS	HS	-	
CD	At 5% = 7.445; At 1%= 9.798						

Superscript a, b, c shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o shows significant difference in within the column (between different group on specific day). ($P<0.05$).

Throughout the experimental period there was highly increase in alanine transaminase values observed in treatment group V on day 28^{th} of experiment. Kim *et al*., (2008) concluded same result in sprague-dawley female rats treated with silver nanoparticles at 30, 300 and 1000 mg/kg. The alanine transaminase values were 44.10, 42.80, 45.30 respectively. Values increase than the control but observed within normal range. Igbinosa *et al*., (2013) also reported non-significant effects in rats treated with methanolic leaves extract *Jatropha curcas* at 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight orally for 21 days. Mahe *et al*., (2017) also recorded similar result in rats treated with leaf extract of *Jatropha curcas* at 100, 200 and 250mg/kg/ body weight, for 28 days oral dosing. ALT values were 26.22, 29.19, 37.37 (U/L) respectively, the showed high activity of

serum ALT in rats at 100 mg/kg, 200 mg/kg and 250 mg/kg of the extract which was proportional to dose, though statistically not significant ($p > 0.05$).

The alanine transaminase values were found increasing as the dose was increase. Though all the values were observed within the normal physiological range but were increased in treatment groups when compared with control group. Hence on this basis it was concluded there was increased in alanine transaminase values in the treatment groups of aqueous extract of *J. curus* coated with silver nanoparticle when compared with control group, suggested sign of mild to severe pattern of toxicity throughout the experiment.

4.5.2 Kidney function test:

4.5.2.a Blood Urea Nitrogen (BUN)

The mean in blood urea nitrogen values in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 20.54±0.48, 20.83±0.72, 19.98 ±0.46 and 20.94± 0.52respectively. In II and III groups the blood urea nitrogen values were 20.73±0.59, 22.97± 0.35, 23.80±0.66, 24.51±0.30 and 20.99± 0.61, 23.60 ± 0.88, 23.99± 0.30, 25.93 ± 0.63 respectively. In groups IV and V, the blood urea nitrogen values were 20.90 ±0.54, 23.76 ± 0.87, 24.70 ± 0.70, 26.03± 0.26 and 20.55 ± 0.26, 24.62± 1.32, 23.19± 0.38, 24.68± 0.52 respectively. The BUN values of control and treatment groups were shown in table 4.15 and figure 4.16.

In group I statistically non-significant difference in blood urea nitrogen values were observed on day 0^{th}, 7^{th} ,$14^{th}$and 28^{th}. However, in treatment groups II, III, IV and V. statistically significant difference in blood urea nitrogen values were observed on 0^{th}, 7^{th}, $14^{th}$and 28^{th}day of experiment.

In group II blood urea nitrogen values were observed increase from 7^{th} day onwards. There was increase in blood urea nitrogen values observed on day 28^{th} as compared with its 0^{th}, $7^{th}$and 14^{th} day values. In group III blood urea nitrogen values were observed increase on day 28^{th} as compared with its 0^{th}, 7^{th} and 14^{th} days values. On day 7^{th} and 14^{th} blood urea nitrogen values was found statistically similar but observed numerically different.

In group IV statistically increase in blood urea nitrogen value found on day 28^{th} as compared to 0^{th},7^{th} and 14^{th} day values and decreased on day 7^{th} as compared to 14^{th} and 28^{th} day values. Statistical, increased in blood urea nitrogen

values in group V observed on day 7thand 28th day as compared with its 0th and 14th day values. Blood urea nitrogen values decreased on 14th day of experiment. Blood urea nitrogen values observed statistical similar but numerically different on day 7th and 28th.

On day 0th there was non-significant difference of blood urea nitrogen values was observed in I, II, III, IV and V group. However, on day 7th, 14th and 28thstatistical, significant difference was observed in group I, II, III, IV and V in blood urea nitrogen values.

On day 7th there was increase in blood urea nitrogen value was observed in group V and decreased in group II as compared to group III, IV and V. On day 14th increase in blood urea nitrogen value were observed in group IV when compared with group I, II, III and V. However, decrease in blood urea nitrogen values was observed in group V as compared to group II, III and IV.

On day 28th statistically significant increase in blood urea nitrogen value was observed in treatment group IV as compared to groups I, II, III and V. The blood urea nitrogen values were observed increased than its normal range on 28th day. In group II and V, the values were observed statistical similar but numerically different.

Throughout the experimental period there was increase in blood urea nitrogen values observed in treatment group IV on 28th day of experiment.

Similar result was recorded by Mahe *et al*., (2017) in albino rats treated with leaf extract of *Jatropha curcas* at 100, 200 and 250mg/kg body weight, for 28 days oral dosing. The blood urea nitrogen values were 9.43, 9.48 and 9.83 mmol/L respectively. High level of urea was obtained in all the treated groups though statistically not significant. Kim *et al*., (2008) also concluded same result in sprague-dawley female rats treated with silver nanoparticles at 30, 300 and 1000 mg/kg. The urea nitrogen values were 21.13, 21.96 and 23.76 respectively. The blood urea nitrogen values were increase than the normal range. Kumar *et al*., (2010) also received the same finding in rats treated with *Jatropha curcas* seed and seed oil with daily oral dosing of *Jatropha curcas* seed @ 4 seed/rat and seed oil @ 1ml / rat for 28 days. The urea nitrogen values were observed increases than control. However, Sulaiman *et al*., (2015) shown the contrast result in his research

on rats treated with silver nanoparticles at 10, 50 and 100 mg /kg. In the research blood urea nitrogen values were decreased than control group and were 20.29, 25.33, 41.81 (mmol/L) respectively. In the present study there was significantly increase in blood urea nitrogen values were observed in all treatment groups. So based on the results and literature it was concluded that there was toxic effect of *J. curus* coated silver nanoparticle was observed in blood urea nitrogen values at different concentration used for 28 days of experiment.

Table 4.15 Mean Serum BUN level (Mean ± SE, mg/dl) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean± S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean± S.E.)	Stat	CD
I	Healthy control	20.54^{am} ± 0.48	20.83^{am} ± 0.72	19.98^{am} ± 0.46	20.94^{am} ± 0.52	NS	At 5% 1.732 At 1% 2.279
II	*Jatropha curcas* leaf extract coated SNP's dose-I	20.73^{am} ± 0.59	22.97^{bp} ± 0.35	23.80^{bo} ± 0.66	24.51^{cn} ± 0.30	HS	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	20.99^{am} ±0.61	23.60^{bn} ± 0.88	23.99^{bo} ± 0.30	25.93^{cno} ± 0.63	HS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	20.90^{am} ± 0.54	23.76^{bn} ± 0.87	24.70^{cn} ±0.70	26.03^{do} ± 0.26	HS	
V	Satellite group	20.55^{am} ± 0.26	24.62^{co} ± 1.32	23.19^{bo} ± 0.38	24.68^{cn} ± 0.52	HS	
Stat		NS	HS	HS	HS	-	
CD	At 5% = 1.732; At 1%= 2.279						

Superscript a, b, c, d shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o shows significant difference in within the column (between different group on specific day). ($P<0.05$).

4.5.2.b Creatinine:

Table 4.16 and figure 4.17 represent the creatinine values for control and treatment group.

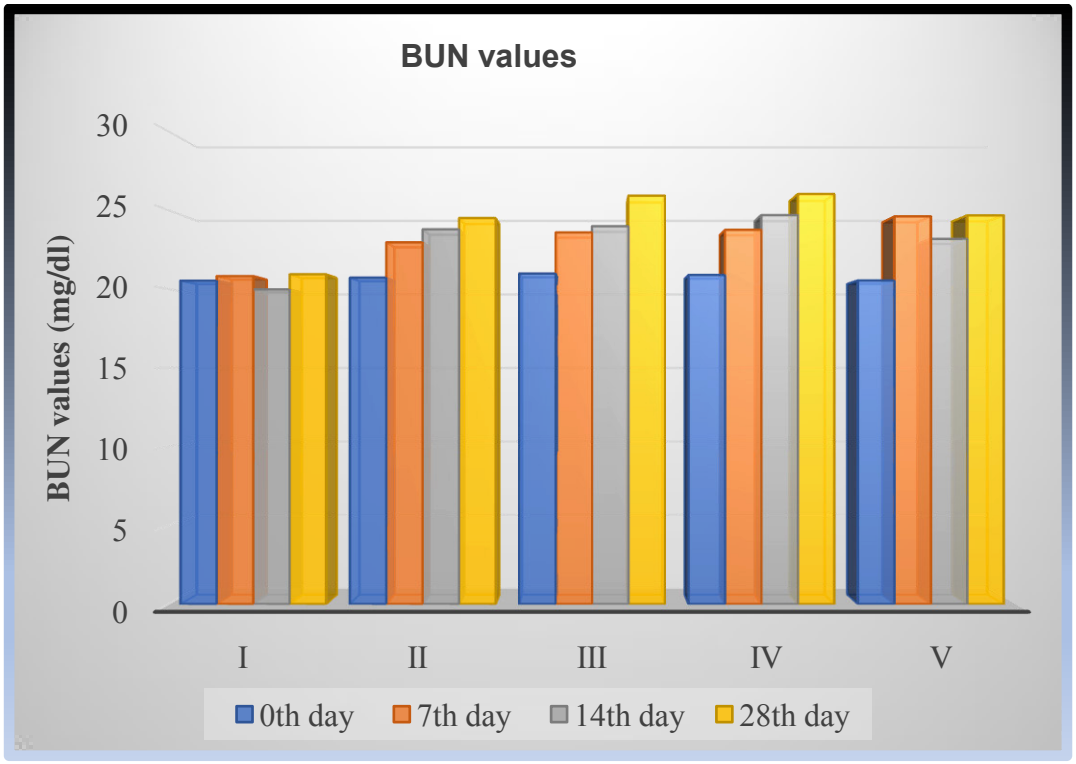


Figure 4.16- Mean BUN values (mg/dl) of experimental groups at different intervals.

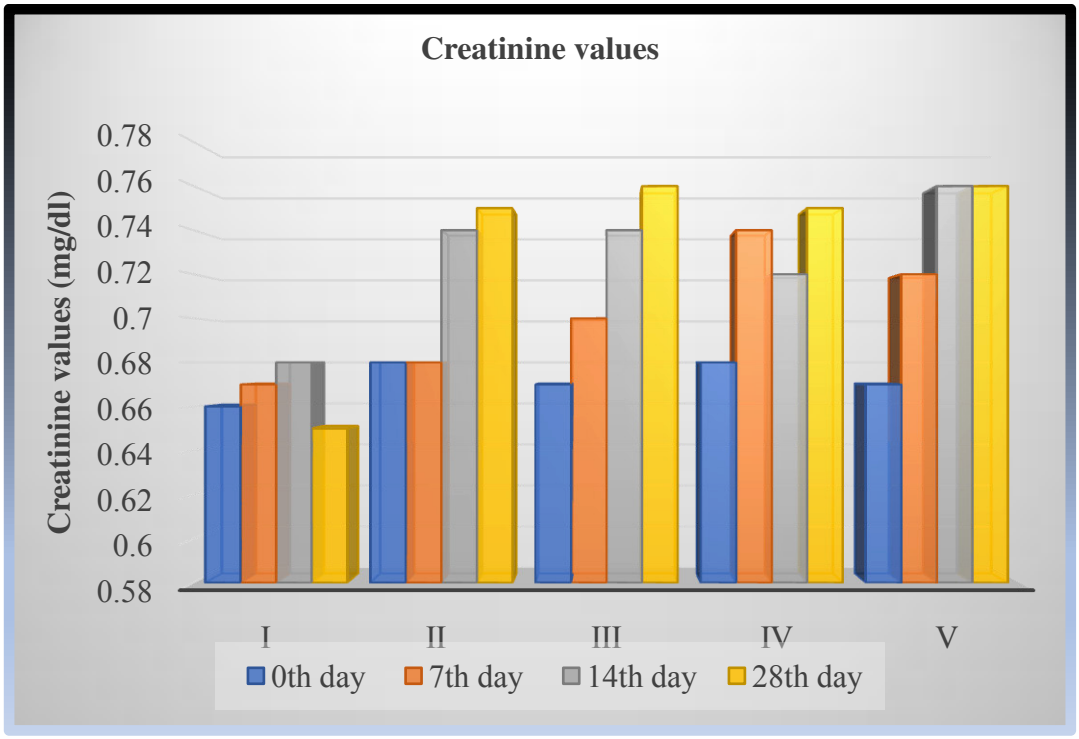


Figure 4.17- Mean Creatinine values (mg/dl) of experimental groups at different intervals.

Table 4.16 Mean Serum Creatinine level (Mean ± SE, mg/dl) on different days in experimental rats of different groups.

Group	Treatment	0^{th}day (Mean ±S.E.)	7^{th} day (Mean± S.E.)	14^{th} day (Mean ±S.E)	28^{th} day (Mean± S.E.)	Stat	CD
I	Healthy control	0.66^{am} ±0.027	0.67^{am} ±0.02	0.68^{am} ± 0.02	0.65^{am} ± 0.02	NS	At 5% 0.061 At 1% 0.085
II	*Jatropha curcas* leaf extract coated SNP's dose-I	0.68^{abm} ± 0.019	0.68^{abm} ± 0.02	0.74^{bn} ± 0.02	0.75^{bn} ± 0.020	S	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	0.67^{abm} ± 0.024	0.70^{bmn} ± 0.01	0.74^{cn} ±0.03	0.76^{cn} ± 0.027	HS	
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	0.68^{am} ± 0.024	0.74^{bn} ± 0.03	0.72^{abmn} ±0.02	0.75^{cn} ± 0.026	S	
V	Satellite group	0.67^{am} ± 0.027	0.72^{abn} ± 0.01	0.76^{bo} ± 0.03	0.76^{bn} ± 0.01	HS	
Stat		NS	S	S	HS	-	
CD	At 5% = 0.061; At 1%= 0.085						

Superscript a, b, c shows the significant difference within the row (between different day in a specific group). (P<0.05).
Superscript m, n, o shows significant difference in within the column (between different group on specific day). (P<0.05).

The mean creatinine values in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 0.66±0.02,0.67±0.02, 0.68±0.03and 0.65±0.02 respectively. In II groups the creatinine values were 0.68 ± 0.01, 0.68± 0.02, 0.74 ±0.02, 0.75± 0.02 respectively. In III group the creatinine values were 0.67± 0.024, 0.70±0.01, 0.74 ± 0.03, 0.76± 0.027 respectively. In groups IV and V, the creatinine values were 0.68±0.024, 0.74±0.03, 0.72±0.02, 0.75± 0.026 and 0.67±0.027, 0.72±0.01, 0.76 ± 0.03, 0.76±0.01 respectively.

In group I there was statistically non-significant difference was observed on day 0^{th} 7^{th}, 14^{th} and 28^{th}. However, statistically significant difference was observed in groups II, III, IV and V at different time intervals. The statistically significant difference was observed on 0^{th}, 14^{th} and 28^{th} day in II, III and V group, however, statistically significant difference was observed on 0^{th} ,7^{th} ,14^{th} and 28^{th} day in IV group of experiment.

In group II and III there was increased in creatinine value was observed on day 28th and decreased on day 7thwas observed as compared to 14th and 28th days values. However, in group IV significant increase in creatinine values observed on day 28th and decreased on day 14th was observed. In group V highly significant increase in creatinine values was observed on day 14thand 28thwhen compared with its 0th and 7thday values.

On day 0th statistically non-significant difference was observed in creatinine values in groups I, II, III, IV and V. However, there was significant difference was observed in creatinine values on day 7th in group I, II, IV and V, on day 14th in groups I, II, III and V and on day 28th in groups I, II, III, IV and V.

On day 7th there was increase in creatinine values was observed in group IV. However, decrease creatinine value was observed in group II as compared to group III and IV and V. Creatinine values were observed increased in all treatment groups compared to control group.

On day 14th there was statistical, increase in creatinine values observed in group V. the creatinine values were found increased in all treatment groups when compared to control group. On day 28th there was increase in creatinine values observed in group II, III, IV and V as compared to group I. Chibuogwu *et al*., (2021) also observed similar finding in mice treated with leaves extract of *Jatropha tanjorensis* at doses of 100, 200, and 400 mg/kg b.wt for 28th day. Creatinine values were 0.82, 0.72, 0.82 respectively. There was observed no significant changes in serum levels of creatinine. Awasthy *et al*., (2010) observed significantly increase in creatinine values than the control group. The increase in creatinine values in rats by feeding the basal diet, and the diet in which the crude protein requirement was supplemented at 25% and 50% levels with *Jatropha* seed powder for short term toxicity study. Similar result was observed by Mahe *et al*., (2017) in albino rats treated with 100, 200 and 250mg/kg/ body weight, leaf extract of *Jatropha curcas* for 28 days of oral dosing. Creatinine values were 1.68, 2.45 and 3.32 respectively. High levels of creatinine in all the treated groups were observed as compared to the control group. Sulaiman *et al*., (2015) reported non-significant effect in rats treated with silver nanoparticles at 10, 50, 100 mg/kg

body weight. Creatinine values were 15.33, 16.37, 15.58 respectively. The creatinine values were decrease than the control.

The creatinine values were increased with increased in dose and time of exposure in treatment groups when compared with the control group. Hence, it was confirmed that aqueous extract of *J. curcas* coated silver nanoparticle revealed the dose depended toxicity pattern in treatment groups as compared with control group of experiment.

4.5.3 Total Protein:

The mean total protein values were recorded and shown in table 4.17 and figure 4.18. The total protein values in control group animals on day 0^{th}, 7^{th}, 14^{th} and 28^{th} were 6.87 $\pm$0.14,7.02$\pm$0.22, 7.11$\pm$0.11and 7.28$\pm$0.14 respectively. In II groups the total protein values were 6.99 $\pm$ 0.11, 7.17$\pm$ 0.22, 7.49 $\pm$0.31, 7.63 $\pm$ 0.10 respectively. In III group the total protein values were 7.15$\pm$ 0.18, 7.75$\pm$0.23, 7.78 $\pm$ 0.14, 8.51$\pm$ 0.31 respectively. In groups IV and V, the total protein values were 7.19 $\pm$0.23, 7.70 $\pm$0.22, 7.71$\pm$0.17, 8.10$\pm$ 0.19 and 7.07$\pm$0.14, 7.89$\pm$0.22, 8.17 $\pm$ 0.21, 8.25$\pm$0.15 respectively.

In treatment group I there was non-significant difference in total protein values was observed on 0^{th}, 7^{th}, 14^{th} and 28^{th} day of experiment and all total protein values were observed within normal range. In group II statistically significant difference in total protein values were observed on day 0^{th},14^{th} and 28^{th} day. In group III, IV and V statistically significant difference in total protein values were observed on day 0^{th}, 7^{th}, 14^{th} and 28^{th} respectively.

In group II and III there was increase in total protein values observed on day 28^{th} as compared to 0^{th} 7^{th} and 14^{th} day values. The Total protein values on 28^{th} day was slightly increased than the normal range in group III.

In group IV and V increase in total protein values was observed on day 28 when compared with its 0^{th}, 7^{th}, 14^{th} day values and observed decrease on day 7^{th} as compared to 14^{th}ans 28^{th} day values. In group V total protein values observed increases from 7^{th} day onwards.

On day 0^{th} statistically non-significant difference was observed in total protein values in all groups. However, statistically significant difference in total protein values was observed in group I, II, III, IV and Von day 7^{th}, 14^{th} and 28^{th}.

On day 7th there was statistical, increased in total protein value observed in group V as compared to other groups, but this value was observed slightly increased than the normal range. In group III and IV the total protein values were observed statistically similar but these values were observed numerically different.

On day 14th statistically increased in total protein values was found in group V, the value was observed slightly increased than the normal range of total protein values. Decrease in total protein values was observed in treatment group II as compared to groups III, IV and V. On day 28th significant increase in total protein values observed in group II, III, IV and V as compared to group I. Highly increased in total protein values was observed in group III. In group III, IV and V total protein values were observed slightly increased than its normal range.

Table 4.17 Mean Serum TP level (Mean ± SE, g/dl) on different days in experimental rats of different groups.

Group	Treatment	0th day (Mean ± S.E.)	7th day (Mean ±S.E.)	14th day (Mean ±S.E)	28th day (Mean± S.E.)	Stat	CD
I	Healthy control	6.87^{am} ± 0.14	7.02^{am} ± 0.22	7.11^{am} ± 0.11	7.28^{am} ± 0.14	NS	At 5% 0.569
II	*Jatropha curcas* leaf extract coated SNP's dose-I	6.99^{am} ± 0.11	7.17^{abm} ± 0.22	7.49^{bm} ± 0.31	7.63^{cm} ± 0.10	S	
III	*Jatropha curcas* leaf extract coated SNP's dose-II	7.15^{am} ± 0.18	7.75^{bn} ± 0.23	7.78^{bn} ± 0.14	8.51^{cn} ± 0.31	HS	At 1% 0.738
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	7.19^{am} ± 0.23	7.70^{bn} ± 0.22	7.71^{bn} ± 0.17	8.10^{cno} ± 0.19	HS	
V	Satellite group	7.07^{am} ± 0.14	7.89^{bo} ± 0.22	8.17^{co} ± 0.21	8.25^{co} ± 0.15	HS	
Stat		NS	HS	HS	HS	-	
CD	At 5% = 0.569; At 1%= 0.738						

Superscript a, b, c shows the significant difference within the row (between different day in a specific group). ($P<0.05$).

Superscript m, n, o shows significant difference in within the column (between different group on specific day). ($P<0.05$).

Throughout the experimental period total protein values was observed increased on day 28th in treatment group III. Similar result was observed by

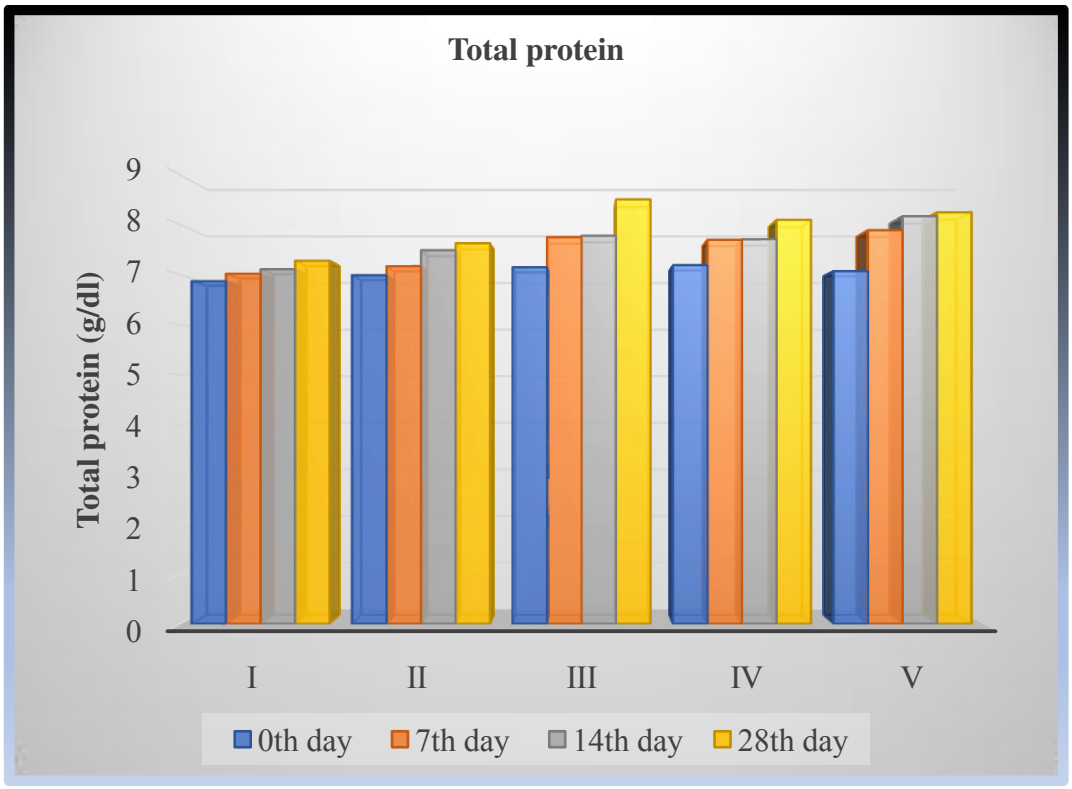


Figure 4.18- Mean Total protein values (g/dl) of experimental groups at different intervals.

Adeyemi and Adewumi, (2014) in rates treated with silver nanoparticles at 100, 1000 and 5000 mg/kg daily for 21 days dosed alternately. Elevation in total protein levels in rat liver was observed with AgNPs. Poon *et al*., (2013) also reported same finding in rats treated with *Jatropha* oil at 0.5, 5, 50 and 500 mg/ kg body weight per day for 28 consecutive days. No significant difference was observed in total protein values, but values observed within normal range. Total protein values were 5.7, 5.8, 5.8 and 6.2 respectively. However, Mahe *et al*., (2017) reported slight variations in total protein values in rats treated with *J. curcas* leaf extract at 0, 100, 200, and 250 mg/kg daily for 28 days. Total protein values were 6.99, 6.59, 6.44, 6.30 respectively. But the total protein values observed within normal range.

So, the present study concluded there was observed effect of aqueous extract of *J. curcas* coated silver nanoparticles on serum total protein values on 28^{th} days of experiments.

4.6 Pathological observation:

4.6.1 Gross Pathological observation:

The gross pathological examination of sacrificed rats of treatment and control groups were shown in figure 4.19 to 4.21. The gross examination of liver revealed pale color, mild focal congestion, multifocal necrosis was recorded in (group V) JcLE coated SNPs (800 mg/kg). Gross morphological structure of heart and kidney were appeared to be normal in entire groups.

4.6.2 Organ Weights:

Mean organ weights of liver, kidney and heart of all the groups were recorded and shown in table 4.18.

The mean organ weight of liver in treatment group I, II, III, IV and V recorded were 7.87±0.82, 7.00±0.20, 6.97±0.77, 6.41±0.22 and 7.21±0.31 respectively. The mean organ weight of heart in treatment group I, II, III, IV and V noted were 0.797±0.012, 0.803±0.015, 0.766±0.043, 0.745±0.036 and 0.761±0.029 respectively. The mean organ weight of kidney in treatment group I, II, III, IV and V were 1.68 ±0.10, 1.62 ±0.03, 1.58 ±0.03, 1.58±0.09 and 1.57±0.12 respectively.

Table 4.18 Relative organ weight (Mean ± S.E., gram) on different days in experimental rats of different groups at the end of study period.

Group	Treatment	Liver	Heart	Kidney
I	Healthy control	7.87±0.82	0.797±0.012	1.68±0.10
II	*Jatropha curcas* leaf extract coated SNP's dose-I	7.00±0.20	0.803±0.015	1.62±0.03
III	*Jatropha curcas* leaf extract coated SNP's dose-II	6.97±0.77	0.766±0.043	1.58±0.03
IV	*Jatropha curcas* leaf extract coated SNP's dose-III	6.41± 0.22	0.745±0.036	1.58±0.09
V	Satellite group	7.27±0.31	0.761±0.029	1.57±0.12
Stat		NS	NS	NS

There was no significant variation were observed in mean relative liver weight in the entire groups. Mean relative weight of liver in group II, III, IV and V was observed non-significantly decrease than group I.

There was non-significant difference was observed in mean relative organ weight of heart in treatment group I, II, III, IV and V. Numerical decrease in the mean relative organ weight of heart was recorded in the group IV as compared with the rest of the treatment group. Non-significant difference was also observed in relative organ weights of kidney in treatment group I, II, III, IV and V. Non-significantly decrease in relative organ weights of kidney was observed in group V.

Similar result was reported by Kim *et al*., (2010) in rats treated with silver nanoparticle at 30, 125 and 500 mg/kg/day for 13 weeks by oral dosing. No significant changes in organ-weights were observed in either the male or female rats except for decreases in the weight of right kidney for the low-and middle-dose female rats. Sawadogo *et al*., (2018) also concluded, no significant effect in organ weights in mice treated with aqueous extract of *jatropha curcas* leaves at 200, 400, 800 mg/kg for 28 days. Sulaiman *et al*., (2015) reported significant effect in rats treated with silver nanoparticles at 10, 50, 100 mg/kg body weight for 30 days. He concluded that the organ weights of heart, liver and kidney were relatively decrease as compared with the control group. But in this research, after binding of silver nanoparticles with *J. curcas* there were no any significant change in organ weight was observed, it confirmed that, the aqueous leaf extract of

jatropha curcas coated silver nanoparticles could not show sign of toxicity on organ weight of any treatment group.

4.6.3 Histopathological examination:

The histopathological examination of liver, kidney and hearts of all the groups were done and recorded in figure 4.22-4.33. Histopathological alterations in experimental rats treated with *jatropha curcas* leaf extract coated SNPs treated group were characterized by congestion, haemorrahges and infiltration of mononuclear cells in liver, heart and kidney of all treated groups and variable in severity except control group.

4.6.3.a Liver

Histopathological alteration in liver of experimental rats treated with *jatropha curcas* leaf extract coated SNPs treated group shown in figure 4.22-4.25. Section of liver on microscopic assessment showed congestion, heamorrhages, dilatation of central vein, necrobiotic changes, fatty degeneration, infiltration of mononuclear cell, dilatation of sinusoidal space and cellular degeneration was observed in experimental group V. Whereas, moderate to severe type of alterations such as congestion, heamorrhages, fatty changes and infiltration of mononuclear cells was also observed in experimental group IV. Experimental group III also showed similar type of lesions in liver as of mild intensity as compare with group IV. Liver revealed similar type of lesions but is of mild intensity noted in experimental group II as compared to experimental group III. Liver showed normal hepatic parenchyma in control group.

Similar finding was also observed by Heydrnejad *et al*., (2015) in BALB/c mice treated with silver nanoparticles at 20 and 50 ppm for 14^{th} days (orally). Histopathological finding of liver organ there were observed vast damage to liver tissue in both sexes which increased with time such as the necrosis, hepatocytic inflammation, and resultant aggregation of lymphocytes in liver tissue. In fact, lymphocyte aggregation was minimal to moderate in all treated animals exposed to silver nanoparticles in the hepatic area. Mice exposed to 50 ppm nanosilver showed more severe alterations than mice exposed to 20 ppm. Mahe *et al*., (2017) also reported similar finding in rats treated with *jatropa curcas* leaf extract @ 100, 200 and 250 mg/kg daily for 28^{th} day. Chibuogwu *et al*., (2021) also reported

similar finding in rat liver when treated with *Jatropha tanjorensis* leaf extract at 100, 200 and 400 mg/kg b.wt. In the present experiment there was observed histopathological changes in liver. Toxicity sign was observed in aqueous extract of *J. curus* coated silver nanoparticles when dosed for 28 days of experiment.

4.6.3.b Kidney

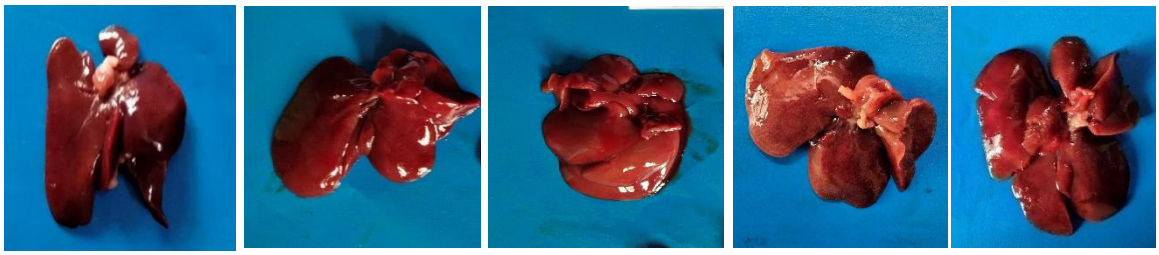
The histoarchitectural observations of kidneys in experimental rats were shown in figure 4.26-4.29. Kidney of control group revealed normal histoarchitecture. The kidney exposed to *Jatropha curcas* coated silver nanoparticles (Experimental group V) revealed severe cellular swelling, multifocal necrosis and focal to multifocal hyaline cast located in the lumen of exposed renal tubules and hydropic degeneration and haemorrhages in intertubular tissue space. The renal tubules of kidney (Experimental group IV) were showed moderate alteration *i.e.,* congestion, necrosis and hydropic degeneration with marked infiltration of mononuclear cells in intertubular tissue space. Similar type of mild to moderate alterations were also observed in kidney (Experimental group III). More or less alterations in histoarchitectural pattern of Kidney were recorded in experimental group II as compared to experimental group III.

Similar finding was also observed by Baraaj and Altaie, (2021) in rat when treated with silver nanoparticles at 0.75 and 1.5 mg/kg body weight for 30 days (intraperitonally). Mahe *et al*., (2017) also reported similar finding in rats treated with *jatropa curcas* leaf extract @ 100, 200 and 250 mg/kg daily orally for 28th day. The kidneys of the 250 mg/kg group revealed minor interstitial haemorrhages and vascular congestion, but the glomeruli, tubules, and vesicles were all normal.

The present study confirmed dose dependent toxicity in histoarchitectural changes in kidney treated with JcLE coated silver nanoparticles. The changes were gradually increases with increasing in dose and duration of exposure.

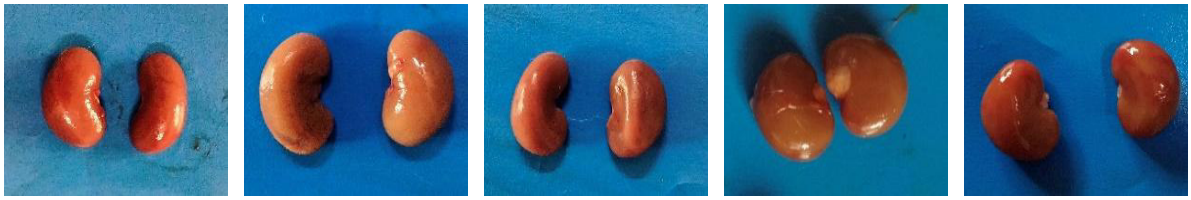
4.6.3.c Heart

The histoarchitectural observations in section of heart of rats of all treated groups revealed infiltration of mononuclear cell, extravasation of blood, and hyaline degeneration. Mild change was observed in group II and III treated with



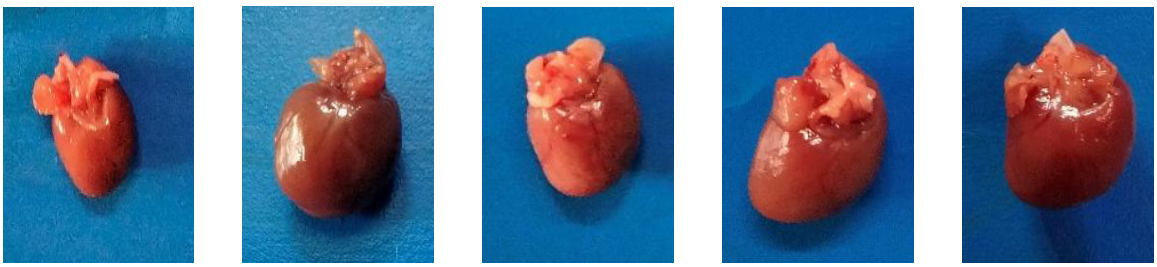
Group-I Group-II Group-III Group-IV Group-V

Figure 4.19- Gross examination of liver



Group-I Group-II Group-III Group-IV Group-V

Figure 4.20- Gross examination of kidney



Group-I Group-II Group-III Group-IV Group-V

Figure 4.21- Gross examination of heart

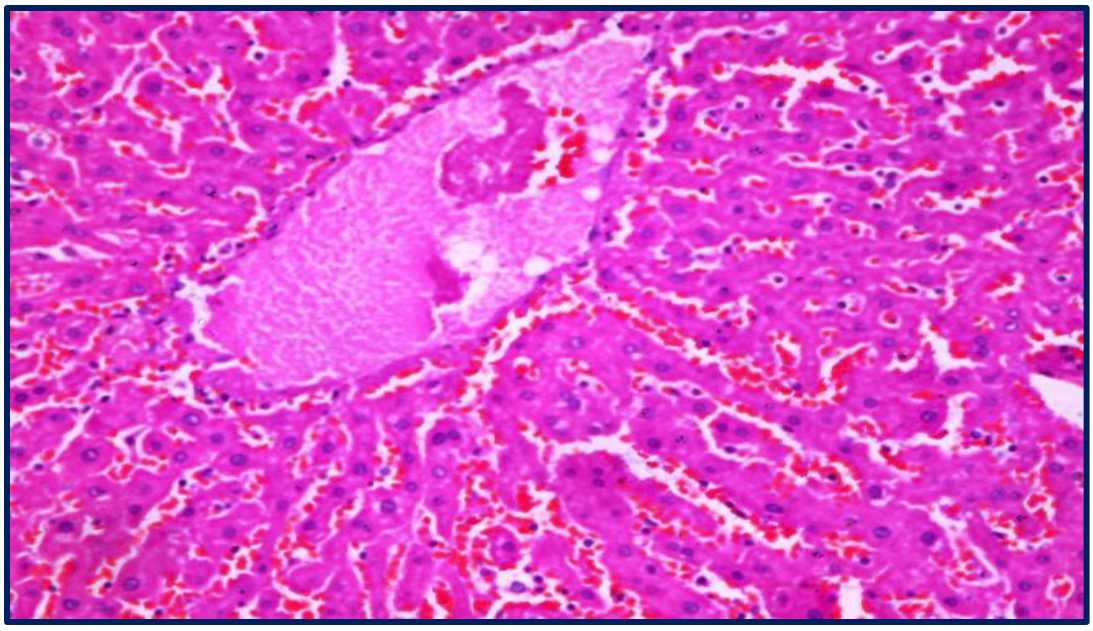


Figure 4.22-Section of liver (II) showing mild congestion of blood vessels and perivascular infiltration of mononuclear cells (H & E 400 X).

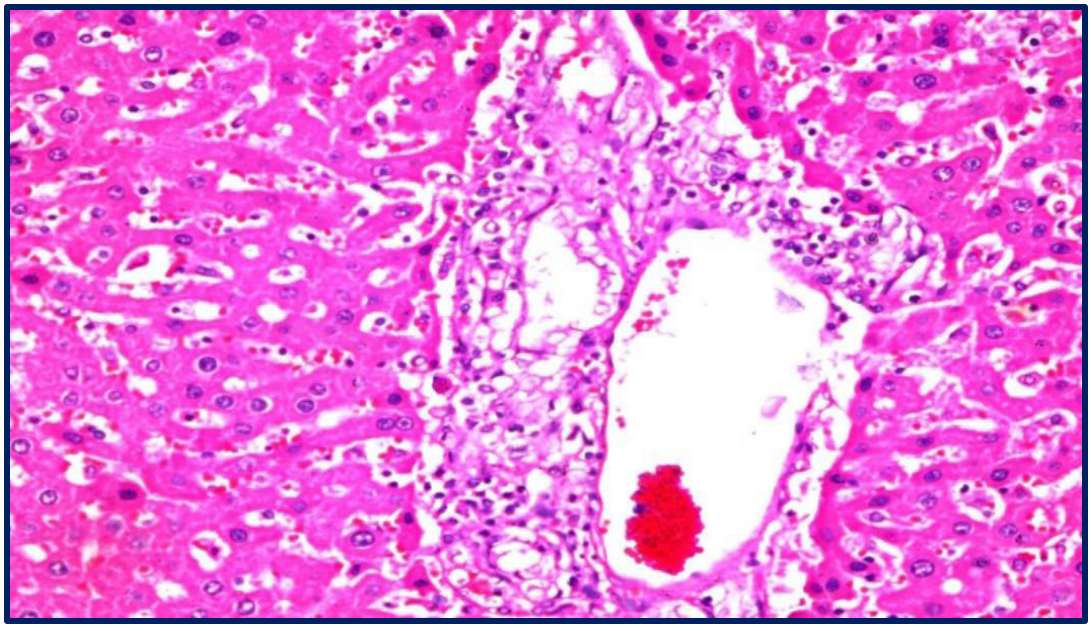


Figure 4.23 Section of liver (III) showing mild haemorrhage (H & E stain, 400X).

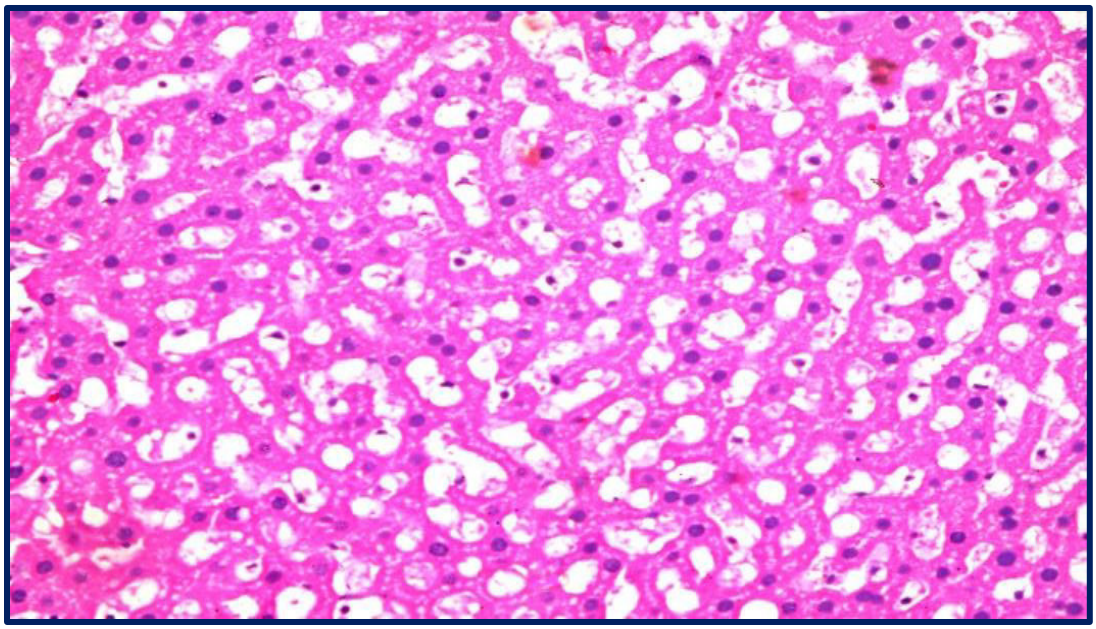


Figure 4.24 - Section of liver (IV) showing fatty degeneration with infitration of mononuclear cells (400 X).

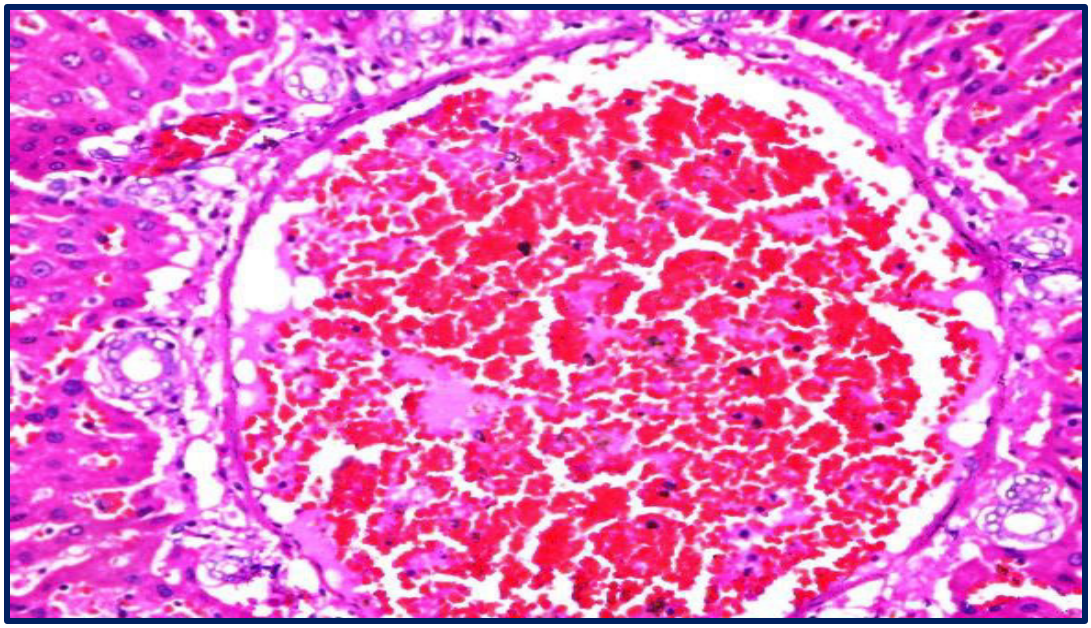


Figure 4.25- Section of liver (V) showing severe dilatation of central vein with congestion (400 X).

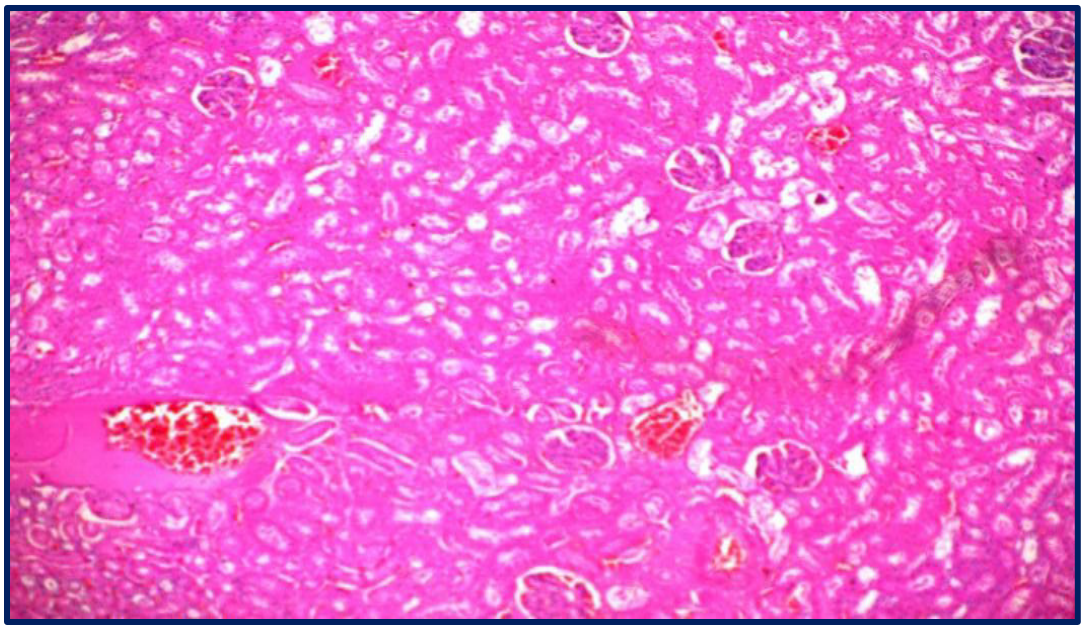


Figure 4.26-Section of kidney (II) showing haemorrhages (H & E stain, 100X).

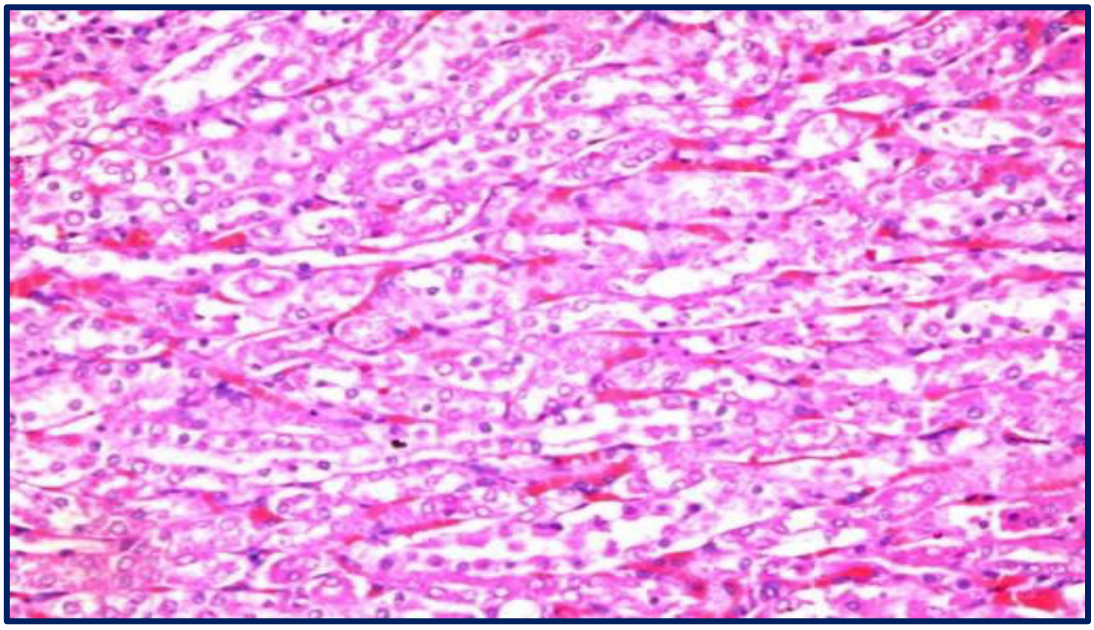


Figure 4.27-Section of kidney (III) showing mild focal hyline casts with necrobiotic changes in lumen of tubules (H & E stain 400 X).

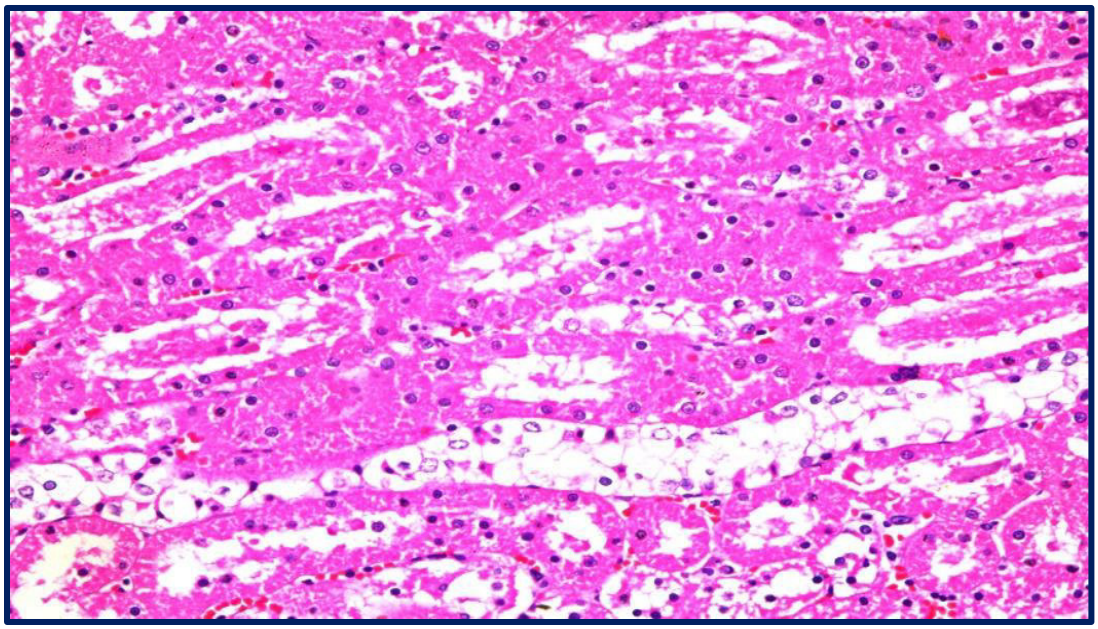


Figure 4.28-Section of kidney (IV) showing hydropic degeneration and marked infiltration of mononuclear cells of tubules (400 X).

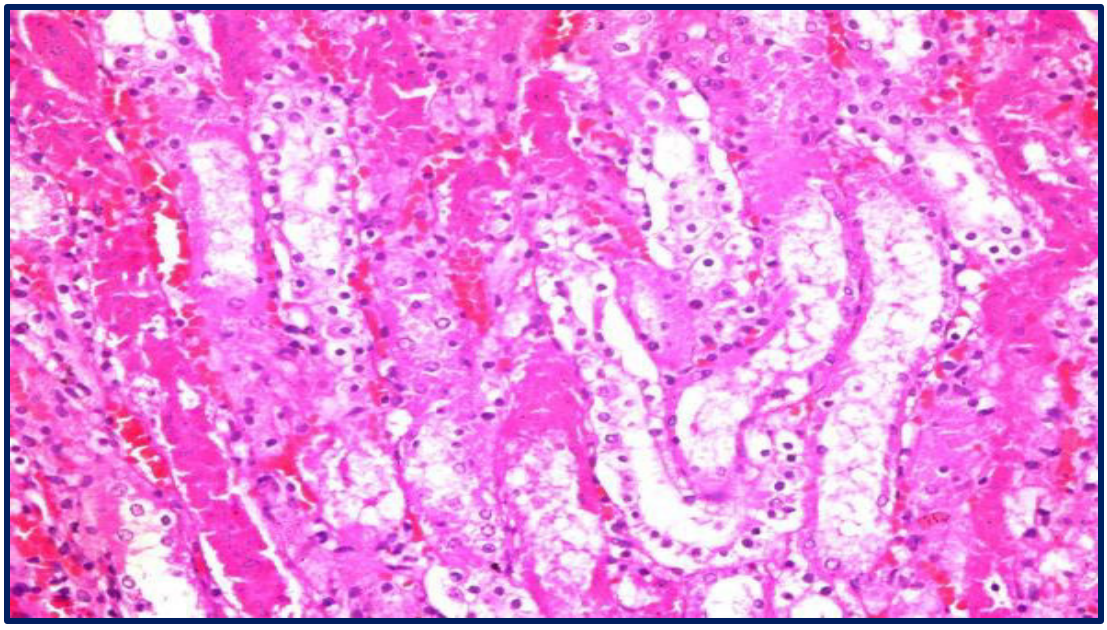


Figure 4.29 -Section of kidney (V) showing marked cellular swelling with necrobiotic changes in renal tubules (H & E stain, 400X).

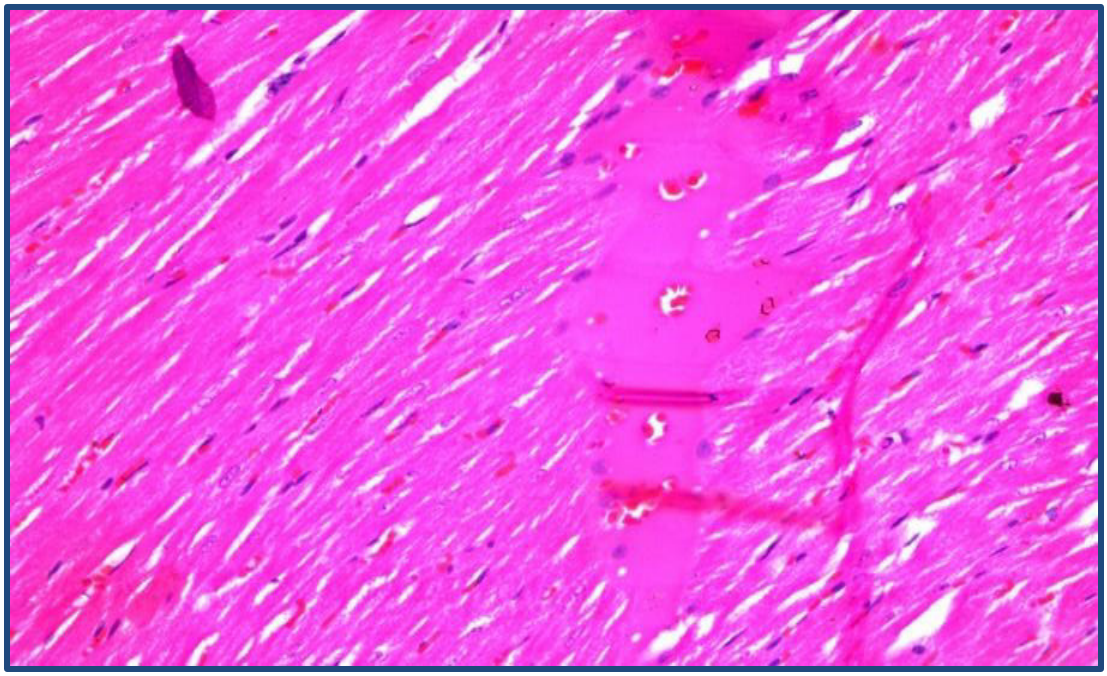


Figure 4.30- Section of heart (II) muscle showing hyaline degeneration (400 X).

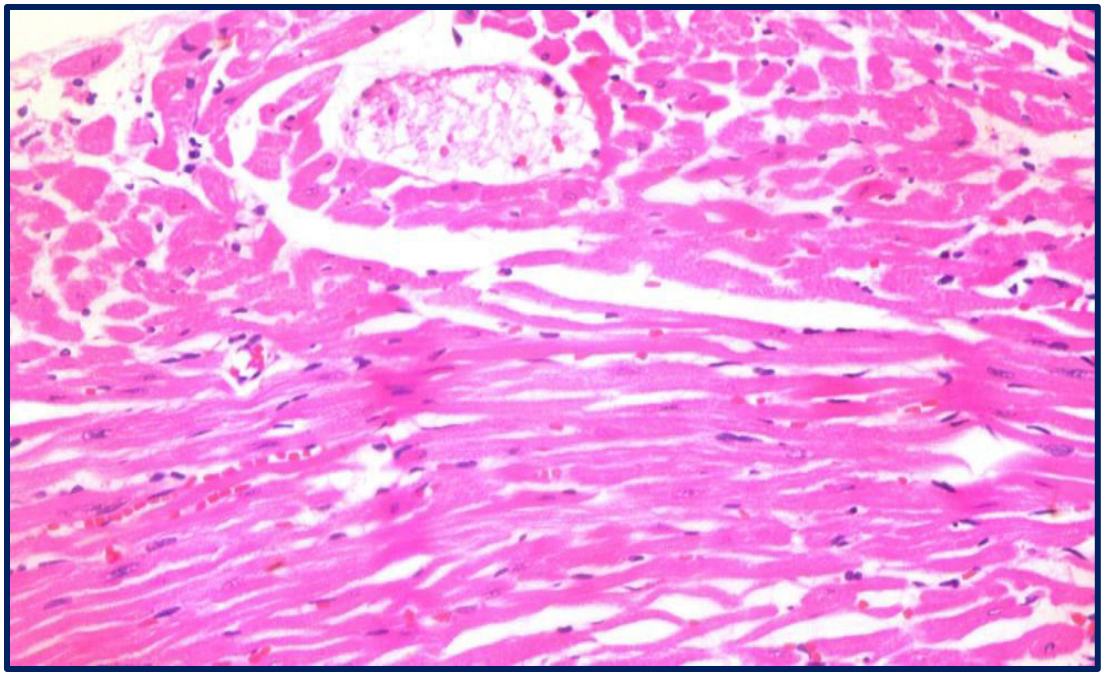


Figure 4.31-Section of heart (III) muscle showing hyaline degeneration along with mononuclear cells infiltration (400 X).

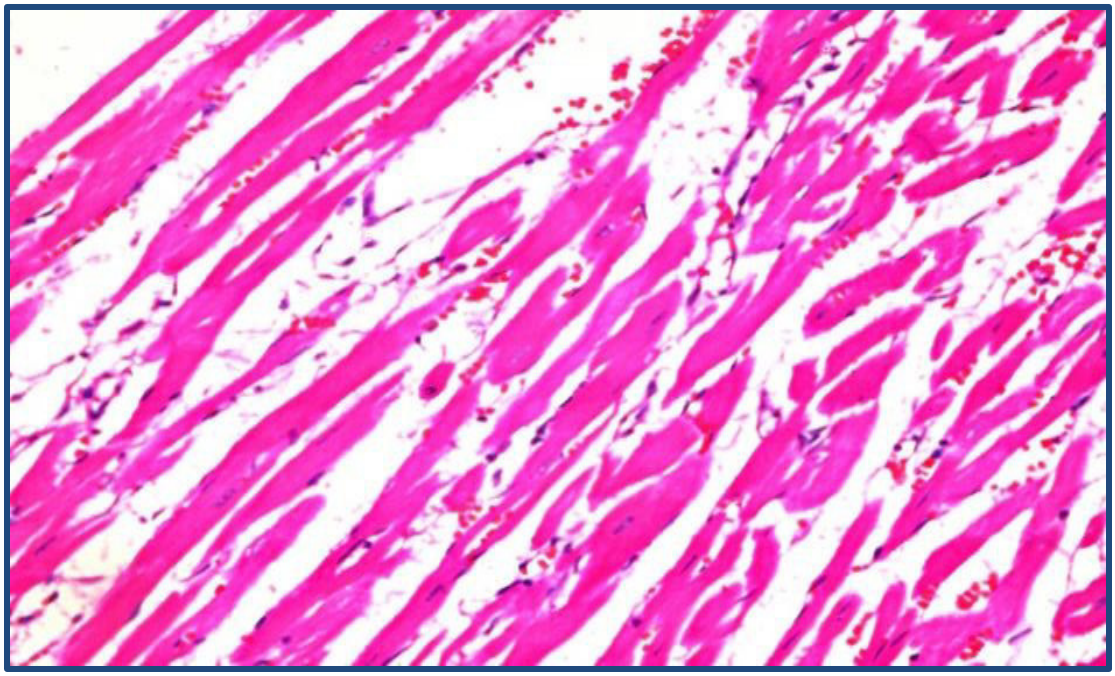


Figure4.32- Section of Heart (IV) showing extravasation of RBCs and infiltration of mononuclear cells (400 X).

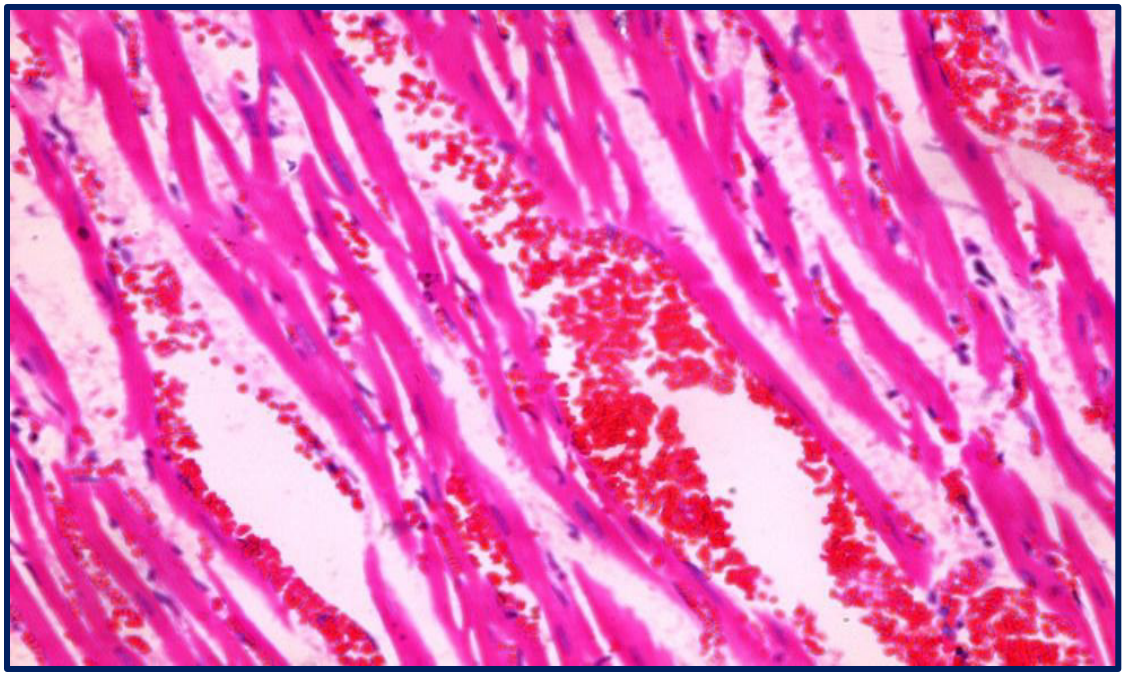


Figure 4.33-Section of heart (V) showing severe extravasation of RBC and infiltration of mononuclear cells (H & E 400 X).

JcLE coated SNPs, in comparison to group IV and V treated with JcLE coated SNPs were found in figure 4.30-4.33. Rats exposed to 800mg/kg JcLE coated SNPs dose showed more severe alterations than rats exposed to 100mg/kg.

Sulaiman *et al*., (2015) observed similar finding in rats treated with silver nanoparticles at 10, 50 and 100 mg/kg body weight orally for 30 days. Azubike *et al*., (2015) also reported similar finding in mice treated with crude aqueous leaf extract of *J. curcas* at dose 10, 100, 1000 and 2000 mg/ kg b.wt in stage I and in stage II 200, 400, 600 and 800 mg /kg b.wt. In heart observed hemorrhage and presence of necrotic myocardial fibers with mild lymphocytic infiltration.

So, the present study concluded that there were mild to severe histopathological changes was observed in group II, III, IV and V. Which confirms the dose dependent toxicity in histopathological changes in heart section as compared with control group of experiment.

Overall, the histopathological analysis revealed dose dependent variation in the liver followed by kidney and heart were recorded in all the treatment groups as compared to control group in experimental rats.

Summary and Conclusions

SUMMARY AND CONCLUSIONS

Present investigation was undertaken to assess the repeated dose oral toxicity of *Jatropha curcas* Linn. leaf extract coated silver nano particles in wistar rats. The subacute oral toxicity of aqueous leaf extract of *Jatropha curcas* coated silver nano particles was evaluated on wistar rats. The different concentrations of *Jatropha curcas* coated silver nano particles were used to find out the sign of toxicities in exposed rats. In depth, the investigation was done by employing standard procedures of extractions and evaluation of toxicity patterned by using the OECD guidelines. The study was planned with the following objectives as a) Preparation of aqueous extracts of *Jatropha curcas* b) Preparation of silver nano particles c) Confirmation of coating of aqueous leaf extract of *Jatropha curcas* and silver nanoparticles d) Evaluation of toxicity in behavioural, body weight gain and feed consumptions in exposed rats e) Assessment of toxicity pattern in haematological and biochemical parameters in rats exposed with different concentrations of aqueous leaf extract of *Jatropha curcas* coated silver nano particles f) Evaluated the delayed/post dosed toxicity in exposed rats g) Pathological evaluation of dose depended toxicity in exposed rats.

The aqueous leaf extracts of *Jatropha curcas* was prepared and the extractability percentage was recorded as 4.66% for 100 gm powder of *Jatropha curcas* leaves. The process of synthesis of of silver naoparticle was carried out by by biological method and was confirmed with the changed colour from pale green to dark brown which indicated synthesis of AgNPs from the leaves. The characterization of synthesis/coating of aqueous leaf extract of *Jatropha curcas* and silver naoparticles was confirmed by Transmission Electron Microscopy (TEM). The TEM images *Jatropha curcas* leaf extract shown their presence specifically around the silver nanoparticles. The average diameter of coated silver nanoparticles was found 31.92 nm. Hence, it was confirmed that *Jatropha curcas* leaf extract has affinity for silver nanoparticles and capable of coating with them.

Different concentrations/doses of aqueous leaf extract of *Jatropha curcas* coated silver naoparticles was used. The doses were confirmed by empolying the

pilot study and were 100, 200, 400 and 800 mg/kg body weight along with 0.17mg/kg SNPs in all the groups except in control group. In general behavioral pattern no any sign of toxicity was observed in the animals of control group as well as treatment groups, the animals remained active and alive throughout the experimental period, however, two mortality was recorded in experimental group. Two mortality was observed on 14^{th} day of experiments in group III and IV. Though the single mortality was there in two groups, the rest animals did not show the sign of toxicity or mortality up-to 28 days of experimental period. Moreover, no delayed mortality was recorded in the satellite group (V) where the rats were maintained for 15 more days. On body weight also there were no significant dose-depended changes were observed in the rats exposed to aqueous leaf extract of *Jatropha curcas* coated silver naopaticles for 28 days. Even, no significant differences in food consumption and water intake was observed in the experimental rats in both treated and control group rats throughout the experimental period. Hence. it was confirmed that, aqueous leaf extract of *Jatropha curcas* coated silver naopaticles did not shown any sign of toxicity on general behaviour, body weight and feed intake ration in exposed rats at any of the concentrations and duration of exposure.

The haematological parameters viz. haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) values were analyzed from blood collected at 0^{th}, 7^{th} ,14^{th} and 28^{th} day of the experimental period. In haemoglobin values there was statistically significant difference was observed in II, III, IV and V groups on 7^{th} ,14^{th} and 28^{th} day of the experimental period. Throughout the experimental period there was highly increase in haemoglobin values was observed on 7^{th} day in group V (satellite group), as compared to other groups. But all the haemoglobin values were observed within the normal physiological range. Hence, it was concluded that, though the binding of silver nanoparticles with *J. curcas* was confirmed but they could not be shown any deviation in haemoglobin values at any of the dose of the experiment. Statistically significant difference in total erythrocyte count (TEC) were observed on day 7^{th} in group III, IV and on day 14^{th} in III and V group. The increase in total erythrocyte count (TEC) values in group IV was observed and

decreased in II and III group as compared to other treatment groups on day 7th of experiment. On day 14th there was increase in total erythrocyte count (TEC) was observed in group III, and decreased in II, IV and V group was observed. The TEC in all groups were observed within normal range. Throughout the experimental period there was lowered in total erythrocyte count (TEC) value was observed on day 7th in group III as compared to other treatment groups, but this value was observed within normal range. Hence, in this research, *J. curcas* coated silver nanoparticles could not shown any sign of toxicity on total erythrocyte count at any of the concentrations used for 28 days. However, throughout the experimental period there was highly increase in total leucocyte count (TLC) was observed in group V on 28th day of experiment. In TLC nonsignificant difference was observed in all the groups, though there was deviation in the TLC values in all the groups at different days no sign of toxicity with respect to increase or decrease in value was observed.

In differential Leucocyte Count, in group I, II and V lymphocyte count values were statistically similar but numerically different, but all lymphocyte values were observed within normal range. Throughout the experimental period there was highly increase in monocyte counts was observed on 28th day of experiment in treatment group III. There was significant increase in monocyte count was observed than the normal range from II to V group of treatment. Similarly, there was increase in neutrophil counts was observed in treatment group IV on day 14th compared to another treatment groups, but the neutrophil values were observed increases than the normal range. No toxicity or pattern of significant difference in eosinophil count at any dose range of aqueous extract of *J. curcas* coated silver nanoparticle with different time intervals was observed. Increase in basophil was increase observed in treatment group IV on 28th day of experiment as compared to other treatment groups but the value was within the normal range only. Throughout the experimental period there was increase in blood clotting time was observed in group V on 28th day of experiment when compared to other treatment groups. The clotting time significantly increase with increasing dose and duration of exposure. Hence, it confirms that the significant increase in blood coating time was dose and duration of exposure dependent. The

biochemical Parameters; Liver function test, aspartate aminotransferase (AST/ SGOT) and Alanine Transaminase (ALT) / SGPT, whereas in kidney function test, blood urea nitrogen (BUN), creatinine and total Protein were analyzed from blood collected at 0^{th}, 7^{th} ,14^{th} and 28^{th} day of the experimental period. On day $7^{th}$and 14^{th} statistically increase in aspartate aminotransferase values was observed in group V and decreased aspartate aminotransferase values was observed in group II. On day 28^{th} statistically increase in aspartate aminotransferase values was observed in group V as compared to other groups. In groups II, III, IV and V aspartate aminotransferase values were observed increased and the values were beyond the normal physiological limit as compared with control group. Throughout the experimental period there was observed increase in aspartate aminotransferase values in group V on 28^{th} day of experiment. However, there was highly increase in alanine transaminase values observed in treatment group V on day 28^{th} of experiment. In kidney function test, on day 7^{th} there was increase in blood urea nitrogen value was observed in group V and decreased in group II as compared to group III, IV and V. On day 14^{th} increase in blood urea nitrogen value were observed in group IV when compared with group I, II, III and V. However, decrease in blood urea nitrogen values was observed in group V as compared to group II, III and IV. Whereas on day 28^{th} statistically significant increase in blood urea nitrogen value was observed in treatment group IV as compared to groups I, II, III and V. In group II and V the values were observed statistical similar but numerically different and throughout the experimental period there was increase in blood urea nitrogen values observed in treatment group IV on 28^{th} day of experiment. The aqueous extract of *J. curcas* coated silver nanoparticles also shown dose depended increased in creatinine values in the treatment groups when compared with the control groups.

The gross examination of liver revealed pale in color, mild focal congestion, slight multifocal necrosis was noted high dose treated groups (V). On heart and kidney, no any gross alteration was observed in all the entire treatment groups. In organ weights, non significant variation were observed in mean relative organ weights of liver, heart and kidney in all the treated groups. The histopathological observation of liver in high dose treated group were showed

severe congestion, heamorrhages, dilatation of central vein with necrobiotic changes, dilatation of sinusoidal tissue space, congestion of blood vessels, fatty degeneration with marked infiltration of mononuclear cell, multifocal to severe congestion, marked diffused necrobiotic changes in hepatocyte and cellular degeneration. Higher dose produced more damaging effects as compared with the respective low dose. Heart were showed infiltration of mononuclear cell, hyperemic blood vessel, extravasation of blood, mild inflammation and cellular degeneration, hyaline degeneration, haemorrhage and presence of necrotic myocardial fibres in group V however, dose dependents changes in the severity of lesion were also recorded in the respective group. Kidney revealed severe cellular swelling, necrosis, congestion, hydropic degeneration, marked infiltration of mononuclear cells in intertubular tissue space, mild to moderate focal and multifocal hyaline cast located in the lumen of exposed renal tubules and hydropic degeneration with haemorrhages in high dose treated group as compared with the rest of the treatment group except control.

The results of the present research conformed the following conclusions:

1. The extractability percentage of aqueous leaf extracts of *Jatropha curcas* was recorded as 4.66% for 100gm powder of *Jatropha curcas* leaves.

2. The changed in colour from pale green to dark brown which indicated synthesis of AgNPs. Hence synthesis of of silver naoparticles by biological method and was confirmed.

3. The characterization of synthesis/coating of aqueous leaf extract of *Jatropha curcas* and silver naopaticles was confirmed by Transmission Electron Microscopy (TEM). The average diameter of coated silver nanoparticles was found 31.92 nm.

4. The aqueous leaf extract of *Jatropha curcas* coated silver naopaticles did not shown any sign of toxicity on general behaviour, body weight and feed intake ration in exposed rats at any of the concentration and duration of exposure.

5. On haematological parameters, no alterations/ deviation in haemoglobin values, total erythrocyte count (TEC) and in total leucocyte count (TLC) was observed in all the treatment groups on 0^{th}, 7^{th} 14^{th} and 28^{th} day of experiment. In differential leucocyte count, only increased in monocyte count was observed than the normal range. However, the lymphocyte count, neutrophil counts, eosinophil count and basophil count values were observed within the normal physiological range throughout the experiment period.

6. The clotting time significantly increase with increasing dose and duration of exposure. Hence, it confirms that the significant increase in blood coating time was dose and duration of exposure dependent.

7. The biochemical Parameters particularly in liver function test, there were gradual increases in aspartate aminotransferase and alanine transaminase values as compared with control groups, confirms the dose dependent toxicity in the treatment groups.

8. In kidney function test, there was significantly increase in blood urea nitrogen and creatinine in all treatment groups. Hence the toxic effect of *J. curus* coated silver nano particles on rats were dose depended.

9. Gross necropsy examination of liver revealed pale in color, focal congestion and necrosis group V. Dose dependent mild changes in liver were also noted in rest of the treatment group whereas, no gross alteration were observed in heart and kidney in the entire treatment group.

10. Dose dependent severe changes in histoarchitecture of liver, kidney and heart were observed in treatment groups as compared with the control group.

Thus, present study suggests for undertaking scientific study for either by selecting the lower doses of silver nanopartical or *J. curcas* and also explore the chronic toxicological pattern of *Jatropha curcas* coated silver nanoparticles.

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Vitae

VITAE

The author Miss. **Bele Sangeeta Sakharam** was born on 5th Feb 1996 at Morwad, Post- Kharwad, Tq- Kalamnuri, Dist- Hingoli of Maharashtra State. She completed secondary school certificate (SSC) examination in 2012 and subsequently completed higher secondary examination in 2014 from M.J.P.V. Kalamnuri. She completed her B.V.Sc. and A.H. degree education from college of Veterinary and Animal Sciences Parbhani under Maharashtra Animal and Fishery Sciences University (MAFSU), Maharashtra.

She actively participated in N.S.S. and animal health camps during graduation. She has special aptitude for animal welfare activities, she is interested in research and development activities related to animals and therefore pursued post graduate studies in Veterinary Pharmacology at college of Veterinary and Animal Sciences, Parbhani.

Thesis Abstract

THESIS ABSTRACT

a)	Title of the thesis	:	**"SUB-ACUTE ORAL TOXICITY EVALUATION OF *Jatropha curcas* LINN. LEAF EXTRACT COATED SILVER NANOPARTICLES IN WISTAR RATS"**
b)	Full name of student	:	**Bele Sangeeta Sakharam**
c)	Name and address of Major Advisor	:	**Dr. N. D. Jadhav** Advisor/Guide Assistant Professor, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, MAFSU, Parbhani.
d)	Degree to be awarded	:	M.V. Sc.
e)	Year of award of degree	:	2022
f)	Major subject	:	Veterinary Pharmacology and Toxicology
g)	Total number of pages in the thesis	:	110
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i)	Signature of Student	:	
j)	Signature, Name and address of forwarding authority (HOD/SH)	:	

Dr. S. R. Rajurkar
Professor and Head,
Dept. of Veterinary Pharmacology & Toxicology,
COVAS, MAFSU, Parbhani.

ABSTRACT

The present study was designed to evaluate "Sub-acute oral toxicity evaluation of *Jatropha curcas* linn. leaf extract coated silver nanoparticles in wistar rats". SNPs were synthesized by biological reduction of silver nitrate with *Azadirachta indica* (neem-5%) leaf extract. The synthesized SNPs were characterized for its coating with JcLE by TEM. In this research 50 wistar rats of either sex were randomly assigned in five groups. One healthy control group with four groups administered JcLE orally at 100 mg/kg, 200mg/kg and 400mg/kg in combination of 0.17 mg/kg SNPs for 28 days. The last group was considered as satellite group dosed at 800 mg/kg JcLE with 0.17 mg/kg SNPs and were kept 15 more days to monitor the delayed/withdrawal effect.

The aqueous leaf extract of Jc coated SNPs did not shown any sign of toxicity on general behaviour, body weight and feed intake rations in exposed rats. No alterations/deviation was observed in haemoglobin, TEC, TLC values in any of the treatment groups. In DLC, only increased in monocyte count was observed, however, the lymphocyte, neutrophil, eosinophil and basophil counts were within normal range. The blood clotting time was increased significantly with increasing dose and duration of exposure. In biochemical analysis, gradual increase in AST and ALT values were observed. The total protein values were significantly increased, and confirm the dose dependent toxicity in liver. The BUN and creatinine values were increased significantly, which concluded the toxic effect of Jc coated SNPs in kidney at different concentration.

Grossly, focal congestion and necrosis recoded in liver whereas, no gross alteration were observed in heart and kidney. Histopathological analysis revealed dose depended alteration in liver followed by kidney and heart were observed in rats treated with JcLE coated SNPs as compared to control groups.

प्रबंध सारांश

प्रबंधाचे नाव	:	**“जट्रोफा कुरकस या पानाच्या अर्काचे वेष्टण केलेल्या रौप्य अतीसुक्ष्मकणांची मात्रा तोंडाद्वारे विस्टार उंदरांमध्ये अतितिव्र देऊन विषाच्या तिव्रतेचे मूल्यांकन.”**
विद्यार्थ्याचे नाव	:	**बेले संगीता सखाराम**
प्रमुख मार्गदर्शकाचे नाव व हुद्दा	:	**डॉ. एन. डी. जाधव,** सहाय्यक प्राध्यापक, पशु औषधी व विषशास्त्र विभाग, पशुवैद्यक व पशुविज्ञान महाविद्यालय , परभणी.
पदवी प्रदान वर्ष	:	२०२२
प्रदान करण्यात येणारी पदवी	:	स्नातकोत्तर पदवी (एम. व्ही. एस. सी.)
मुख्य विभाग	:	पशु औषधी व विषशास्त्र विभाग
प्रबंधातील एकुण पृष्ठे	:	११०
सारांशातील एकुण शब्द संख्या	:	२८९
विद्यार्थ्याची स्वाक्षरी	:	
पुढे पाठवणाऱ्या अधिकाऱ्याचे नाव	:	

डॉ. एस. आर. राजुरकर,
प्राध्यापक व प्रमुख,
पशु औषधी व विषशास्त्र विभाग,
पशुवैद्यक व पशुविज्ञान महाविद्यालय,
परभणी

सारांश

प्रस्तुत प्रयोग "जट्रोफा कुरकस या पानाच्या अर्काचे वेष्टण केलेल्या रौप्य अतिसुक्ष्मकणांची मात्रा तोंडाद्वारे उंदरांमध्ये अतितिव्र देऊन विषाच्या तिव्रतेचे मूल्यांकन." करण्यात आले. 5% कडूलिंबाच्या पानाचा अर्क सिल्वर नायट्रेट मध्ये मिसळून जैविक विघटणाद्वारे रौप्य अतिसुक्ष्मकण तयार करण्यात आले. जट्रोफा कुरकस पानाच्या अर्क वेष्टण तयार झालेल्या रौप्य अतिसुक्ष्मकणांसोबत इलेक्ट्रॉन मायक्रोस्कोप द्वारे निश्चीतिकरण करण्यात आले. या अभ्यासात प्रतेक गटात १० उंदीर या प्रमाणे ५० उंदराचे ५ गट बनवण्यात आले. त्यापैकी १ गट सुद्रुढ व चार गटांतील उंदरांना अनुक्रमे १०० मिलिग्राम / किलोग्राम, २०० मिलिग्राम / किलोग्राम आणि ४०० मिलिग्राम /किलोग्राम, जट्रोफा कुरकस या पानाच्या अर्क सह ०.१७ मिलिग्राम / किलोग्राम, वेष्टीत केलेलया रौप्य अतिसुक्ष्मकणांची मात्रा तोंडाद्वारे 28 दिवसांसाठी दिले. चौथा गट हा सॅटलाइट गट म्हणून ठेवण्यात आला. त्या गटाला ०.१७ मिलिग्राम/किलोग्राम, रौप्य अतिसुक्ष्मकणांसोबत ८०० मिलिग्राम / किलोग्राम, जट्रोफा कुरकस पानाच्या अर्काचे मिश्रण इतर गटांपेक्षा १५ दिवस जास्त देऊन प्रभावाचे निरीक्षण व परिणाम तपासण्यात आला.

जट्रोफा कुरकस जलीय पानांचा अर्क वेष्टीत केलेल्या रौप्य अतिसुक्ष्मकणांचा उंदरांमध्ये त्यांच्या वागणुकी मध्ये , शरीराच्या वजनावर आणि खाद्य सेवनावर विषारीपणाची कोणतीही लक्षणे दर्शविली नाहीत. रक्त घटकाची तपासणी केली असता , कोणत्याही उपचार गटातील हिमोग्लोबिन , लाल पेशी , पांढऱ्या पेशी मूल्यांमध्ये कोणतेही बदल आढळले नाही. डीएलसीमध्ये, केवळ मोनोसाइट्सच्या संख्येत वाढ दिसून आली . लिम्फोसाइट, न्यूट्रोफिल, इओसिनोफिल आणि बेसोफिल गणना मूल्ये सामान्य श्रेणीमध्ये होती. वाढत्या डोस आणि एक्सपोजरच्या कालावधीसह रक्त गोठण्याची वेळ लक्षणीयरीत्या वाढली. रक्तजलातील घटकांमद्धे, एएसटी आणि एलटि मूल्यांमध्ये हळूहळू वाढ झालेली दिसुन आली. तसेच एकूण प्रथिने मूल्यांमध्ये देखील लक्षणीय वाढ दिसून आली , जे यकृतातील डोसवर अवलंबून विषारीपणाची पुष्टी करतात . बी.यू.एन. आणि क्रियाटिनिन ची मूल्ये लक्षणीयरीत्या वाढली , त्यामुळे वेगवेगळ्या डोसला मूत्रपिंडात जट्रोफा कुरकस वेष्टीत रौप्य अतिसुक्ष्मकणां चा विषारी प्रभाव

असल्याचा निष्कर्ष निघाला. शवविच्छेदन पाहणीत यकृतावर मध्यम रक्तसंचय दिसून आले. हृदय आणि किडनीवर , कोणतेही बदल दिसुन आले नाहीत. सुक्ष्म दरक्षिके मधून केलेल्या परिक्षणाद्वारे यकृतामधे प्रमुख बदल दिसुन आले. तसेच सुद्रुढ गटांच्या तुलनेत जट्रोफा कुरकस पानाच्या अर्क वेष्टीत रौप्य अतिसुक्ष्मकणांची मात्रा दिलेल्या उंदरांमध्ये मूत्रपिंड आणि हृदयातील यकृतामध्ये देखील मोठ्या प्रमाणात बदल झाल्याचे दिसून आले.