EFFECTS OF *Carica papaya* ON CARDIAC PARAMETERS IN WISTAR RATS

T H E S I S

Submitted

In partial fulfilment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE VETERINARY PHARMACOLOGY AND TOXICOLOGY

BY

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Enrolment Number **:** V/13/210

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **EVALUATION OF EFFECTS OF *Carica papaya* ON CARDIAC PARAMETERS IN WISTAR RATS** or diploma of any University, nor 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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Miss**. MORE KSHITIJA JAGDISH** has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, **"EVALUATION OF EFFECTS OF *Carica papaya* ON CARDIAC PARAMETERS IN WISTAR RATS."** submitted by her is the result of 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.

We also certify that the thesis or part thereof has not been previously submitted by him/her for a degree of any other University.

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF *Carica papaya* ON CARDIAC PARAMETERS IN WISTAR RATS"** submitted by Miss. **MORE KSHITIJA JAGDISH** 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 oral examination in collaboration with the External Examiner.

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Pharmacology and Toxicology, COVAS, Parbhani.

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ABBREVATIONS

@	At the rate of
Ad-lib	Adlibitum
ALT	Alanine transaminase
AST	Asparatate transaminase
b.wt	Body weight
BUN	Blood Uria Nitrogen
CAD	coronary artery disease
CpAE	*Carica papaya* seeds extract
CD	Critical difference
CVD	Cardiovascular disease.
CHD	Cardiac heart failure.
CRD	Complete Randomized Design
DLC	Differential leucocyte count.
EDTA	Ethylene Diamine Tetra Acetic Acid
g	Gram.
g/dl	Gram per deciliter
GST	Glutathione Transferase
GPx	Glutathione peroxidase
fig.	figure.
hr	hour.
H & E	hematoxylin and Eosin stain.
Hb	Hemoglobin
HDL	High density lipid
IU/L	international unit per liter
LDL	Low density lipid.
Mg	Milligram per deciliter
N	total number.
P.O	Per orally
RBC	Red blood cells.
ROS	Reactive Oxygen Species
SCr	Serum Creatinine
SGOT	Serum glutamate transaminase
SGPT	
TC	Total cholesterol
TG	Triglyceride
TEC	Total erythrocyte count
TLC	Total leucocyte count

Introduction

CHAPTER I

INTRODUCTION

Today our lifestyle has changed, Junk food, lowered physical activities, lockdown, work from home and stress have aggravated the risk of hyperlipidemia and obesity and thus it has also increased the cardiovascular complications.

Agents such as low-density lipoprotein, free radicals, homocysteine, and nicotine are also considered as leading factors. Hyperlipidemia, formation of atherosclerotic plaque involves accumulation of low-density lipoprotein, low-density lipoprotein oxidation, uptake of oxidized low-density lipoprotein by macrophage scavenger receptors, influence of macrophages on foam cells, and stabilization of plaque. In all steps of atherosclerosis, inflammatory cytokines are involved and make this process a chronic inflammatory disease.

When the blockage of the coronary arteries reaches more than 75%, usually the symptoms of angina will gradually appear. Blood clot usually develops on the irregular surfaces of arteries, which then may become detached, thus blocking the downstream blood flow. Heart attacks and strokes are usually caused by such blood clots. Moreover, the atherosclerotic blood vessels are generally weak and can burst.

The best treatment in diseases such as atherosclerosis is prevention. Drugs are also used to lower cholesterol levels or blood pressure; however, most of them possess considerable side effects.

Using alternative treatments, especially medicinal plants and their complements, to treat different diseases such as hyperlipidemia, diabetes and cardiovascular diseases has increased over the recent decades in majority of countries worldwide.

One of the important problems faced by doctors and also users of medicinal plants is lack of enough information in the field of drug safety and its effect on disease. Extensive studies have been done on the effectiveness of medicinal plants used in traditional medicine over the past 30 years and some of their efficiencies and deficiency have been recorded.

Plants as a natural source of antioxidants and has all the herbal and natural properties of treating many diseases naturally, one of the important plant which have medicinal value in all the products of it, is *Carica papaya* and its seeds contains antihyperlipidemic properties and can be useful in treatment of atherosclerosis. *Carica papaya* is a sweet topical fruit with musky undertones and a distinctive aroma. Everything in papaya plant such as leaves, peel, roots, latex, flower, fruits and seeds have their nutritional and medicinal significance.

The papaya seeds can be considered as a treatment for atherosclerosis, as it contains antihyperlipidemic properties. It contains small amounts of proteins and almost free from cholesterol and fats. Considering these facts, the present study has been planned to evaluate the protective and medicinal effect of *Carica papaya* seeds in treatment of atherosclerosis in Wistar rats with the following objectives.

Objectives:

1. To induce Hyperlipidemia in Wistar rats using high fat diet.
2. To study the effect of *Carica papaya* on blood biochemical analysis, lipid profile and hematological parameters in Wistar rats.
3. To study the behavioral effect of *Carica papaya* in Wistar rats.
4. To study the gross and histopathological changes in heart and various affected organs.

1.1 Atherosclerosis

One of the important disease these days observed is Atherosclerosis, It is the result of hyperlipidemia and lipid oxidation and has always being the major cause of mortality in many countries. It is the disease of vascular intima in which all the vascular system from aorta to coronary arteries can be involved and is characterized by intimal plaques. The term atherosclerosis is of Greek origin, meaning thickening of intimal layer of arteries and accumulation of fat. Formation of these plaques starts with deposition of small cholesterol crystals in the intima and its underlying smooth muscles.

1.1.2 Aetoilogy

Important and most common factors of Atherosclerosis are metabolic disorders are Reactive oxygen species (ROS) or the superoxides which are generated due to peroxidation process of the lipid. Dyslipidemia and inflammation, hypertension and inflammation, diabetes and inflammation, obesity and inflammation by Nayak (2007).

1.1.3Treatment

Low fat diet therapy

Physiothrapy

Synthetic medicinal treatments

1. LDL lowering therapy
2. Cholesterol absorption inhibitors
3. Bile acids sequetrants

Triglycerides lowering therapy

1. Statins.
2. Omega-3-fatty acids.

   Surgical treatment.

   Nutraceuticals treatments.

1.2 Statin

Statin were observed that they have beneficial effects in the serum lipid profile, it has significantly reduced the serum total cholesterol, tryglycerides, LDL-C levels properly after drug treatment. (Khurana *et al.,* (2015).

1.3 Indications

These drugs are used in the conditions like, Cardiovascular diseases, Atherosclerosis, Arteriosclerosis, hence these conditions are responsible for the increase in cholesterol levels and triglycerides.

1.4 *Carica papaya* seeds

In recent era, plant research has got very effective for various diseases due to its curative effect and safety measures, and a good evidence have shown the excellent ability of the medicinal plants used in various traditional systems. (Omotade, 2011).

Plants as a natural source of antioxidants and all the herbal and natural properties of treating many diseases naturally, one of the important plant which have high medicinal value in all the parts of the plant is *Carica papaya*.

Proper experimental researches has demonstrated that the use of the seed extract of papaya is a nutritional tonic for the heart, has analgesic properties used in the treatment of stomach and gastric problems and has antioxidant properties. (Giove and Nakazawa, 1996; Indran *et al.,* 2008).

The papaya can be considered as a nutrient dense food, as it provides many more nutrients. Calorie basis as compared to other food, It contains small amounts of proteins and almost free from cholesterol and fats and here it shows its antihyperlipidemic effect.

Considering these facts, the present study has been planned to evaluate the protective and medicinal effect of *Carica papaya* seeds extract in treatment of atherosclerosis in Wistar rats.

Carica papaya has a rich antioxidant profile which plays a very effective protective role against the production of reactive oxygen species and lipid peroxidation by-products. Recent studies have proven Reactive Oxygen Species (ROS) in the pathogenesis of many human diseases. (Repetto and Lleshy, 2002).

In view of the above background, the present study has been undertaken to evaluate the effect of *Carica papaya* on serum lipids in Wistar rats fed with high fat diet (HFD) and to compare it with a standard hypolipidaemic drug statin. (Singh and Jialal, 2005; Choi and Hwang, 2005).

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

2.1 Atherosclerosis progression and vulnerability

Heart is considered to be one of the most vital organs that pumps blood around the body. It carries deoxygenated blood to lungs where it loads up the oxygen and unloads carbon dioxide, and waste products of metabolism. Atherosclerosis is known as the bland lipid storage disease, due to inflammation.

Among the first to invent and explain the atherosclerosis was Leonardo da Vinci (1452-1519) Davies, (2004). He stated that "vessels in the elderly restrict the transit of the blood through thickening of the tunics". Some years later, in 1799, Caleb Hillier Parry, a British physician (1755-1822) made a major contribution to medicine by the recognition of the cause of angina.

The experiment was conducted in sheeps to estimate the circulation and the effects of impairment of the vascular supply. During the examination of autopsy he found a plaquey substance in the coronary arteries. His interpretation of the study was that there was the plaster like substance inside the layer of arteries, this explaination was ignored for the next 100 years and now has become the most commonest disease among the people.

The atherosclerosis is the result of a long term, low grade inflammation that results to an reaction between the cells of the immune system, proliferation of the smooth muscle cells, and formation of fibrous tissue. Atherosclerosis is far the most frequent underlying cause of Heart disease that considered to be one of the most vital organs that pumps blood around the body. It carries deoxygenated blood to lungs where it loads up the oxygen and unloads carbon dioxide, and waste products of metabolism.

Hyperlipidemia is a major contributor to cardiovascular disease (CVD). The risk factors of it includes elevated blood pressure, high blood cholesterol, and diabetes.

Atherosclerosis existed from the decades and have the historical background. It is the accumulation of fatty plaque inside the blood vessels, which results in the hardening of the blood vessels and leads to narrowing of the blood vessels, the plaque is formed with the accumulation of the deposits of calcium and cholesterol particles in the vessels.

The chronic condition Atherosclerosis may remain asymptomatic for years and mainly produces two main conditions those are:

1. The atheromatous plaques in the arteries which are accumulated, after sometime may lead to plaque ruptures and can form the clots inside the lumen of the arteries due to the ruptures. The clots formed can easily be healed but the eventually shrink and that may results in the stenosis of artery locally and around its branches that leads to closure and hence there is insufficient blood supply to the tissue and organs.
2. If the artery enlargement process is progressive, then anuerysm may formed.

Other severe conditions may take place called infarction, this is due to the sudden rupture of the soft plaque accumulated and may lead to thrombus formation that will instantly slow or stop the blood flow, leading to the death of tissues in few minutes.

Maton, Anthea, *et a*l (1993) explained atherosclerosis and its severity, progression, and the conditions which may lead to death.

Kahlon *et al* (1996) reported that the micro vessels may rupture due to plaque and lead to haemorrhaeges, hence there is high risk of rupture.

Groneberg *et al* (2001) stated that statin also induces thrombocytopenia.

Repetto and Lleshy (2002) explained the mechanism of reactive oxygen species, the ROS is mechanism that have beneficial effects as this inhibits the ageing process, this may help from damaging the cell and reduce the risk of cardio vascular diseases.

Thampson, (2003) reported that statin are also associated with myopathy and rhabdomyolysis as a view to adverse effects.

Singh and Jialal (2005) Reported that on the basis of experimental study the curative effect of *Carica papaya* seeds extract has shown good effect on the lipid profile.

Mayerl *et al* (2006) explained the sequence from past to present affects, changes, advancements now in the atherosclerosis, and explained that there was presence of atherosclerosis since long.

Akatsu, (2006) explained that the depositions observed in the inner layer of arterial wall were derived primarily from the fibrin and the other blood contents. Hence the atheroma is derived from the degeneration of the fibrin and other blood proteins and lastly these deposits were modified towards the pulpy mass containing the cholesterol crystals and fatty globules.

Ridkar, *et al;* (2009), Explained the reason of atherosclerosis with the association with cholesterol and immune system, due to hypercholesterolemia results in deposition of cholesterol in macrophages and other immune cells, due to which it helps in inflammatory responses, and production of monocytes and neutrophils in bone marrow and spleen and this may be one of the reasons which may result or help in progression of atherosclerosis.

Slijkhuis, (2009) stated based on the histological characteristics of atherosclerotic lesions that the atheroma was the product of an inflammation within the intimal and the fibrous thickening emerged as a result of a reactive fibrosis induced by proliferating connective tissue cells within the intima.

Barlovic, (2011), Explained the role of diabetes, in progression of atherosclerosis, as diabetes is associated with obesity and obesity is the common factor for progression of atherosclerosis and diabetes , so there are chances again they can be together.

Khurana *et al;* (2015), stated that statins are very much beneficial and effective in lowering the serum cholesterols, triglycerides, LDL-c levels.

Ebadi, (2020), stated the description of the immune and inflammatory responses and mechanism of atherosclerosis.

Risk factors and etiology of atherosclerosis.

Beaglehole, and Magnus (2002), reported that, Epidemiological studies has enlisted over some 4-5 decades has stated that there may be various different factors for the progression of atherosclerosis. These can be classified in 2 forms the Genetic and environmental form. Atherosclerosis is usually determined by the presence of atheromas, patchy intimal plaques.

Commonly the location of accumulation of the plaque is lumen of medium to large arteries. The plaque is formed with contents like, inflammatory cells, smooth muscles cells, fibrous component like connective tissues, and fat components like lipids.

The danger and utmost risk factors are hypertension, dyslipidemia, diabetes, obesity, unhealthy life style, genetic history and smoking these are some reasons that may progressively help in developing atherosclerosis.

Some of the symptoms which may lead to atherosclerosis are, bleeding, intraplaque rupture, thrombosis and stenosis. Diagnosis can be done on the basis of imaging and tests.

Kawsara *et al*; (2018) reviewed that the Atherosclerosis is the disorder which generally occurs due to the chronic and form of the inflammatory reaction in the inner epithelial linings that cause the multifocal plaque development of the Arterial vascular wall.

2.1.2 Pathogenesis of atherosclerosis

Atherosclerosis is the progressive disease of the arteries that results in the development of heart disease and stroke. The current structure for understanding how atherosclerotic lesion was initially proposed by Russel Ross (1929-1999). The pathogenesis of the atherosclerotic lesions are remarkably complex and convulated, it involves multiple stages and many cell types and systems.

Angiogenesis in Atherosclerotic Plaques

The development of the atherosclerotic plaque is associated with the appearance and growth of the vasa vasorum. Plaque microvessels are immature and delicate and hence may lead to intra plaque haemorrhages, hence plaque increases the risk of rupture. Kahlon, (1996).

Platelets and Atherosclerosis

Adhesions due to platelets is also the cause of atherosclerosis occurs due to high stress, as in the stenotic arteries, they develop the arterial thrombosis. Platelets ultimately initiates the progression of the atherosclerotic plaques via their secretary functions. Huo, (2004).

Chronicity in the inflammatory condition is the primary confirmation of the atherosclerosis.

Atherosclerosis, is no doubt to be a cholesterol deposition disease, but the recent evidences show that the involvement of the immune system and chronicity in inflammation gives rise to the progression of atherosclerotic lesions, Ridker (2009),which is related to the M phenotype which are the specific source of proinflammatory mediators that evoke the supplementary macrophages together with immune cells hence continuously progresses the inflammation of plaque Ebadi,(2020).

Hyperglycemia and Atherosclerosis

For the growth of atherosclerosis, presence of glucose in the environment may play a vital role for the atherosclerosis, hence diabetes severely is responsible for atherosclerosis. Hyperglycemia related to Type 1, or Type 2of diabetes is a strong and specific risk factor for atherosclerosis and cardiovascular disease. Borlivic, (2011).

Atherosclerosis main trigger cause is Hypercholesterolemia, the unwanted level of increased plasma cholesterol levels leads to disturbance in the impermeability of arterial endothelial cells that allows the migration of lipids, specifically LDL-C particles, inside the arterial wall. This ultimately causes sticking of monocytes to the endothelial cells that leads to adhesion of IL-4, IL-13

and irritates an alternative anti-inflammatory response and reparatory M2 phenotype.

2.2. *Carica papaya*

Carica papaya is one of the richest source of vitamins, antioxidants and many other nutritional components and every part or plant is important an d has medicinal value in it. Papaya plant is also refferedas a "tree of health" and its fruit is termed as a "fruit of long life". The leaves, stem, and unripe fruit of papaya release a whitish milky fluid (latex) that consists of proteins, alkaloids (mainly carpaine), starches, sugars, oils, tannins, resins, pectins and gums, which coagulate on exposure to air. Unripe papaya is rich in papain and chymopain.

The seeds inside are edible and have a sharp, spicy taste. The ground seeds are sometimes used as a substitute for black pepper. In papaya plant such as roots, leaves, peel, latex, flower, fruit and seeds have their nutritional and medicinal significance. Papaya can be used as a food, a cooking aid, and in medicine. Parle (2011).

Medicinal values

Topping, (1980) reported that the main activity is of saponin which lowers the serum cholesterol level by causing resin like action, due to reduction of the enterohepatic circulation of bile acids.

Brocklehurst *et al*., (1985) have shown that *Carica papaya* plant is having medicinal value and is best for consumption for human and animals and contains phytochemicals like, alkaloid, carpain, nicotine, tanins, and terpinins and also enzymes like papain and chymopapain which are very much effective in hypolipidemia.

Adeneyea, (2009) reported the alkaloids, flavonoids, saponins, anthraquinones, elicit wide range of biological activities in hypolipidemia.

Giove and Nakazawa, (1996), stated that papaya seed extract effectively cures the cardiovascular problems and also have analgesic properties and also have antioxidant properties

Luximon *et al;* (2003) have shown papaya as the top-ranking fruit in terms of its carotenoids, flavonoids, fiber, vitamin A, ascorbic acid, folate, niacin, thiamine, riboflavin, iron, calcium, and fiber contents per serving (USDA, National Nutrient Database for Standard Reference, 2006.

Ali *et al*; (2011) the nutrients in papaya helps to prevent oxidation of cholesterol, hence oxidized cholesterol sticks to the internal lining of blood vessels, which forms dangerous plaques that eventually causes heart attacks or strokes. Results from various studies state that dietary vitamins E and vitamin C preventing the oxidation of cholesterol because of their suggested association with paraxonase, enzyme that inhibits the oxidation of LDL and HDL cholesterol.

Anandhi *et al* (2013) studied that regulation of cholesterol and its homeostasis, intestinal absorption plays a very vital role.

Many medicinal plants are available in the world which are used as the antihyperlipidemic, antioxidant and antiatherosclerosis nutraceutical agent. Large number of medicinal plants are studied for these properties which are present in them by many scientific worker (Joshi *et al*., 2016 & 2017: Abdalla and Abdelgadir 2016, Zhao and Ma.,2016)

2.2.3 Active principles present in *Carica papaya* responsible for antihyperlipidemic activity.

Trease and Evans (1983) mentioned that the presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars in the extract were tested for using simple and standard qualitative methods.

Brocklehurst (1985) reported papaya is also a good source of carpaine. It is one of the major alkaloid components of papaya leaves that have been studied for its cardiovascular effects in male Wistar rats. Increasing dosages of carpaine from 0.5 mg/kg to 2.0 mg/kg resulted in progressive decrease in systolic, diastolic, and mean arterial blood pressure.

Misra (1996) reported that the hexane fraction of fruits (collected from India) exhibited activity against Klebsiella sp. 5-Nonatetracontanone, 2-

hentriacontanone, triacontane, 16-hentriacontanone and beta-sitosterol were isolated from the hexane fraction.

Bennett *et al* (1997) reported that because of its high phytochemical contents, it shows significant antioxidant activities and also studied that papaya seeds represent a rich source of biologically active isothiocyanates and the n-hexane extract of papaya seeds homogenate which was highly effective in inhibiting the superoxide generation and apoptosis induction in HL-60 cells, the activities of which are comparable to those of authentic benzyl isothiocyanate. In contrast, the papaya pulp contained an undetectable amount of bezyl-glucosinolate. They showed that papaya seeds and not the papaya pulp is a rich source of biologically active isothiocyanate, especially the BITC and its precursor glucosinolate, which are as high as those in Brassica vegetables. BITC is formed from benzyl glucosinolate in papaya seeds.

Nguyen and Schwartz, (1999) have shown that papaya also contains many bioactive phytochemicals with diverse structure and functional properties which have not yet been fully exploited for their potential health benefits. It contains substantial amounts of carotenoids, flavonoids and polyphenols. It contains relatively high levels of beta-carotene, which the body converts to vitamin A. Papaya contains about 6% of the level of beta carotene found in carrots (USDA, National Nutrient Database for Standard Reference, 2006). Red flesh papaya has been reported to contain significant quantities (4.1 mg/100g flesh) of lycopene.

Maria *et al*; (2006) stated that the papaya lipase is currently considered as a “naturally immobilized” biocatalyse.

Ezike *et al*; (2009) studied that the extracts of unripe papaya have been reported to contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides and steroids.

Ali (2011) concluded that carpaine directly affects the myocardium. The effects of carpaine may be related to its macrocyclic dilactone structure, a possible cation chelating structure.

2.2.4 Hypolipidemic effect and properties of *Carica papaya*

Historical use of the dry seeds of *Carica papaya* is the traditional management of the suspected patients of obese and diabetic patients by Yoruba herbalists (South – West Nigeria),

In this view the study is designed to evaluate the hypolipidemic and cardioprotective potentials of the seed extracts in the normal Wistar rats.

The pattern of weight gain decreases with dose of the extract in treatment. This extract can therefore be used in dyslipidemia and obesity alike. It is well established that there is a strong link between diabetes mellitus, dyslipidemia, obesity, hypertension and ischemic heart disease. (Modan *et al* 1985; NCEP, 1990. Previous human studies have shown papaya to slow down the heartbeat, and reduce blood pressure. (Gupta *et al* 2001).

The hypolipidemic effect of *Carica papaya* could be related to its chemical composition, which shows the presence of alkaloids, flavonoids, saponin and cardiac glycosides.

All these components are known to reduce serum lipid level in animals, the reduction in the serum total cholesterol levels following the administration of the extract may be attributed to reduction in the concentration of acetyl CoA resulting from decreased β-oxidation of fatty acids since acetyl CoA is a key substrate in the biosynthesis of cholestesterol. The reduction observed in the serum triglyceride level can be adduced to inhibition of lipolysis. It can also be attributed to the antioxidant activities of saponins which might have interfered with the oxidation of fatty acid, the significant reduction in the LDL is understandable since a reduction in total cholesterol should normally result in reduction of LDL. The increase in high density lipoprotein following the administration of aqueous extract of *Carica papaya* can be clinically beneficial. It has been demonstrated that an increase in the concentration of HDL correlates inversely with coronary heart disease. This is because HDL removes cellular cholesterol and transports it to the liver where it is converted to bile acids and eventually excreted from the body, and as such will reduce the risk of coronary artery disease.

The study revealed that Aqueous extract of *Carica papaya* seeds at doses given has shown its effects on hypolipidemic, cardioprotective parameters in normal male Wistar rats for 30 days.

2.2.5 Properties of *Carica papaya*

Papaya also be called as powerhouse of nutrients. Its properties are as follows :

It is rich source of vitamin C, A and E the potent antioxidants, the minerals (magnesium, potassium), vitamin B, pantathonic acid, folic acid and fibres. Papaya also contains the digestive enzyme papain that heals the traumas, allergies and injuries. All the nutrients and minerals present in the papaya are responsible for the improvement of overall health of cardiovascular system and protect the heart from the disease like heart attack, strokes and prevent the colon cancer and also the fruit is rich source of beta carotene, that prevents damaging of free radicals that may lead to some types of cancers. It also helps in the curing the heart disease caused due to diabetes.

Papaya lowers the high cholesterol levels as it is an good source of fiber. The plant *C. papaya* possess potent medicinal activities like antioxidant, anti-hypertensive, wound healing, hepatoprotective, anti-inflamatory, antimicrobial, antifungal, anti-fertility, histaminergic, diuretic, anti amoebic, anti-tumor, anthelmintic, effect on smooth muscles, antimalarial, hypoglycemic activity, immunomodulatory activity, anti-ulcer activity, anti-sickling activity. (Aravind *et al* 2013).

2.2.5.1. Antioxidant activity

The methanolic extract of *C. papaya* was studied *in vivo* for its potential effect on the activities of some antioxidant enzymes which contains glutathione peroxidase, glutathione transferase (GST), glutathione reductase, catalase and glucose-6-phosphate dehydrogenase in mice treated with oral dose of 100 mg/kg. There was statistical significant increase in the activities of glutathione reductase, GST, glucose-6-phosphate dehydrogenase due to the ethyl acetate fraction.

Significant decrease in GPx was observed in kidney following administration of ethyl acetate fraction. (Oloyede, 2005)

2.2.5.2 Anti-hypertensive activity

The extract of ripe fruit of *C. papaya* was used for the anti-hypertensive activity, the basal mean arterial blood pressure in the normotensive, renal and DOCA-salt hypertensive animals. Both hydralazine and ethanolic extract of unripe fruit of *C. papaya* produced statistical significant depression of MAP in normotensive, renal and DOCA-salt hypertensive animals groups as compared to control. The study resulted that the unripe fruit of *C. papaya* had a potent anti-hypertensive activity. (Eno *et al* 2000).

2.2.5.3 Wound healing activity

The aqueous extract of *C. papaya* fruit for the wound healing property in streptozotocin-induced diabetic rats using excision and dead space wound models. The aqueous extract has shown the 77% reduction in the wound area when compared to 59% contraction to wound of the controls. Thus the result estimated that the aqueous extract of C. papaya had a potent wound healing property. (Nayak *et al* 2007).

2.2.5.4. Hepatoprotective activity

The aqueous and ethanol extract of dried fruit of C. papaya for its hepatoprotective activity in rats against CCl4induced hepatotoxicity in rats has shown the significant effect extracts of *C. papaya* as an hepatoprotective agent by lowering the biochemical parameters such as SGPT, SGOT, serum bilirubin, akaline phosphatase (Sadeque and Begum, 2010).

2.2.5.5 Anti-sickling activity

The methanolic extract of *C. papaya* in vitro has efficiently reduced hemolysis and protected erythrocyte membrane integrity under osmotic stress conditions. (Imaga *et al* 2009).

2.2.5.6 Administration dependent antioxidant effect

Seeds are the important content in the fruit in this study. It has estimated that the antioxidant activities of the *C. papaya* seeds water extract against hydrogen peroxide (H2O2) oxidative stress in human skin Detroit 550 fibroblasts. *C. papaya* seeds water extract is not toxic and acts as a potent free radical scavenger, providing protection to Detroit 550 fibroblasts that underwent H2O2 oxidative stress. The more protective effect is achieved by the administration of the extract. The extract in presence of an oxidative stress does not increase catalase activity and prevents the release of cytochrome C and the inner mitochondrial transmembrane potential loss. The extract is more efficient than vitamin C to hamper the oxidative damage, the purified extract of the seeds water extract exert the same antioxidant effect of whole extract. It has interpreted that *Carica papaya* seeds water extract is potentially useful for protection against oxidative sress,(Panzarini *et al* 2014).

2.2.6 Mechanism of anti-hyperlipidemic activity of *Carica papaya* seeds extract

Several studies have reported that the use of seed extract of *Carica papaya* can be a tonic for the heart, an analgesic and treatment for stomach ache (Giove and Nakazawa, *et al* 1996) and contains huge amount of rich antioxidant properties (Rahmat *et al* 2004), that has ultimately beneficial to the efficient protection against the oxidative stress, to the body tissues which have very effective role in hyperlipidemia (Indran *et al* 2008).

All the antioxidant systems, minerals, vitamins present in *Carica papaya* play a protective role opposite to the production of reactive oxygen species and lipid peroxidation by-products. Some recent studies have shown to have an implicated Reactive Oxygen Species (ROS) in the pathogenesis of many human diseases.

2.3 Statins as standard drug

Cardiovascular diseases (CVD) are the most now a days prevalent cause of death and disability in both developed as well as developing countries. Lipid imbalances may also result lead to high saturated fat/cholesterol diet, obesity,

caloric excess, stress and subclinical hypothyroidism interacting with a predisposing genetic factor or susceptibility gene (multifactorial hypercholesterolemia).

Studies and various trials have demonstrated that the statin therapy statistically and significantly potently effective in lowering the lipid levels, and the incidences in the coronary events and lowering the cholesterol levels, (ALLHAT, 2002).

Mevastatin was the very first HMG-Co A reductase inhibitor and was derived from Penicillumcitrinum. Other statins like Simvastatin, Lovastatin and Pravastatin are also fungal derivatives, while Atorvastatin, Cerivastatin, Fluvastatin, Pitavastatin and Rosuvastatin are the synthetic substances. (Wierzbicki 2003).

Use of statins is the most effective treatment for curing the increased in LDL-C levels in the patients.

2.3.1 Mechanism of action of Statins

Statins mainly blocks the HMG-CoA reductase enzyme, which ultimately lowers the cholesterol synthesis. All statins present are the competitive inhibitors of HMG-Co A reductase. Statins are easily metabolized by the cytochrome P450 (CYP450) enzyme, (Bottorff and Hansten 2000).

The main metabolite product after metabolizing the atorvastatin is, 2-hydroxy- and 4-hydroxy-atorvastatin acid, explained by Jacobsen, *et al.,* 2002).

2.3.2 Adverse effects of the statin

Normally, statins are well tolerated by everyone but some adverse effects are reported. The statin has shown some of the serious adverse effects like myopathy, which may lead to progressive to fatal or non fatal rhhabdomyolysis.

Some studies have revealed that statins also induces thrombocytopenia. (Groneberg *et al*., 2001).

Puberty phase have restricted their use in children during the prepubertal stage. Further, fat-soluble vitamins are taken by lipoproteins, their reduction by

statins has been suspected to lead to vitamin deficiencies. (Arambepola *et al*., 2007).

The study also revealed that atorvastatin may lead to pancreatitis (Prajapati *et al*., 2010)

2.4 Medicinal plants having antihyperlipidemic activity.

Extra production of reactive oxygen species (ROS) and other radicals have been implicated as for induction in tissue injury in various pathological conditions such as inflammation (Choi 2005) and other condition related to this is atherosclerosis, the lowering in the tissue antioxidant enzyme activity that may lead to the increase in lipid peroxidation.

The various number of traditional plants have studied in the literature, but there has been some scientific effort to explain and validate their effects. Over some the last few years many research studies have focused on plants with therapeutic properties. One of the most effective and recent approaches to disease prevention involves the use of specified nutrients and vitamins to protect tissue against toxic injury and degenerative diseases (Pryor 1991). Atherosclerosis is the condition caused not only by the deposition of cholesterol but also by inflammation. Polyunsaturated fatty acids of the membrane are peroxidized by free radical-mediated reactions and lipid peroxidation is the autocatalytic process, which is the consequence of cell death. The biological effects of ROS are controlled *in vivo* by a wide spectrum of enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (vitamins A, C, E and reduced glutathione) defence mechanisms.

Anti inflammatory drugs treating arthritis may also simultaneously lower the risk of heart disease by keeping the arteries limber, which means that inflammation plays an important role in heart disease.

Chenevard *et al.,* (2003) reported that the cyclooxygenase-2 (COX-2) inhibition is effective in improvement of endothelium-dependent vasodilation and lowers low-grade chronic inflammation and oxidative stress in coronary

artery disease, recommended that selective COX-2 inhibition has the potentially effective in the protective outcome in patients with cardiovascular disease.

This is due to the antioxidant nutrients such as vitamin E, vitamin C, carotenoids and the various number of the polyphenolic compounds directly acts on reactive oxidants, they are hypothesized to constitute a vital endogenous defence against oxidative cell and tissue injury caused by toxic and carcinogenic chemicals. Pharmacological modulations, including lipid lowering and antioxidant agents have been shown to improve endothelial function in humans.

There is rapid growing interest in the protective effects and biochemical function of natural antioxidants included in dietary plants. Phytochemical evidently play an important role as dietary antioxidants for the inhibition of oxidative damage caused by active oxygen radicals in living systems.

However, they can be safe and be used as curative drugs for some of the oxidative damage inducing diseases. Some of the studies are reported that tradional use of the various medicinal plants against the oxidative stress and changes associated with oxidative tissue injuries, has shown good effects. Some of the plants like, (*Piper cubeba, Physalis angulata and Rosa hybrida*) are studied on the basis of the preventive action on the nitrite deposition of macrophages and antioxidant actions *in vitro*.

In this, the antioxidant and lipid-lowering effects of these plant extracts were examined *in vivo*.

2.5 High fat diet:

Hyperlipidemia was progressively induce in this project by feeding high fat diet to the animals the diet content was diet (40%) fat / cholesterol (5%0) for two months in rats. The high-fat diet constituted was cholic acid (0.35%) to enhance the enteral absorption of lipids. The occurrence of hyperlipidemia was determined by measuring lipid profile (total cholesterol, triglycerides and HDL). The hyperlipidemic animals were only used. (Gröne , *et al;* 1989).

HFD is a major risk which can lead to disease condition including cardiovascular disease, and other metabolic disorders. a significant risk factor for

many disease states, including cardiovascular, and metabolic disorder and this has lead to weight gain. (Buettner *et al*.,2007; Panchal *et al*.,2012).

The high fat diet significantly has increased the weight gain in the experimental animal which has lead to rise in their lipid profiles. The hypercholesterolemic diet (HD) included 2% cholesterol, 0.6% sodium deoxycholate added to grinded ND has successfully induced atherosclerosis and weight gaining in lab animals in between 2-3 weeks of time period. (Kamesh, *et al*., 2012).

2.6 Effect of high fat diet on Blood and biochemical parameters

Mayes (1996), reported that the because of high fat diet there may be decrease in the blood parameters like Hb, TEC and increase in cholesterol and other lipid parameters.

Susiji and Roshitha (2013), gave the report that the *Carica papaya* is helpful in the increase in blood parameters.

2.7 Effect of high fat diet and aqueous extract of papaya seeds on biochemical and lipid profile.

All these components are known to reduce serum lipid level in animals, the reduction in the serum total cholesterol levels following the administration of the extract may be attributed to reduction in the concentration of acetyl CoA resulting from decreased β-oxidation of fatty acids since acetyl CoA is a key substrate in the biosynthesis of cholesterol. The reduction observed in the serum triglyceride level can be adduced to inhibition of lipolysis. It can also be attributed to the antioxidant activities of saponins which might have interfered with the oxidation of fatty acid, the significant reduction in the LDL is understandable since a reduction in total cholesterol should normally result in reduction of LDL. The increase in high density lipoprotein following the administration of aqueous extract of *Carica papaya* can be clinically beneficial. It has been demonstrated that an increase in the concentration of HDL correlates inversely with coronary heart disease. This is because HDL removes cellular cholesterol and transports it to the liver where it is converted to bile acids and eventually excreted from the body, and as such will reduce the risk of coronary artery disease.

Serum cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), LDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), BUN and Creatinine were evaluated. And has shown the desirable effect on the parameters before and after the treatments given groups group III and group IV. (Kim *et al*.,2012)

2.7 Effect on histopathology:

The study revealed that, oral administration of graded doses of *Carica papaya* seeds over a time period of dosing there can be evidently seen the difference and improvement in liver and kidneys histopathology. The fatty degeneration and fat cells size accumulated can be seen decrease in size after treatment. (udoh 2005).

Materials and Methods

CHAPTER - III

MATERIAL AND METHODS

This experimental study was planned to estimate the active potential of the aqueous seeds extract of *Carica papaya* in the hyperlipidemia. As it has been proven for its hypolipidemic effect and tonic properties for heart. This study have been carried out on the Wistar rats which were fed on high fat diet by the intention of inducing atherosclerosis and then the curable effect of *Carica papaya* seeds extract effect was evaluated by examining the Heamatological parameters, and Biochemical analysis of the serum samples and for the comparison the standard group was also included which has been treated with the Atorvastatin which is also effective in the Atherosclerosis.

Materials and chemicals

The materials and chemicals required for the evaluation of the study was *Carica papaya* seeds extract, distilled water, gauze, syringe and atorvastatin.

The standard drug atorvastatin was used in the experiment for the comparison and evaluation of the effects of papaya seeds extract and the standard drug.

3.1*Carica papaya* seeds extract

Table 1 :**Taxonomyof *Carica papaya* :**

1	**Kingdom**	Plantae
2	**Subkingdom**	Tracheobionta
3	**Super Division**	Spermatophyta
4	**Division**	Magnoliophyta
5	**Class**	Magnoliopsida
6	**Sub Class**	Dilleniidae
7	**Order**	Violales
8	**Family**	Caricaceae
9	**Genus**	Carica
10	**Species**	*Carica papaya*

3.1.2 Botanical description

Parle M, Gurditta *et al*,(2011)

Table 2: Indian synonyms

Sr.no	Languege	Vernacular name
1.	Bengoli	Papaya, Pepe, Papita
2.	Punjabi	Papita
3.	English	Papaya
4.	French	Papayer
5.	German	Kaneel
6.	Gujrati	PapaiyaPhala
7.	Hindi	Papaya, Papita
8.	Kannada	Papaihannu
9.	Malayalam	Omakai
10	Marathi	Papai
11.	Oriya	Amritabhanda
15	Tamil	Pappali
16.	Telugu	Boppaipamdu

Plant and stem :*Carica papaya* is having a stem which is observed to be cylindrical and hollow in shape and it is reffered herb which is tree like which usually grows 2-10 meters in height.

Leaves: Leaves of this plant is outward and projected downwards and are assymetrical finger like and consists of the lobes which are 5-7 in numbers.

Flowers : The flowers are trimorphous in nature, and normally unisexual dioecious, and the male flowers bloom in together with many in bunch and are hairy. Female flowers are large, solitary.

Fruits : The fruit is a huge berry elongated circular in shape and varies in size from elongate to globe like with large central large berry elongated round shaped, varying widely in size.

Seeds : Seeds are tuberculous and black, with a clear aril around them.

Plate 1 : *Carica papaya* seeds from fruit

Annegowda 2016, gave the above botanical description of the plant.

3.1.3 Phytochemical analysis of *Carica papaya* seeds extract

The present study was carried out for the detection of the antihyperlipidemic efficacy of the *Carica papaya seeds extract in rat*s. The active constituents present in the seeds extract were detected by carry out some chemical tests which helped to know the Anti-hyperlipidemic activity of the *Carica papaya* seeds extract having chemical constituent. These have saponins, tannins, flavonols, glycosides, terpenoids, fats, alkaloids, reducing sugars, steroids, proteins, and polyphenols.

In 2016, Annegowda, reported the chemical composition of C. papaya to include; alkaloid, αcarpaine, β-D-glucosides, β-sitosterol, papain, choline, carotene, riboflavin, vitamin C, phenyethyl- β- D- glucosides, amongst others.

3.1.4 Phytochemical constituents of *C. papaya* seeds aqueous extract

Table: 3 Phytochemical constituents of *C. papaya* seeds aqueous extract

Phytochemical constituent	Level
Saponins	+
Tannins	+++
Flavonols	++
Glycosides	+
Terpenoids	++
Alkaloids	+++
Reducing sugars	+
Amino acids	++
Fats	+
Proteins	++
Phenols	+++
Vitamins	+++
Sterols and Triterpenes	++

Key: +++ abundant, ++moderate, +trace

Papaya is also a rich source of carpaine. It is the most important alkaloid components of papaya that have been for its cardiovascular effects in male Wistar rats. Dosages of carpaine on the regular basis can give an excellent and progressive decrease in systolic, diastolic, and mean arterial blood pressure, which can ultimately decrease and improve the lipid profile in the atherosclosis It was studied that carpaine directly affects the myocardium. The effects of carpaine may be related to its macrocyclic dilactone structure, a possible cation chelating structure (Ali 2011). The extracts of papaya have been concluded that it contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides and steroids (Ezike *et al.,*2009). The papaya lipase is currently considered as a "naturally immobilized" biocatalyst (Maria *et al*.,2006).

3.1.4 Toxicity and adverse effects of the *Carica papaya*

Generally *Carica papaya* plant does not really have any kind of the side effect or adverse effects on the animal, but when taken in excessive amounts, the papaya also have some other anti-nutrient compounds such as phytate, oxalate and tannins. The levels of these anti-nutrients (phytate, oxalate, hydrolysable tannins and condensed tannins) and antioxidants (vitamin C, tocopherols, total phenols, and carotenoids) contents of papaya can decrease with the storage.

The World Health Organization (WHO) Global Report on Traditional and Complementary Medicine (2019) reported that the safety of the herbal medicine is also often required to be examined and estimated thoroughly, for the further use. The herbal medicines for traditional use are also subjected to thorough assessment of the specific regulatory requirements based on the verification of recorded scientific research. In general, the toxicity of herbs is broadly categorised as intrinsic or extrinsic. Intrinsic toxicity leads to adverse reactions associated with the pharmacological nature and bioactive phytochemicals of herbs, while extrinsic toxicity refers to impurities and potential toxicants introduced externally through agricultural practices.

There is an evidence that excessive dosage of *Carica papaya* seeds aqueous extract may lead to liver damage and also kidneys damage,

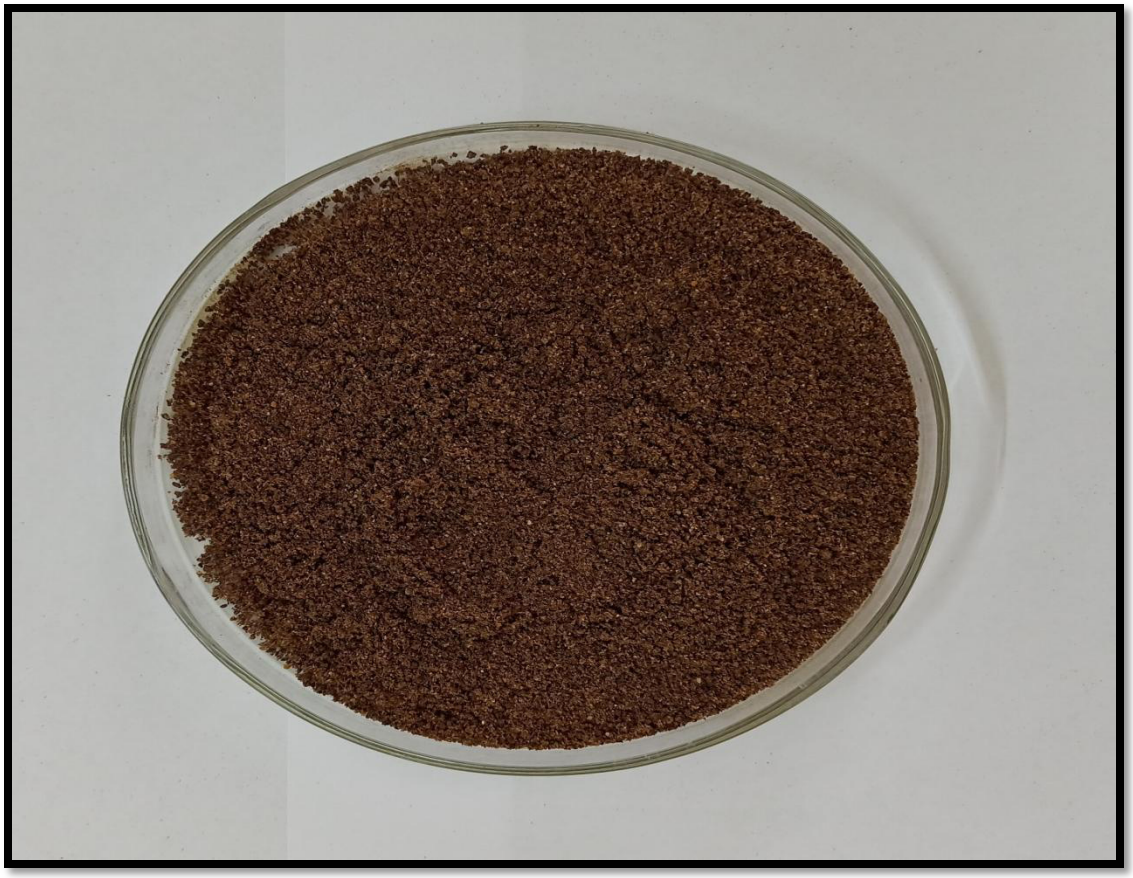

Plate 2 : Dried seeds powder

Plate 3. Extracted powder seeds

Plate 4. Blood collection and handling of animals

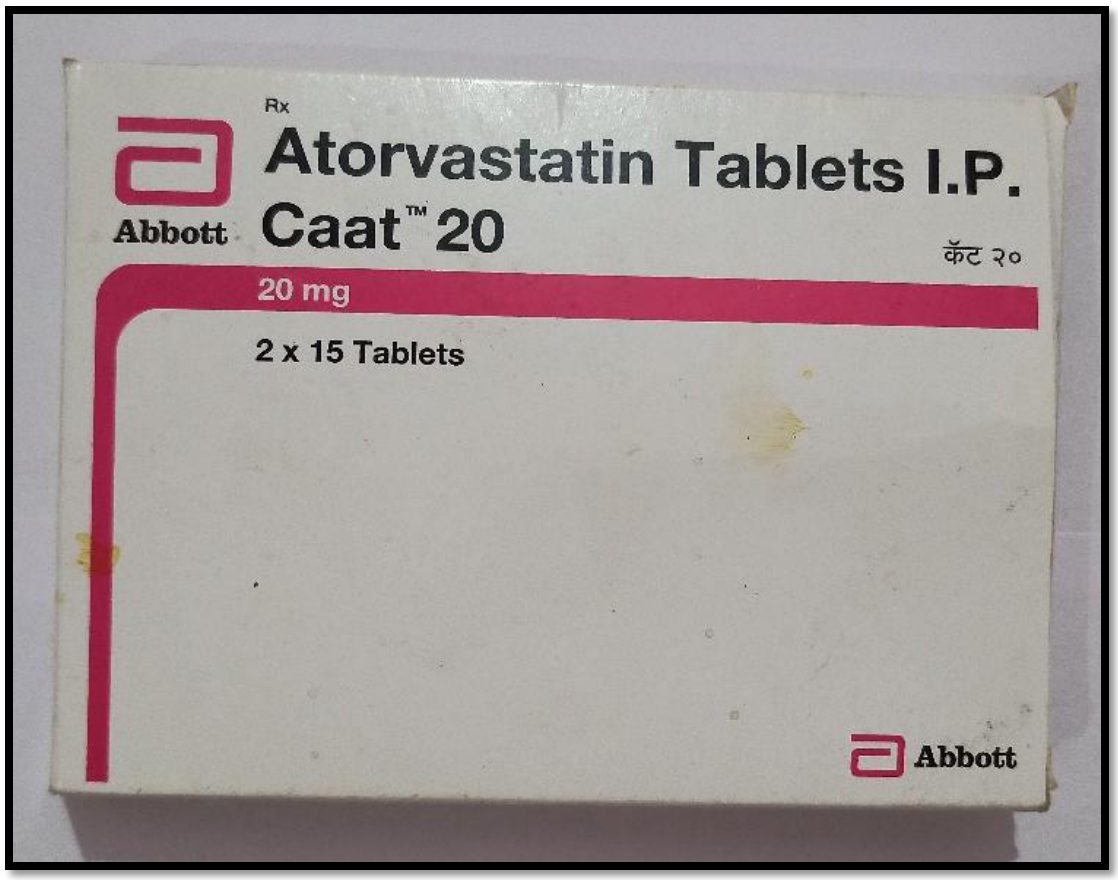

Plate 5. Atorvastatin

gastrointestinal disturbances were also reported, some showed the rashes on skin associated with the allergic reactions.

3.2 Processing of plant material.

3.2 .1 Collection and identification of plant material

The papaya fruit was collected from the local market of Parbhani, the fruit was washed cut in two parts and the seeds were collected in petri dish and also the seeds were washed and dried at room temperature for 8 days.

The seeds were then grinded in the grinder and the powder was soaked in the distilled water overnight, next day the soaked seeds were boiled with the water and then with the help of muslin cloth it was filtered and the filterate was again dried upto the total moisture evaporates for 5 days, then the extract dried extract was collected. (Olagunju *et al*.,1995).

3.2.2 Organoleptic Character of *Carica papaya* seeds

Table 4: Organoleptic Character of *Carica papaya* seeds

Sr.no	Variables	Result
1)	Colour of Seed	Blackish brown
2)	Dried seed colour	Brown
3)	Powdered seed	Brown
4)	Taste	Multivitamin powder

3.2.2 Atorvastatin

Atorvastatin was used as standard oral hypolipidemic drug

Compound name : Caat -20

Chemical name : Atorvastatin calcium

Mfg. Lic. No. : 23/UA/2007

Company : Abott Healthcare Pvt. Limited

Physical appearance : Tablet

Storage condition : Stored at cool and dry place

3.2.3 Preparation hot aqueos extract of *Carica papaya*

3.2.4 Collection and storage of Extract.

At the last fine brownish coloured powder was obtained after the evaporation of the liquid portion of the extract which separated from the petri plate with the help of spatula and collected in the dispensing bottles for the storage at 25 °C, and refrigerated till use for the oral dosing.

3.2.5 Phyto profile and Yield of Extract

Table 5 Phyto profile and Yield of Extract

Plant material (gm)	Solvent (ml)	Total yield	Extractability (%)	Consistancy	Nature
10gm	100ml	3-5%	3-5%	Sticky	Amorphous

3.3 Experimental animals

The Wistar rats were chosen for the experimental purpose for this study as they are very much useful lab animals in case of the hyperlipidemia and atherogenic studies. The animals were observed for change in bodyweight, behavioral changes, haematological and biochemical parameters after inducing hyperlipidemia and treatment.

The experimental animals employed in present study were procured from CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals), authorized breeder Dhule after got approval of the experimental protocol by IAEC (Institutional Animal Ethical Committee). The Present Investigation was Carried on the 32 Wistar rats. The Animals were selected After the Physical and Behavioral Examination the range of 200-300gm of their body weight. All animals were kept in the cages provided by the Department of Pharmacology and toxicology, COVAS, Parbhani. Institutional Animal Ethical committee had approved the protocol of the experiment which fulfil the guidelines as per the guidelines of CPCSEA (Committee for the purpose of

Control and Supervision of Experimental Animals) with the resolution number **2/20** on dated **10.1.2020**. All the Animals were kept in the observation of About **15 days** before the commencement of the Experimental tasks. All required precautions and preventive measure were followed to keep animals free from stress and Diseases.

3.3.1 Location

The present studies were carried out at the small animal laboratory house, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani. MAFSU, Nagpur, Maharashtra.

3.3.2 Animal housing

All lab animals were housed in small animal lab house in the room no.1 and 2 as per the norms of CPCSEA of the College of Veterinary and animal sciences, Parbhani, Maharashtra. The animals were kept in the cages as per the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) norms. The cages were changed every three alternate days in a week throughout the experimental period.

3.3.3 Husbandry

Table: 6 Husbandry

Housing	Cage system, four Animals in Single Cage of Same Sex
Temperature	22±3 °C.
Relative humidity	30-70%
Photoperiod	12 hours light and 12 hours dark period.
Light intensity	120-400 Lux
Food	Pelleted diet high fat for all animals except the Positive Control Which are fed on Natural diet.
Water	Clean and safe Drinking water for the Animals is provided

3.3.4 Environmental conditions

All the Wistar rats were kept in the environmental controlled system in which the temperature of the lab animal house was kept at 22 $\pm$ 3°C and relative humidity of 30-70 % of environmental condition.

All animals were kept in the lab animal house for the 12/12 hr. light and dark condition throughout the experiment from the acclimatization up to the sacrifice.

The floor and walls of the room were cleaned, mobbed and swept with proper disinfectant available in the lab animal house every day.

3.3.5 Acclimatization of the Animals

Acclimatization of the animals was carried out for the 20 days i.e. 10 days resting period from their arrival and 10 days after grouping according to their body weight, prior to the commencement of the experiment at ideal lab environmental condition. They were provided with the natural diet along with electrolyte powder for the rehydration of the animals. The animals were provided with the safe drinking water ad-libitum for the drinking purposes.

3.3.6 Drinking water and diet

During the acclimatization all animals were provided the natural diet as per the requirement of the animals. From the initiation of the experiment positive control group animals were kept on the natural diet and remaining all animal groups were kept on the high Fatty diet till the End of the Experiment. All animals were provided the filtered reverse osmosis water for the drinking through the experiment.

3.3.7 Grouping of animals.

Animal Grouping:

The experimental animals (32 Wistar rats) were divided into 4 different groups. Each group had 8 rats, (4 male and 4 female). Wistar rats as detailed as below.

1) Group I: (Positive Control)

This group was comprised of 4 males and 4 female Wistar rats, which were maintained on conventional laboratory animal diet, as per (NRC 1994) and (Hammond 1996). All the animals were provided with pure and safe drinking water *ad-libitum*.

2) Group II (Negative Control)

This group was comprised of 4 males and 4 female Wistar rats, which were maintained on high fat diet, as per the daily nutritional requirement of the animals and were provided with pure and safe drinking water *ad-libitum,* no treatment was given to this group.

3) Group III (Standard treatment)

This group was comprised of 4 males and 4 female Wistar rats, which were maintained on high fatty diet, as per the daily nutritional requirement of the animal, and were treated with standard hypolipidemic compound statin from day 29^{th} of experiment. All the animals were provided with pure and safe drinking water *ad-libitum.*

4) Group IV (*Carica papaya* seeds aqueous Extract Treatment)

This group was comprised of 4 male and 4 female Wistar rats, which were maintained on high fat diet, as per the daily nutritional requirement of the animal, and were treated with the aqueous extract of seeds of *Carica papaya*. All the animals were provided with pure and safe drinking water *ad-libitum.*

3.3.8 Animal Identification and Numbering

All the animals were separated by the individual body weight and sex in each group four animals were kept of same sex, they were identified with proper marking, cage no, group details, body marking and type of treatment given to animals according to the specific group of animals like head, back, tail and unmarked like markings were given to animals with the help of Picric acid solution.

3.3.9 Feeding schedules

All the animals of experimental studies were given the feed at scheduled time of feeding i.e; at morning 8 A.M and at evening 6 P.M as per their required amount of feed during the studies was divided into twice a day.

All the experimental rats, except in group I were given standard pelleted high fat diet *ad-libitum* throughout the experimental period.

3.3.10 Induction of the Atherosclerosis and hyperlipidemia

The animals other than the positive control group were given the HFD feed, after the completion of their acclimatization period. They were maintained on the High fat diet at the rate of 10% of their body weight or the minimum 15-20 gm for daily basis to produce the atherosclerosis in their body the animals were constantly monitored for their behavioral changes and eating habits during the induction of the atherosclerosis. The weight gain of animals was observed for the examination of their health status and their atherosclerosis development.

3.3.10.1 Composition of experimental diets in different periods of the experiment

The high fat diet consisted of 1% cholesterol.

3.3.11. Handling and Restraining

Handling of animals was done during the routine procedures like grouping, sexing, weighing, blood collection, sacrifice like activities.

For restraining of animals during crucial procedures like blood collection all animals were given chloroform as gaseous anaesthetic, the animals were kept in desicator with the cotton sprinkled with chloroform to avoid any physical injury during the procedure.

3.3.12. Dosing and blood collection

The animals of Group I and II were left without treatment and without dosing.

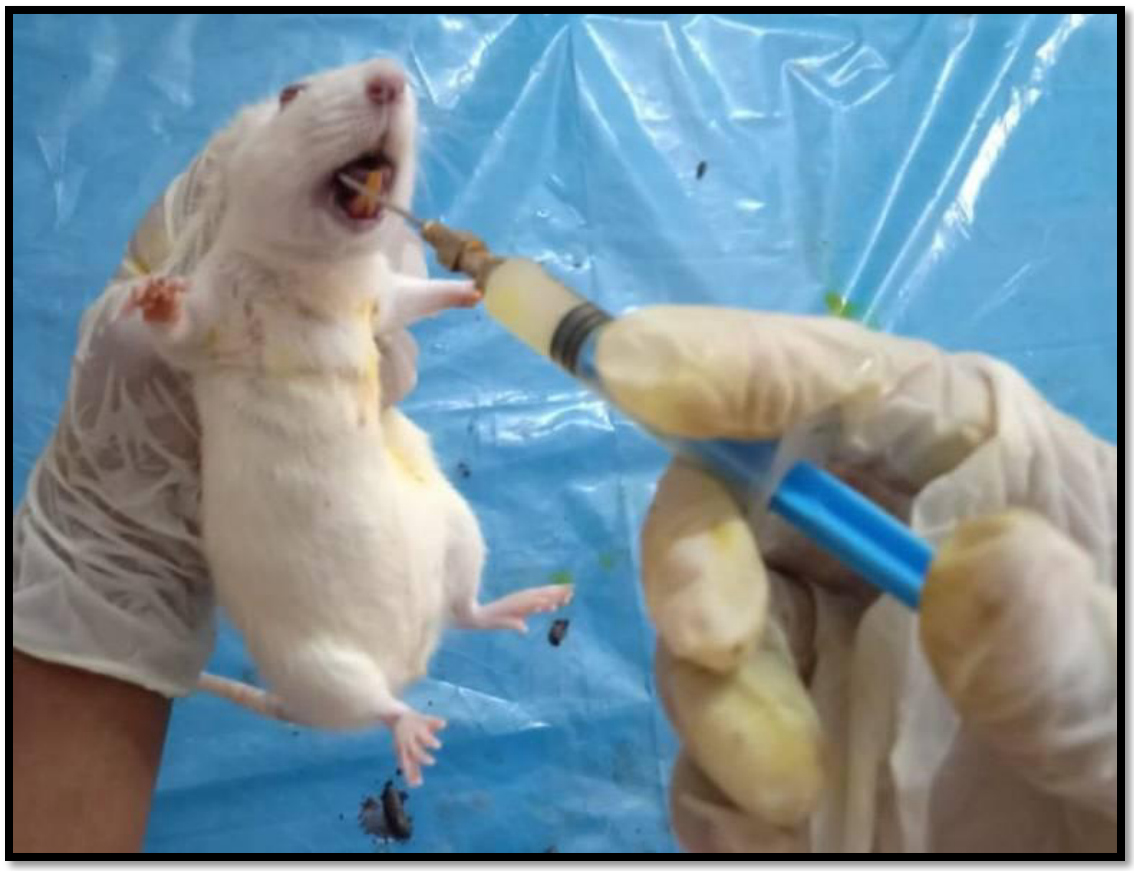

Plate 6. Dosing

The animals of Group III and IV were given the standard statin and *Carica papaya* seeds extract as a treatment drug via oral dosing in the distilled water, respectively.

The blood collection of animals was done from the retro orbital plexus of eye in all animals.

3.3.13. Selection of vehicle for the dosing

The vehicle used for the dosing of standard and test drug was selected by trial and error method which occurs due to some reasons as follows

1. Distilled water was used as vehicle for proper mixing of the standard drug statin so that is easily administered via oral route to the rats through gauze.
2. Distilled water was used as a vehicle again for the proper solubility with the powdered *Carica papaya* seeds extract and administered via oral route through the gauze.

3.3.13 DOSE DETERMINATION

The determination of the dose of standard and treatment were considered according to the previous cited literature available on the experimental data of the *Carica papaya* seeds extract. The Statin grouped drug i.e. Atorvastatin was used for the standard group treatment as per the guidelines and doses which are determined for its Procedure. The dose of Atorvastatin is determined by taking reference of 0.5 mg/kg BW (Mohammad 2014) is given to the animals by the oral dosing with the help of gauge.

The dose of *Carica papaya* seeds extract was decided by the taking range between 60mg/kg of the body weight with references of the previous studies carried out on this dose.

Distilled water was used as the vehicle for the dosing of the animals at the proportion of volume by adjusting the 1ml of dose with appropriate concentration of standard and treatment drug for the animals.

3.3.14. Anaesthesia / Euthanasia.

Rats were anesthetized by using diethyl ether during blood collection on 0^{th},14^{th} and 28^{th} days of experiment and blood was collected for hematological and biochemical estimations. Finally, the rats were euthanatized in ether chamber after the completion of experiment for study of oxidation stress on heart and detailed pathological studies**.**

Table 7: Routine of the animals during experimental period.

Group	Treatment	Feeding and treatment
Group I	Positive control	Fed on the normal laboratory pellet diet @ 10-15mg/animal daily and *ad-libitum water* throughout the experiment and without any treatment given.
Group II	Negative control	Fed on the High Fat diet @ of 10-15 gm/ animal/ day throughout the experiment with *ad-libitum* water.
Group III	Standard treatment statin	Fed on high fat diet @ 10-15gm /animal/day and *ad-libitum* water throughout the experiment + standard Antihyperlipidemic treatment of **Atorvastatin** @ 0.5mg/kg via oral route dosing per day from 29 th day of experiment. Vehicle used was distilled water.
Group IV	*Carica papaya* seeds extract Treatment	Fed on high fat diet @ 10-15gm /animal/day and *ad-libitum* water throughout the experiment + Treatment of aqueos seeds extract of ***Carica papaya*** seeds extract @ 60mg/kg oral route dosing in the vehicle of distilled.

3.4 Parameters studied

3.4.1. Programme of research work:

The present investigation has been planned to study the Antihyperlipidemic effect of *Carica papaya* in atherosclerotic rats which is going to be induced in rats by feeding them high fatty diet.

Oral administration of seed aqueous extract at different dose rate for 28 days in Wistar rats and also standard drug statin will be administered to the animals.

At different time intervals the blood will be collected for hematological and biochemical studies, gross and histopathological alterations of the experimental rats will be studied at the end of trial.

3.4.1 Duration of experiment

Total 56 days duration of experiment was comprised of first 28 days duration to induce hyperlipidemia and atherosclerotic changes if any and later treatment were started on 29 day, which was considered as the day 1.st

3.4.3 General examinations

Body weights of individual experimental rats were assessed.

All the experimental rats were observed regularly for behavioral changes and mortality changes if any throughout the experimental period.

3.4.4 Hematological parameters:

The blood samples were analyzed for estimation of Hemoglobin (Acid Hematin method using Sahli's haemoglobinometer) Jain (1986), estimation of TEC (Hemocytometer), estimation of TLC (Hemocytometer) (Yakubu 2008), estimation of DLC (Wright's staining) Weiss and Wardrop (2010). For all groups at 0, 14th and 28th day of study. The blood samples were collected from the retro-orbital plexus from the rats in clean, dry and sterilized EDTA vials and were processed as per standard methods.

The blood samples were analyzed for following hematological parameters:

Hemoglobin (Hb)	: Acid Hematin method
Total Erythrocyte Count (TEC)	: Hemocytometer
Total Leucocyte Count (TLC)	: Hemocytometer

3.4.5 Biochemical Studies:

On 0, 14th and 28th day of experiment, the blood samples about 2-3 ml volume was collected from the rats into clean, dry and sterilized test tubes without anticoagulant and used for separation of serum.

The serum samples were analyzed for estimation of liver function tests- AST and ALT, kidney function test- creatinine, BUN. The lipid profile- cholesterol, triglycerides, HDL, LDL. The lipid profile of all the animals was studied on day 0 (before induction). Day first of experiment, day 14th, and day 28th.

AST	: UV kinetic method (Teitz 1976, Najmi 2005)
ALT	: UV kinetic method (Teitz 1976, Najmi 2005)
BUN	: Berthelot method (Chaney and Shils 1962)
Creatinine	: Alkaline picrate method (Bowers 1980)
Triglycerides	: Fossati and Prencipe (Ali 2009)
Total cholesterol	: CHOD/POD method (Allain, 1974)
HDL Cholesterol	: CHOD/POD method (Allain, 1974)

3.4.6 Relative organ weight:

The relative organ weights of heart, liver, kidney were measured at the end of trial to know effect of aqueous seed extract.

At the end of the experiment the organ of concern were examined for the gross observations and relative organ weight of the animals.

The relative organ weights of liver, heart, kidney was measured at the end of trial to know effect of extract.

3.4.7 Histopathological studies:

3.4.1 Gross pathology

Gross necropsies of the organs like heart, liver, kidney from sacrificed animals were observed for the presence of any gross abnormal pathological change, on completion of the experiment.

3.4.2 Histopathological changes

Heart, liver and kidney tissues of the experimental Wistar rats were collected in 10% buffered formalin Luna, (1968) and were dehydrated in ascending grade of the alcohol, cleared in xylene and then was processed by routine paraffin wax embedding technique and stained with 4-5-micron thick sections were stained with routine Hematoxylin and eosin-staining method and examined and was examined for presence of histoarchitectural alterations if any.

All the rats in each group were sacrificed on the termination day (56^{th} day) of the experiment and the organs viz. liver, heart, kidney were subjected to gross and microscopic examinations.

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

The present study was planned to study the antihyperlipidemic effects of aqueous extract of *Carica papaya* seeds.

The rats were fed with specially prepared high fat diet containing 1 per cent cholesterol for the induction of hyperlipidemia and producing the atherosclerotic changes in the Wistar rats for a period of 28 days prior to the start of the experiment and for 28 days (Total 56 days) during the treatment period.

From day 1st of the experiment i.e. after the induction period of 28 days rats in treatment groups were daily administered with aqueous seed extract of *Carica papaya* at the dose rate of 60 mg per kg body weight orally to Group IV rats, against the Healthy control (Group I), negative control (Group II) and standard drug treated group (Group III), were treated with standard drug Statin at the dose rate of 0.5 mg per kg body weight orally to assess and compare the efficacy of aqueous extract of *Carica papaya* seeds.

4.1 Phytochemical properties of *Carica papaya seeds*

The aqueous extract of *Carica papaya* was assessed for presence of phytochemical constituents using different analysis tests.

The qualitative analysis of aqueous extracts of seeds of *Carica papaya* was carried out by employing various phytochemical tests as described by Treas and Evans (1985).

The phytochemical constituents present in aqueous extracts of seeds of *Carica papaya* mentioned in table 8.

Table 8: Phytochemically active constituents of aqueous seed extract of *Carica papaya*

Sr. no.	**Test**	Observation
1	Alkaloids	**Positive**
2	Flavonoids	**Positive**
3	Tannins	**Positive**
4	Glycosides	**Positive**
5	Anthroquinones	**Positive**
6	Saponins	**Positive**
7	Sugar	**Positive**

The aqueous seed extract of *Carica papaya* showed presence of Alkaloids, Flavonoids, Tannins, Glycosides, Anthraquinines, saponins and Sugar.

4.2 Behavioral changes

During the experiment all the rats from all the groups did not show any significant abnormal behavioural changes all the rats were observed to be alert, normal and healthy.

4.3 Body weight changes

All the animals in control as well as treatment groups were observed for the body weight changes on day '0', 14th and 28th of the experiment. The results obtained are summarized in table 9 and fig 1.

The mean body weight in group I, II, III and IV on day 0, 14^{th},28^{th} ranged between 182.31 to 254.87, 182.83 to 287.25, 190.62 to 292.25 and 184 to 285.93 gm, respectively.

Statistical significant increase in the mean body weight in group no I (Healthy control) was observed on day 14^{th} (200.48 gm) and day 28^{th} (254.87 gm) when compared with 0 day values. All the values were observed to be within normal physiological limits.

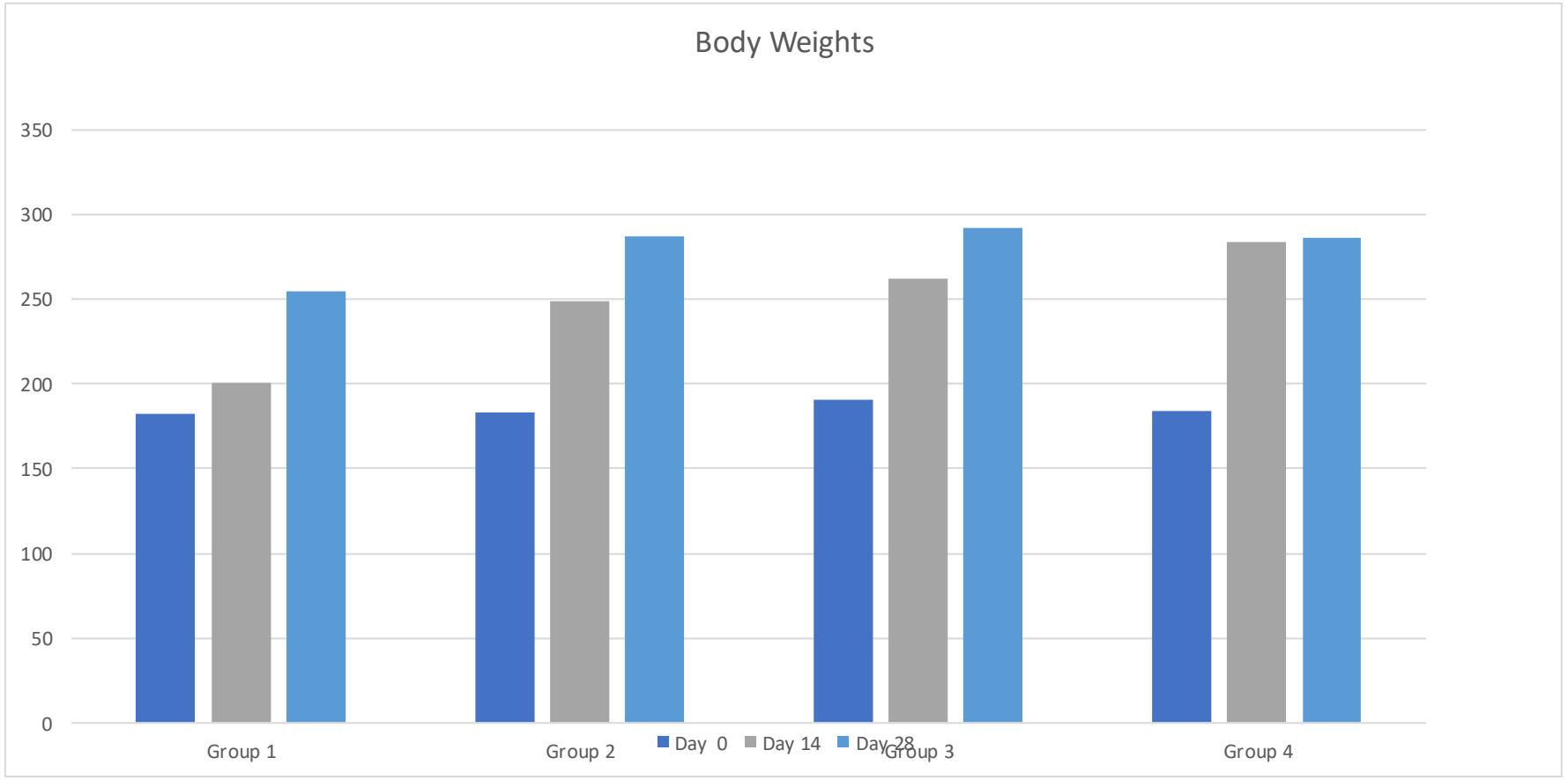

Fig. 1. Body weight in (g) of the experimental animals

Statistically significant increase was observed in group II on day 14th and 28th as against its day 0 value. But the values were in normal were in normal physiological limits.

In group III and IV, significant increase in mean body weight was observed on day 14th and 28th as compared to 0 day, respectively.

In group III animals, the weight measured was observed to be 261.87 and 292.25 on day 14th, 28th and in group IV the values on 14th and 28th day were 283.62 and 285.93

Statistically significant increase in weight gain was observed on day 14th. But there was decrease in weight observed on day 28th of group III than of group IV as an effect of treatment. (Bugajski *et al;* 2007) Explained that high fat diet and hyperlipidemia and obesity.

Table 9 : Body weight (G) in experimental rats.

Groups of rats	Body weights(gm)		
	Intervals of study		
	0th day	**14th day**	**28th day**
I	182.31±1.78	200.48 ±1.15 [c]	254.87 ±7.98 [b]
II	182.83±2.28	249.17 ±9.96 [b]	287.25 ±6.061 [a]
III	190.62±1.153	261.87 ±9.43[b]	292.25±6.48 [a]
IV	184±2.14	283.62 ±5.16 [a]	285.93 ±12.47 [a]
Stat	NS	**S**	**S**
CD values		**CD(0.01) = 28.740** **CD(0.05) = 21.303**	**CD(0.05)= 24.998**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4 Haematological examination

4.4.1 Haemoglobin

Table 10, Fig 2 summarizes the mean haemoglobin (Hb) level in all the experimental rats at different experimental days.

The mean Hb concentration in group I (healthy control) animals on day 0 ranged between 13.31 to 14.12gm/dl though significant reduction is observed on

day 14th and day 28th when compared with its respective day '0' values. All the values were observed within normal physiological limits.

The mean haemoglobin levels in group II, III and IV ranged between 12.5 to 14.56, 13.62 to 15.25 and 12.12 to 14.81 gm/dl, respectively during the experimental period of 28 days.

Statistically significant reduction was observed in group II on day 14th and 28th as against its day 0 value. But the values were observed within normal physiological limits.

In group III and IV, significant increase in mean Hb level was observed on day 14th and 28th as against the Hb level observed on its respective day and '0' . where the values in group III were observed to be 15.06 and 15.25 gm/dl as against Hb level of 13.62 on its day '0' reading.

In group IV animals, the values were observed 13.87 and 14.81 gm/dl as, respectively as against 0 day value.

Statistically non significant elevation in mean Hb level was observed on day 14th, 28th of treatment in group III and IV as against group II and I values indicating positive response of statin and CpAE on erythropoiesis.

Though some alterations in the mean Hb level in all the control and treatment groups were observed on day 28th i.e. on the termination day of the experiment all the alterations were observed to be statistically non significant and also the mean Hb values in all the group animals were observed to be within normal physiological limits.

Though statistically non significant but elevation in the mean haemoglobin level in group III animals treated with drug Statin at the dose rate of 0.5 mg/ kg body weight orally and in group IV animals treated with the aqueous seed extract of *Carica papaya* at the dose rate of 60 mg/ kg body weight orally was observed when compared with its respective day '0' values and also against the control and group II animals.

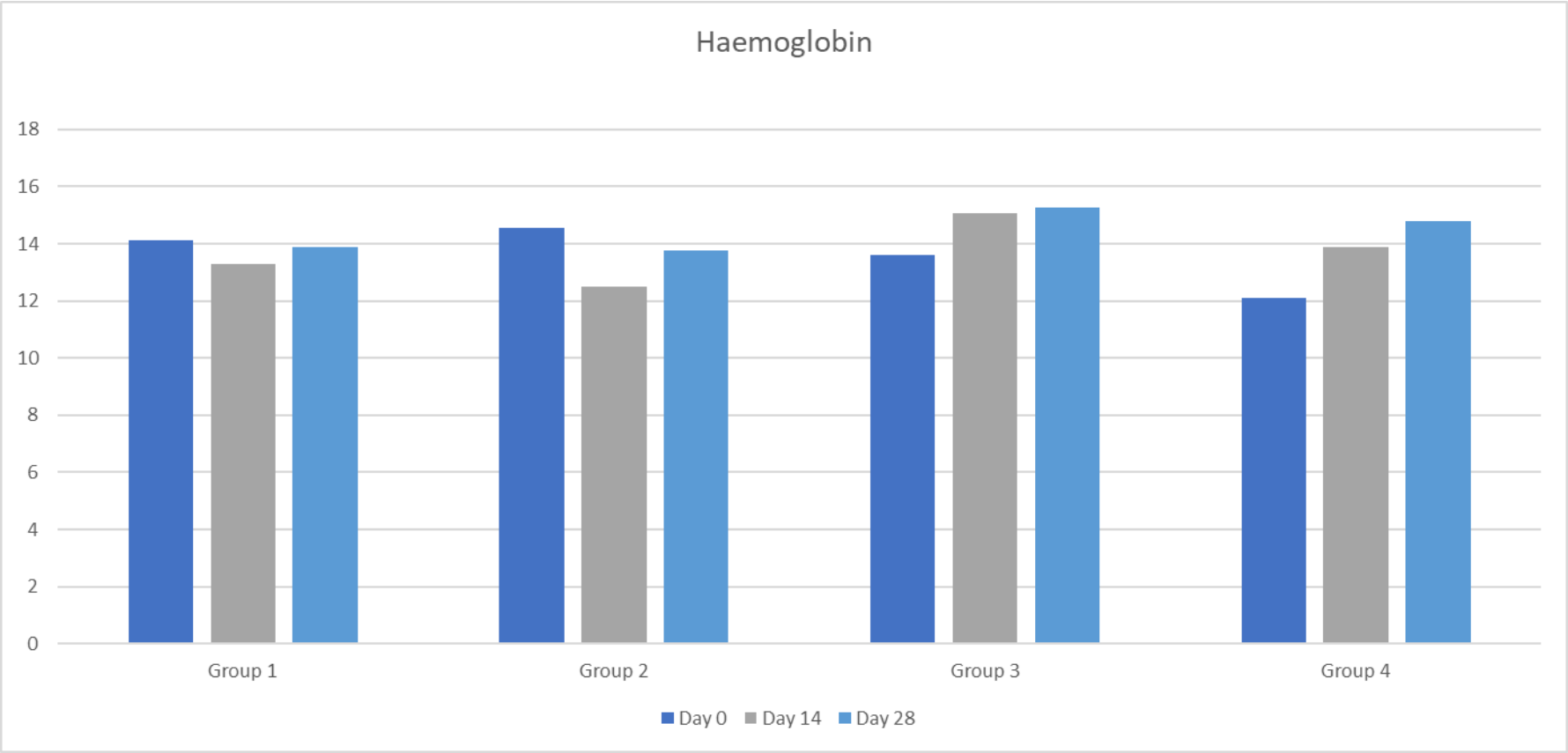

Fig. 2Mean hb value rats at the different intervals

Similar observations were also reported by Ahmad *et al*.,(2016) who has reported the statistically significant elevation in Hb level in *Carica papaya* treated rats.

An increase in the mean Hb value may be due to erythropoiesis stimulating effect of *Carica papaya*.

Table 10: Mean Haemoglobin (Hb) level (g/dl) in different treatment groups of rats.

Gr.	Group and treatment	Intervals of study		
		0^{th} day	**14^{th} Day**	**28^{th} Day**
I	Group I (positive control)	14.12 ± 0.44^{a}	13.31 ± 0.66	13.87 ± 0.63
II	Group II (Negative control)	14.56 ± 0.41^{a}	12.5 ± 0.53	13.75 ± 0.36
III	Group III (Statin)	13.62 ± 0.59^{ab}	15.06 ± 0.40	15.25 ± 0.25
IV	Group IV (CpAE Treatment)	12.125 ± 0.83^{a}	13.87 ± 0.85	14.81 ± 0.46
Stat		**S**	**NS**	**NS**
CD	**Treatments found Significant at 5% level of Significance CD(0.05)= 1.724**			

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.2 Total Leukocytic Count

The Mean Total Leukocyte Count (Thousand / cu mm) in different experimental group animals on day 0, day, 14^{th} and 28^{th} of treatment is depicted in table 11.

The mean TLC concentration in group I healthy control on day 0, 14^{th},28^{th} ranged between 9.19 to 10.20 thousand/cu mm, respectively.

No statistically significant alterations were observed in group I animals i.e. healthy control group on day 14th and 28th of treatment when compared with its day'0' values.

The TLC values in group II, III and IV ranged between 9.42 to 10.04, 9.16 to 10.38 and 9.26 to 10.63 thousand/cu mm.

Statistically significant increase was observed in group II, III and IV on day 14th when compared between the groups.

Statistically non significant alterations were observed on day 28th when compared between the groups.

Though slight increase in the mean TLC level was observed in group II animals on day 14th and day 28th of treatment. It was observed to be statistically nonsignificant when compared with its day '0' value. Also all the values were in normal physiological limit.

In group III and IV, non significant increase on day 14th and then non significant decline in mean TLC level on day 28th was observed when compared with its day '0' value.

An increase in the mean TLC values in all the groups on day 14th of experiment may be due to change in the diet. However all the alterations in TLC level in all the group animals were observed to be within normal physiological limit.

Table11: Mean Total Leukocyte Count (Mean ± S.E. thousand /cu mm) in experimental rats.

Groups of rats	Total Leukocyte Count (Mean ± S.E. thousand /cumm)		
	Intervals of study		
	0th day	14th day	28th day
I	9.19 ± 0.20	9.31 ± 0.20^{c}	10.20 ± 8.45
II	9.42 ± 0.12	9.91 ± 0.20^{bc}	10.04 ± 2.82
III	9.16 ± 0.22	10.38 ± 0.30^{ab}	9.96 ± 0.26
IV	9.26 ± 0.22	10.63 ± 0.25^{a}	10.29 ± 0.29
Stat	NS	S	NS

4.4.3 Total Erythrocyte Count

The Mean Total Erythrocyte Count (million / cu mm) in different experimental group animals on day 0, day, 14th and 28th of treatment is depicted in table 12 and Fig4.

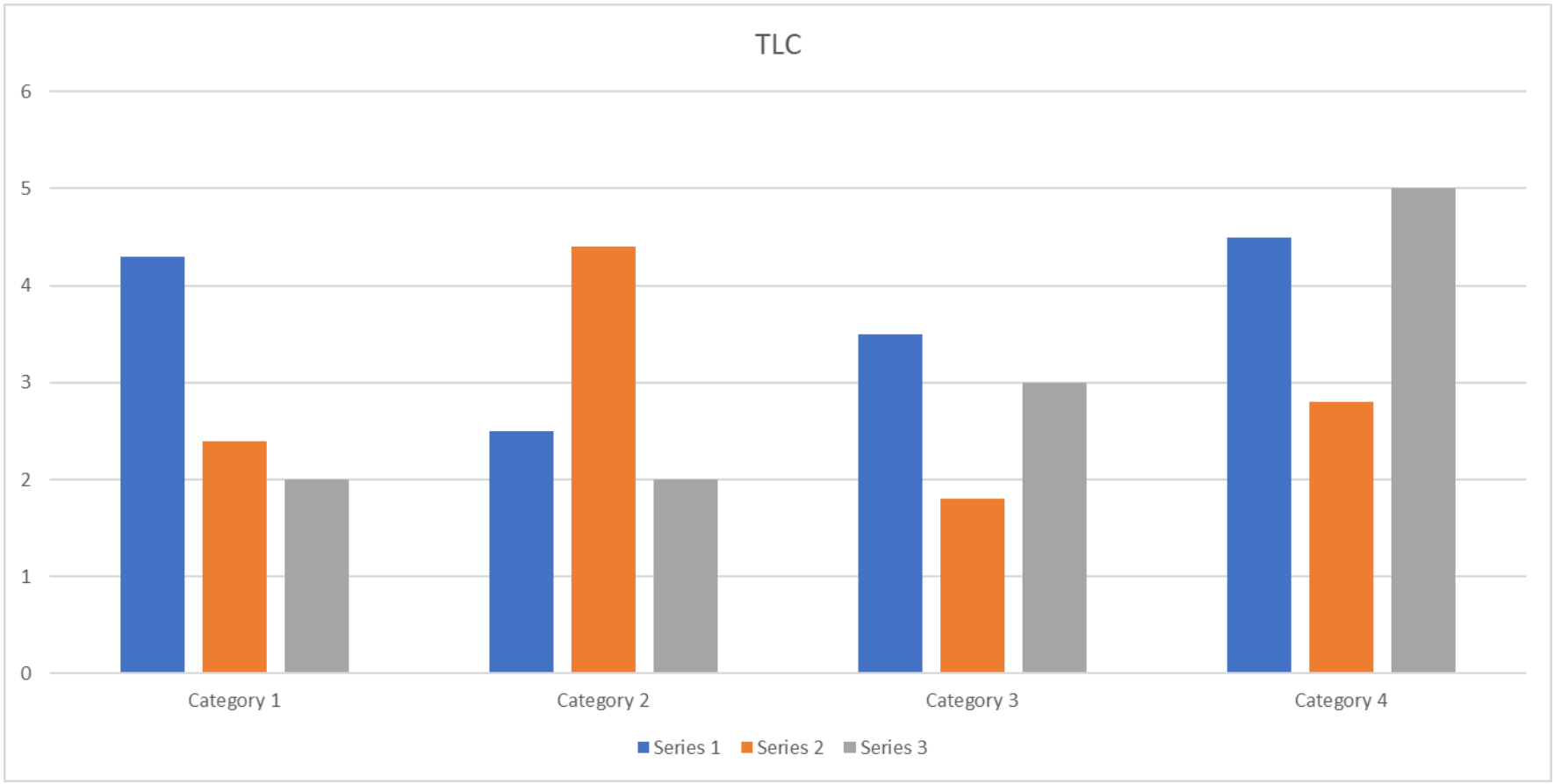

Fig 3 Mean TLC value at the different intervals

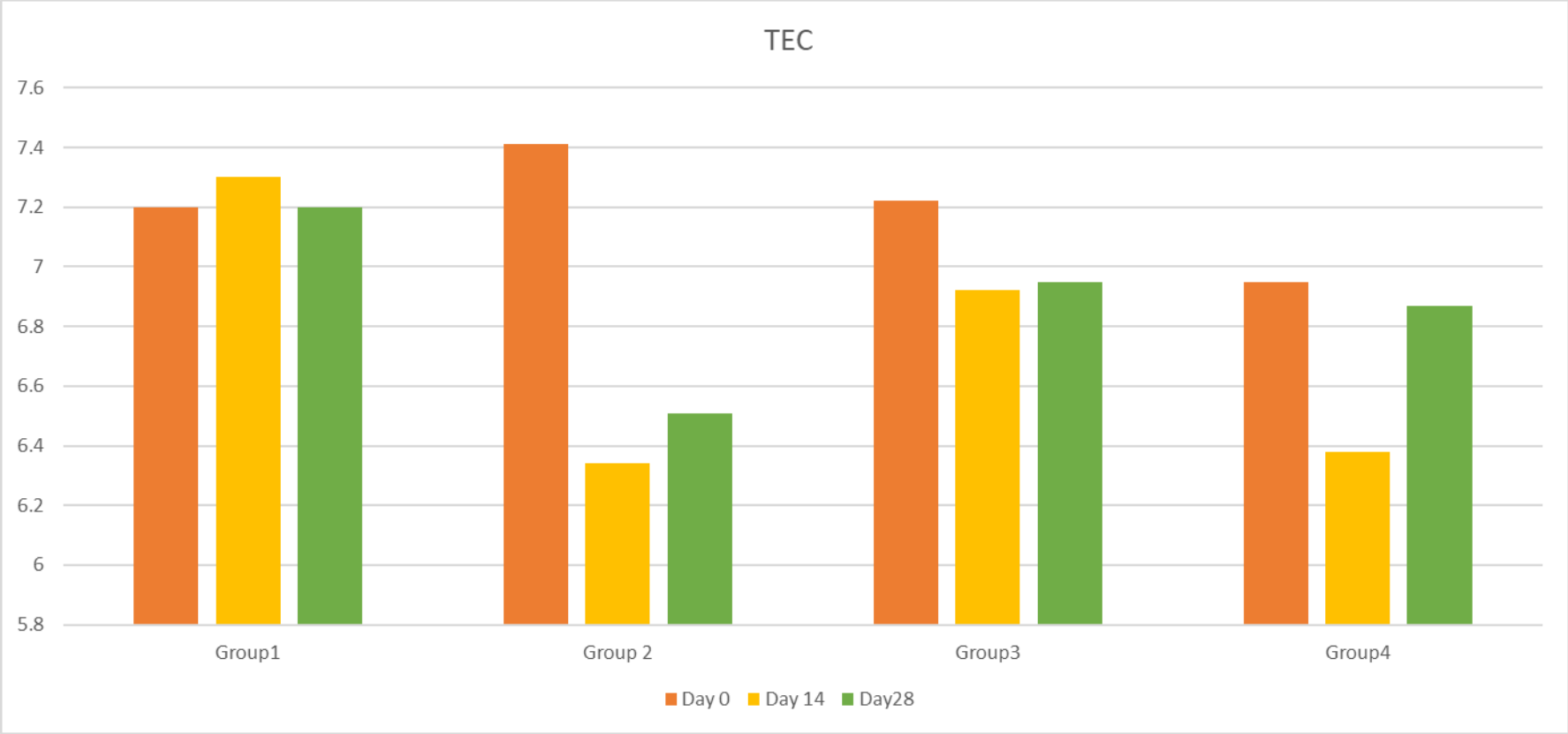

Fig.4 Mean TEC value at the different intervals

The mean TEC mean concentration in group I, II, III and IV on day 0, 14th,28th ranged between 7.20 to 7.30, 6.34 to 7.41, 6.92 to7.22, and 6.38 to 6.95 milli/cu mm respectively.

Though some alterations were observed in all the group animals on different days of treatment No statistically significant changes were observed in control and all the treatment group animals when the respective values on day 14th and 28th were compared with their day '0' values.

The mean TEC values on day 14th of the experiment in group II, III and group IV animals were observed to be significantly lowered when compared with control group values. but on day 14th of the experiment statistically non significant elevation was observed.

Table 12: Mean Total Erythrocyte Count (millions/cu mm) in experimental rats.

Groups of rats	Total Erythrocyte Counts (Mean ± S.E. millions/cumm)		
	Intervals of study		
	0th day	**14th day**	**28th day**
I	7.20 ± 0.21	7.30 ± 0.65[a]	7.20 ± 0.14
II	7.41 ± 0.21	6.34 ± 0.10[b]	6.51 ± 0.15
III	7.22 ± 0.22	6.92 ± 0.15[b]	6.95 ± 0.18
IV	6.95 ± 0.25	6.38 ± 0.15[c]	6.87 ± 0.23
Stat	**NS**	**S**	NS
CD values		**CD(0.05)0.360**	

TEC Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.3 Differential Leucocyte Count (DLC)

The DLC counts (%) in different experimental group animals on day 0, day, 14th and 28th of treatment is depicted.

4.4.3.1 Mean Neutrophils count (%)

Table13 summaries the mean Neutrophil count in all the experimental animals on different days of the experiment.

The mean neutrophil count in control group rats ranged between 28.25 to 31.62 per cent on different experimental days.

In group II, III and IV the neutrophil count ranged between 38.25 to 38.75, 36.12 to 37.50 and 32.82 to 39.12 percent, respectively on '0', 14th and 28th day of the experiment.

Though slight alterations are observed in mean neutrophil count in all the group animals no significant alterations were observed in any treatment group when compared within the groups.

The mean neutrophil count on day 14th and 28th of treatment within group II, III and IV were observed to be at par However the values were observed to be higher in group II, III and IV when compared against the control group animals. However, all the values were observed to be within normal physiological limits.

Table 13: Mean Neutrophils count (%) in experimental rats.

Gr.	Group and treatment	Intervals of study		
		0^{th} day	**14^{th} Day**	**28^{th} Day**
I	Group I (positive control)	31.62 ± 1.879^{b}	30.00 ± 2.07^{b}	$28.25\ c \pm 1.66^{c}$
II	Group II (Negative control)	38.75 ± 0.67^{a}	38.50 ± 0.62^{a}	38.25 ± 0.79^{a}
III	Group III (Statin)	37.5 ± 0.46^{a}	36.12 ± 1.007^{a}	35.50 ± 0.42^{ab}
IV	Group IV (CpAE Treatment)	39.12 ± 0.58^{a}	32.82 ± 0.98^{a}	34.62 ± 1.01^{b}
Stat		**S**	**S**	**S**
CD		**CD (0.01) = 4.163 CD(0.05) = 3.086**	**CD(0.01) = 5.055 CD(0.05) =3.747**	**CD(0.01) = 4.201 CD (0.05) = 3.114**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.3.2 Mean Lymphocyte count (%)

Table 14. summaries the mean Lymphocyte count in all the experimental animals on different days of the experiment.

The mean lymphocyte count in control group rats ranged between 57.50 to 62.50 per cent on different experimental days.

In group II, III and IV the lymphocyte count ranged between 57.12 to 65.87, 57.37 to 64.5 and 57.00 to 65.25 percent, respectively on '0', 14th and 28th day of the experiment.

Though slight alterations are observed in mean lymphocyte count in all the group animals no significant alterations were observed in any treatment group when compared within the groups.

The mean lymphocyte count on day 28th of treatment within group II, III and IV were observed to be at par. However the values were observed to be higher in group II, III and IV when compared against the control group animals.

The alterations in mean lymphocyte count in all other groups on all other treatment days was observed to be statistically non significant. However, all the values were observed to be within normal physiological limits.

Table 14: Mean Lymphocyte count (%) in experimental rats.

Gr.	Group and treatment	Intervals of study		
		0^{th} day	**14^{th} Day**	**28^{th} Day**
I	Group I (positive control)	57.5 ± 0.77	62.5 ± 0.77	61.25 ± 0.61[b]
II	Group II (Negative control)	57.12 ± 0.66	62.25 ± 0.52	65.87 ± 0.44[a]
III	Group III (Statin)	57.37 ± 0.53	62.12 ± 0.47	64.5 ± 0.5[a]
IV	Group IV (CpAE Treatment)	57.00 ± 0.51	62.75 ± 0.64	65.25 ± 0.45[a]
Stat		**NS**	**NS**	**S**
CD				**CD(0.01)= 1.986** **CD(0.05)= 1.472**

4.4.3.3 Mean Eosinophils count (%)

Table 15 summaries the mean Eosinophil count in all the experimental animals on different days of the experiment.

The mean Eosinophil count in control group rats ranged between 1.5 to 4.13 per cent on different experimental days.

In group II, III and IV the Eosinophil count ranged between 1.75 to 2.50, 1.62 to 3.62 and 1.50 to 1.75 percent, respectively on '0', 14th and 28th day of the experiment.

The mean eosinophil count in all the group animals on day '0' was observed to be at par.

The mean eosinophil count in group II animals on day 14th and 28th of treatment were observed to be significantly higher then the value observed in control group animals. However the mean Eosinophil count in animals from group I, III and IV on day 14th and 28th was observed to be at par.

The mean eosinophil count in all the treatment group animals on all the different treatment group animals were observed to be within normal physiological limits.

Table 15: Mean Eosinophils count (%) in experimental rats.

Gr.	Group and treatment	Intervals of study		
		0^{th} day	**14^{th} Day**	**28^{th} Day**
I	Group I (positive control)	1.5 ± 0.42	3.75 ± 0.36^{a}	4.13 ± 0.29^{a}
II	Group II (Negative control)	2.00 ± 0.26	1.75 ± 0.25^{b}	2.50 ± 0.26^{ab}
III	Group III (Statin)	1.62 ± 0.37	3.00 ± 0.46^{a}	3.62 ± 0.26^{a}
IV	Group IV (CpAE Treatment)	1.75 ± 0.36	1.50 ± 0.32^{a}	1.75 ± 0.52^{b}
Stat		**NS**	**S**	**S**
CD			**5% level of Significance CD(0.05) = 1.145**	**5% level of Significance CD(0.05) = 1.262**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.3.4 Mean Basophils count (%)

Table 16 summaries the mean basophil count in all the experimental animals on different days of the experiment.

The mean basophil count in control group rats ranged between 1.25 to 1.75 per cent on different experimental days.

In group II, III and IV the basophil count ranged between 1.00 to 1.50, 1.25 to 1.75 and 1.37 to 1.62 percent, respectively on '0', 14th and 28th day of the experiment.

Though slight alterations are observed in mean basophil count in all the group animals no significant alterations were observed in any treatment group when compared within the groups and between the groups on different experimental days. However, all the values were observed to be within normal physiological limits

Table 16: Mean Basophils count (%) in experimental rats.

Gr.	Group and treatment	Intervals of study		
		0^{th} day	14^{th} Day	28^{th} Day
I	Group I (positive control)	1.75 ± 0.16	1.37 ± 0.26	1.25 ± 2.54
II	Group II (Negative control)	1.00 ± 0.26	1.25 ± 0.31	1.50 ± 0.26
III	Group III (Statin)	1.25 ± 0.31	1.75 ± 0.16	1.62 ± 0.26
IV	Group IV (CpAE Treatment)	1.62 ± 0.18	1.50 ± 0.18	1.37 ± 0.26
Stat		**NS**	**NS**	**NS**
CD	-			

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.3.5 Mean Monocyte count (%)

Table 17 summaries the mean monocyte count in all the experimental animals on different days of the experiment.

The mean monocyte count in control group rats ranged between 3.00 to 4.13 per cent on different experimental days.

In group II, III and IV the monocyte count ranged between 1.75 to 2.50, 1.75 to 3.62 and 2.00 to 3.62 per cent respectively on '0', 14th and 28th day of the experiment.

The mean monocyte count in all the group animals on day '0' was observed to be at par.

The mean monocyte count in group II animals on day 14th and 28th of treatment were observed to be significantly lower than the values observed in control group animals. However the mean monocyte count in animals from group III and group IV on day 14th and 28th was observed to be at par but were observed to be significantly higher on these days when compared as against group II animals.

The mean monocyte count in all the treatment group animals on all the different treatment group animals were observed to be within normal physiological limits.

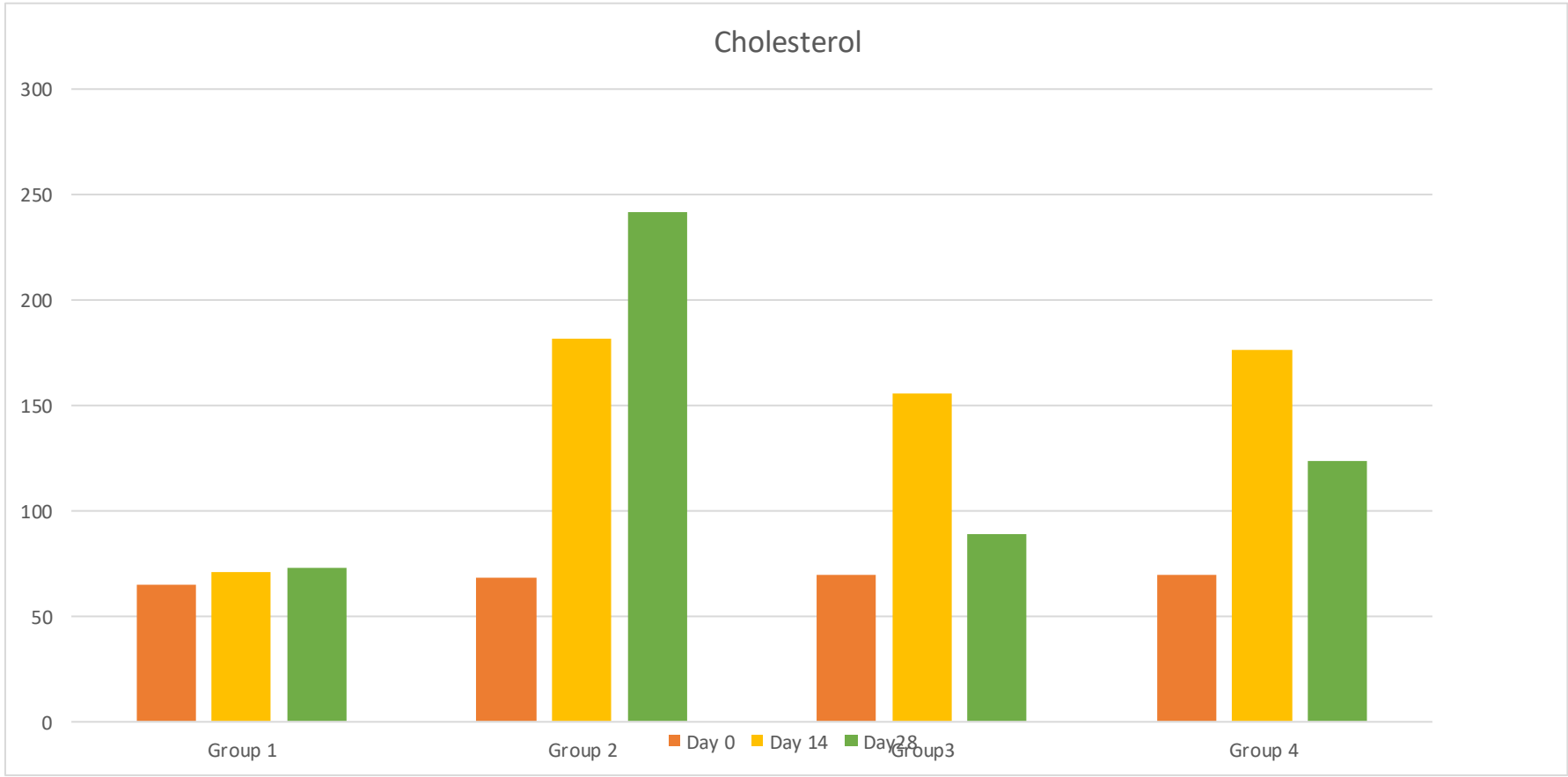

Fig.5 Mean total cholesterol value at the different intervals

Table 17: Mean Monocyte count (%) in experimental rats.

Gr.	Group and treatment	Intervals of study		
		0^{th} day	**14^{th} Day**	**28^{th} Day**
I	Group I (positive control)	3.00 ± 0.42	3.75 ± 0.36[a]	4.13 ± 0.29[a]
II	Group II (Negative control)	2.00 ± 0.32	1.75 ± 0.25[b]	2.50 ± 0.26[b]
III	Group III (Statin)	1.75 ± 0.31	3.00 ± 0.46[a]	3.62 ± 0.26[a]
IV	Group IV (CpAE Treatment)	2.00 ± 0.32	2.75 ± 0.31[ab]	3.62 ± 0.26[a]
Stat		**NS**	**S**	**S**
CD			**CD (0.01) = 1.394 CD (0.05) = 1.033**	**CD (0.01) = 1.065 CD (0.05) = 0.789**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.5 Blood Biochemical examination

4.5.1 Total Cholesterol count

The mean cholesterol level (mg/dl) in all the experimental animals is summarized in Table 18 and fig 5.

The mean Cholesterol concentration in group I, group II, group III, group IV on day 0, 14^{th},28^{th} ranged between 64.91 to 72.79, 68.61 to 241.93 , 69.51 to 155, and 69.47 to 176.18 mg/dl.

An increase in the mean cholesterol level in the experimental animals is an indicative of effect of the high fat diet.

Non significant elevation in the mean cholesterol level on day 14th and 28th of the experiment as against its day '0' level was observed in group I animals and the elevated level was observed to be within normal physiological levels only.

The mean cholesterol level in group II animals (negative control group) significantly roused from 68.61 to 181.87 mg/dl on day 14th of the experiment and further increased to 241.93 on day 28th of the experiment. Statistically

significant elevation in the mean cholesterol was observed in group II, III and IV as against group I animals on day 14th of the experiment.

However the group III and group IV animals significant reduction on day 28th as compared to the observation in group I animals on respective day.

The mean cholesterol level in group III, IV animals on day 28th of the experiment was observed to be significantly lowered when compared with the day 28th values in group II animals indicating the hypolipidemic effect of the standard drug statin and the aqueous seed extract of *Carica papaya.*

Reduction in the mean cholesterol level in group III and IV was observed on day 28th of the experiment when compared against their respective day 14th values where the reduction from 155.42 to 89.16 and from176.18 to 123.77 mg/dl was observed, respectively.

An elevation in the mean cholesterol level in all the experimental animals from group II to IV is due to the high fat diet. The disturbance in the lipid profile is an indicative of atherosclerotic changes. Similar observations are also reported by Nwangwa (2013)

Table 18 : Mean value of total cholesterol (mg/dl) in experimental rats

Groups of rats	**Mean value of total cholesterol (mg/dl)**		
	Intervals of study		
	0^{th} day	**14^{th} day**	**28^{th} day**
I	64.91 ± 1.27^{b}	70.73 ± 1.44^{b}	72.79 ± 1.63^{d}
II	68.61 ± 1.05^{a}	181.87 ± 23.27^{a}	241.93 ± 3.92^{a}
III	69.51 ± 1.46^{a}	155.42 ± 12.25^{a}	89.16 ± 7.68^{c}
IV	69.47 ± 1.13^{a}	176.18 ± 8.81^{a}	123.77 ± 0.84^{b}
Stat	S	S	S
CD values	**CD (0.05) = 3.593**	**CD (0.01) = 54.265** **CD (0.05) = 40.222**	**CD (0.01) = 17.237** **CD (0.05) = 12.777**

a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

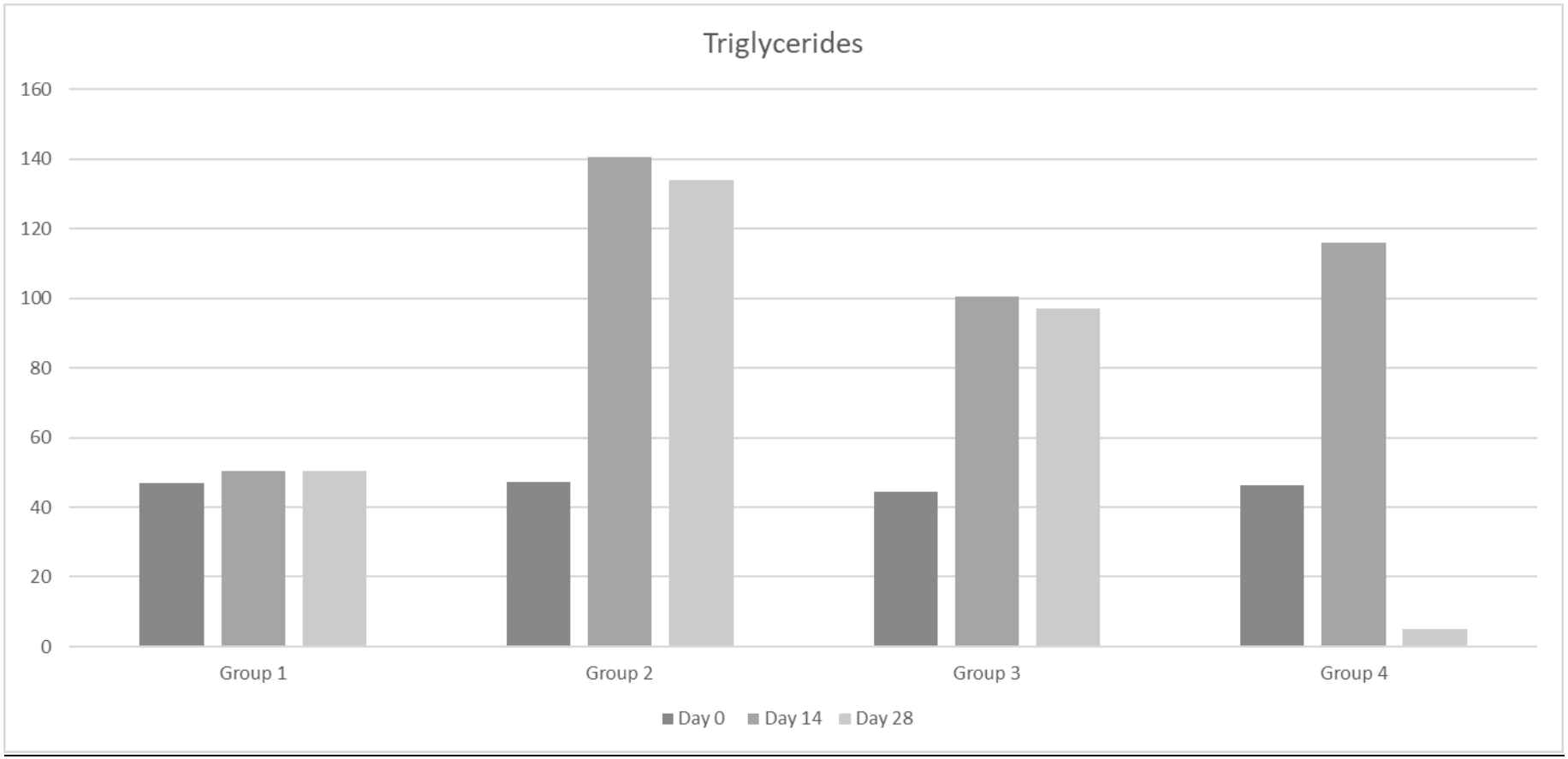

Fig. 6 Mean Triglyceride value at the different intervals

4.5.2 Serum triglycerides

The mean serum triglyceride level (mg/dl) in all the experimental animals is summarized in Table 19 and Fig.6

The mean serum triglyceride level in group I, group II, group III, group IV on day 0, 14^{th},28^{th} ranged between 47.00 to 50.54, 47.33 to 140.62 , 44.39 to 100.37, and 46.39 to 115.99 mg/dl .

An increase in the serum triglyceride level in the experimental animals is an indicative of effect of the high fat diet.

Non significant elevation in the mean serum triglyceride level on day 14th and 28th of the experiment as against its day '0' level was observed in group I animals and the elevated level was observed to be within normal physiological levels only.

The mean serum triglyceride level in group II animals (negative control group) significantly roused from 47.37 to 140.62 mg/dl on day 14th of the experiment. Further slight reduction in the serum triglyceride level to 133.95 was observed on day 28th of the experiment. Statistically significant elevation in the mean serum triglyceride was observed in group II, III and IV as against group I animals on day 14th of the experiment.

However the group III and group IV animals significant reduction on day 28th as compared to the observation in group I animals on respective day.

The mean serum triglyceride level in group III, IV animals on day 28th of the experiment was observed to be significantly lowered when compared with the day 28th values in group II animals indicating the hypolipidemic effect of the standard drug statin and the aqueous seed extract of *Carica papaya.*

Reduction in the mean serum triglyceride level in group III and IV was observed on day 28th of the experiment when compared against their respective day 14th values. where the reduction from 100.37 to 96.93 and from 115.99 to 104.19 mg/dl was observed.

An elevation in the mean serum triglyceride level in all the experimental animals from group II to IV is due to the high fat diet. The disturbance in the lipid

profile is an indicative of atherosclerotic changes. similar observations are also reported in past in that the significant elevation in the serum total cholesterol level was found mainly because of high fat diet.

Table 19: Mean value of Serum triglycerides (mg/dl) in experimental rats

Groups of rats	Serum triglycerides (Mean ± S.E. mg/dl)		
	Intervals of study		
	0th day	**14th day**	**28th day**
I	47.00 ± 1.37	50.54 ± 0.95[c]	50.52 ± 1.11[d]
II	47.37 ± 0.67	140.62 ± 12.7[a]	133.95 ± 0.97[a]
III	44.39 ± 1.53	100.37 ± 1.06[b]	96.93 ± 1.31[c]
IV	46.39 ± 1.57	115.99 ± 1.06[b]	104.19 ± 1.94[b]
Stat	**NS**	**S**	**S**
CD		**CD (0.01) = 25.175** **CD (0.05) = 18.661**	**CD (0.01) = 5.431** **CD (0.05) = 4.026**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.5.3 Serum High Density Lipoproteins (HDL Cholesterol)

The mean HDL cholesterol level (mg/dl) in control as well as all the experimental animals is summarized in Table 20 and Fig.7

The mean HDL concentration in group I, group II, group III, group IV on day 0, 14th,28th ranged between 35.63 to 46.05, 32.21 to 44.52, 36.51 to 43.22 and 35.78 to 42.50. respectively.

Statistically non significant alterations in the mean HDL values were observed in group II, III and IV animals on day '0' and 28th of the experiment.

The mean HDL level in group II, III and IV was observed to be lowered on day 14th of the experiment when compared with the group I levels indicating impairment in the lipid profile due to high fat diet. The mean HDL level in these three groups viz. group II, III and IV was observed to be at par with each other on day 14th of the experiment.

On day 28th of the experiment mean HDL levels in group II further declined to 32.21 mg/dl from 38.21 mg/dl indicating further detonation of the

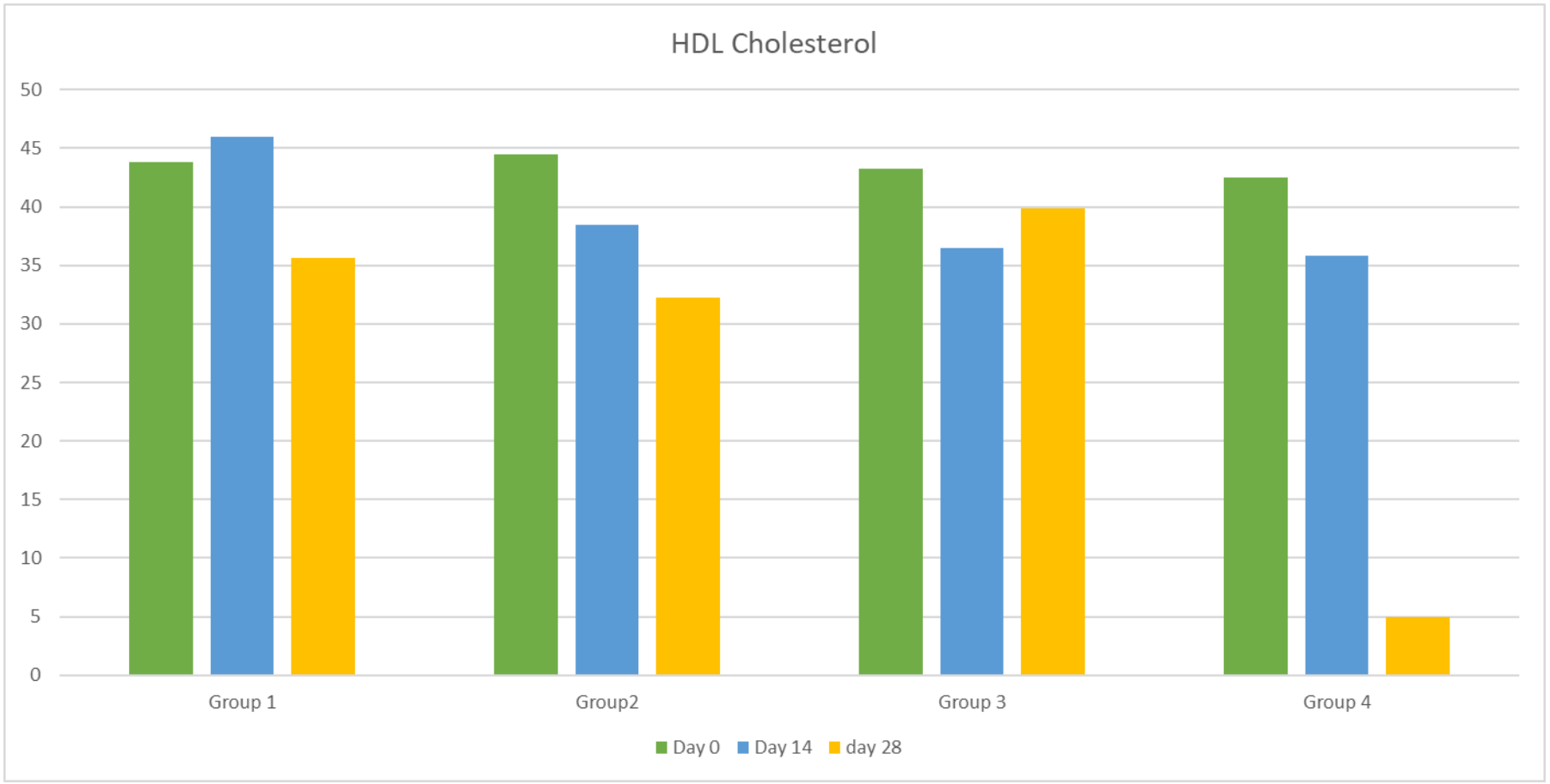

Fig.7 Mean HDL value at the different intervals

lipid profile. However elevation in the HDL level was observed in group III and IV on day 28th as against day 14th values where the mean HDL levels were 39.86 and 37.03 on day 28th as against 36.51 and 35.78 on day 14th, respectively.

Elevation in this level is indicative of repair in the lipid profile due to treatment with standard drug Statin and aqueous seed extract of *Carica papaya.*

Decrease in HDL cholesterol level might be due to excessive accumulation of cholesterol on the liver resulting to down regulation of LDL receptors which carry cholesterol thus leads to bad cholesterol being re-circulated in the blood . Nwangwa,(2013) Our results are same as reported by other.

Table 20: Mean value of serum HDL CHOLESTEROL (mg/dl)

Groups of rats	Mean value of serum HDL CHOLESTEROL (mg/dl)		
	Intervals of study		
	0th day	14th day	28th day
I	43.83 ± 2.27	46.05 ± 1.08[a]	35.63 ± 6.85
II	44.52 ± 2.11	38.45 ± 2.23[b]	32.21 ± 2.94
III	43.22 ± 1.73	36.51 ± 2.31[b]	39.86 ± 2.10
IV	42.50 ± 1.55	35.78 ± 1.98[b]	37.03 ± 1.40
Stat	NS	S	NS
CD values		CD(0.01) = 7.684 CD(0.05) = 5.696	

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$).

4.5.4 Serum Low Density Lipoproteins (LDL Cholesterol)

The mean LDL cholesterol level (mg/dl) in control as well as in all the experimental animals is summarized in Table 21 and Fig.8

The mean LDL concentration in group I, group II, group III, group IV on day 0, 14th,28th ranged between 15.43 to15.95 , 15.91 to16.91, 14.83 to24.63 and 17.17 to 24.66 mg/dl, respectively. No statistically significant alterations were observed in day '0' values of LDL in all the group animals.

Though few alterations in mean LDL level were observed in group I and group II animals on different days when compared within the groups all were observed to be within normal physiological limits.

The mean LDL values in group I and II on day 14th and 28th of the experiment were observed to be non significant. Also the mean LDL values in group III and IV on day 14th and 28th of the experiment were observed to be non significant. However significant increase in the mean LDL values in group III and group IV was observed on day 14th which further increased on day 28th of the experiment as against group I and II values.

An increase in the LDL values in treatment group animals is indicative of positive result of the treatment and all the observations in the present investigation are in association with the observations of. (Nwangwa, 2013)

Table 21 Mean value of serum LDL cholesterol (mg/dl) in experimental rats

Groups of rats	Serum LDL cholesterol (Mean ± S.E. mg/dl)		
	Intervals of study		
	0^{th} day	**14^{th} day**	**28^{th} day**
I	15.43 ± 0.94	15.95 ± 0.89^{b}	15.94 ± 1.197^{c}
II	16.91 ± 1.22	15.91 b $\pm 0.82^{b}$	15.94 ± 1.197^{c}
III	15.83 ± 0.75	17.23 ± 2.1^{a}	24.63 ± 0.59^{c}
IV	17.17 ± 0.74	18.85 ± 2.13^{a}	24.66 ± 0.57^{c}
Stat	**NS**	**S**	S
CD values		**CD(0.01) = 6.390** **CD(0.05) = 4.737**	**CD(0.01) = 4.859** **CD(0.05) = 3.602**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.5.5: Serum Creatinine (mg/dl) in experimental rats

The mean creatinine concentration in group I, group II, group III, group IV on day14^{th},28^{th} ranged between0.77 to 0.83 , 0.82 to 0.83, 0.82 to 0.83 and 0.83 to 0.85mg/dl, respectively.

No statistically significant alterations were observed in the mean creatinine level in control as well as any of the treatment group on different experimental days.

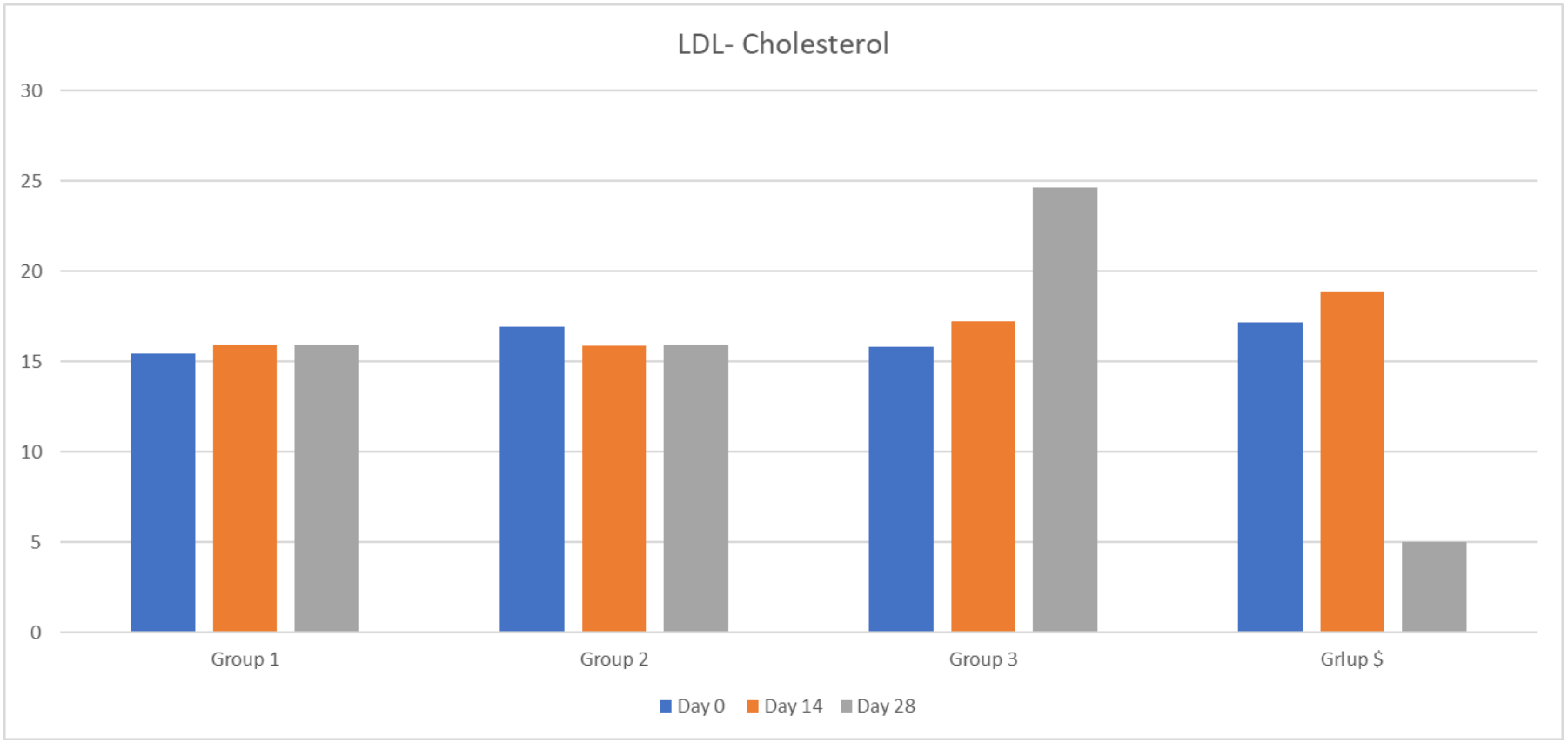

Fig.8 Mean LDL value at the different intervals

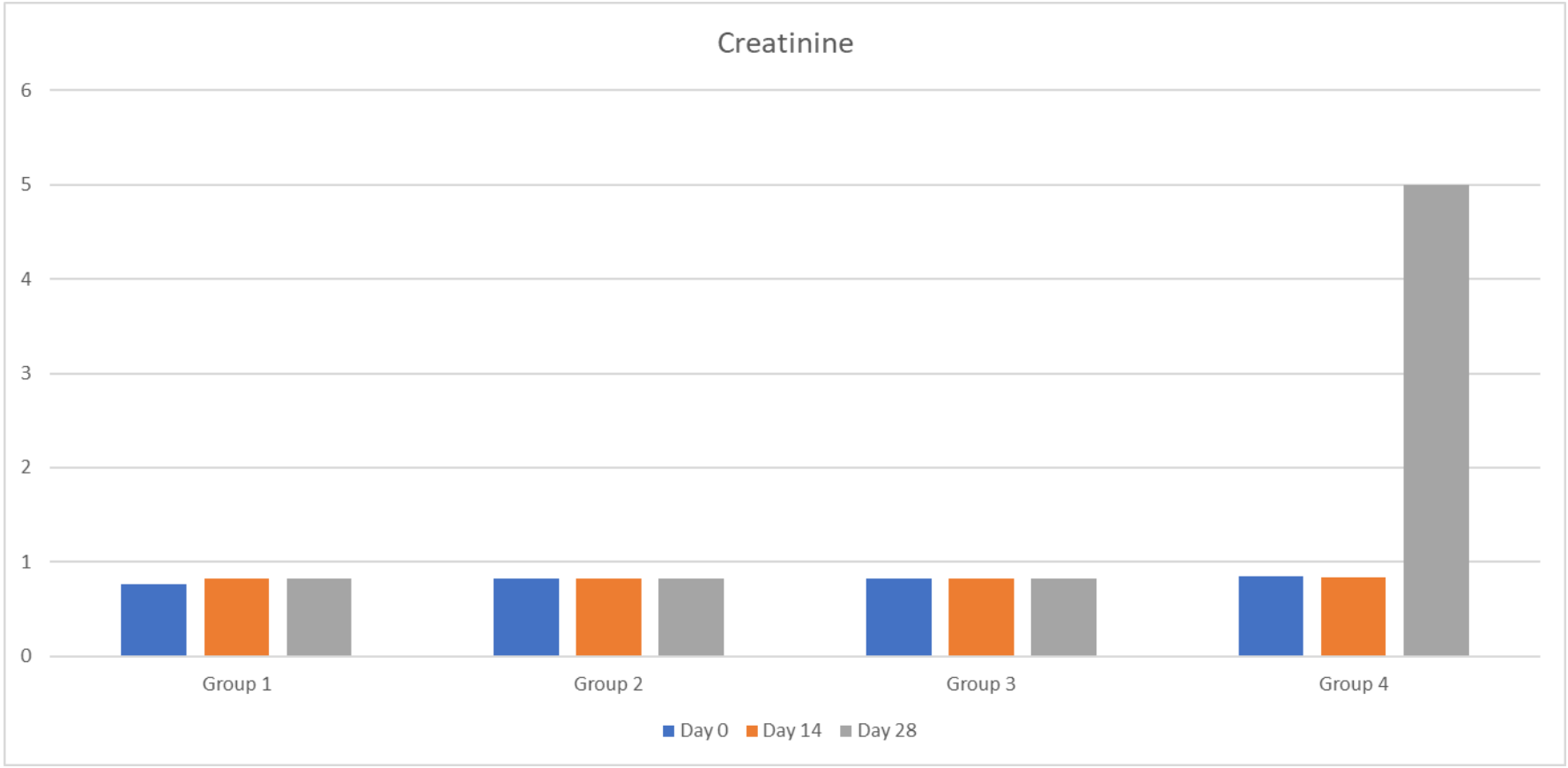

Fig.9 Mean serum Creatinine value at the different intervals

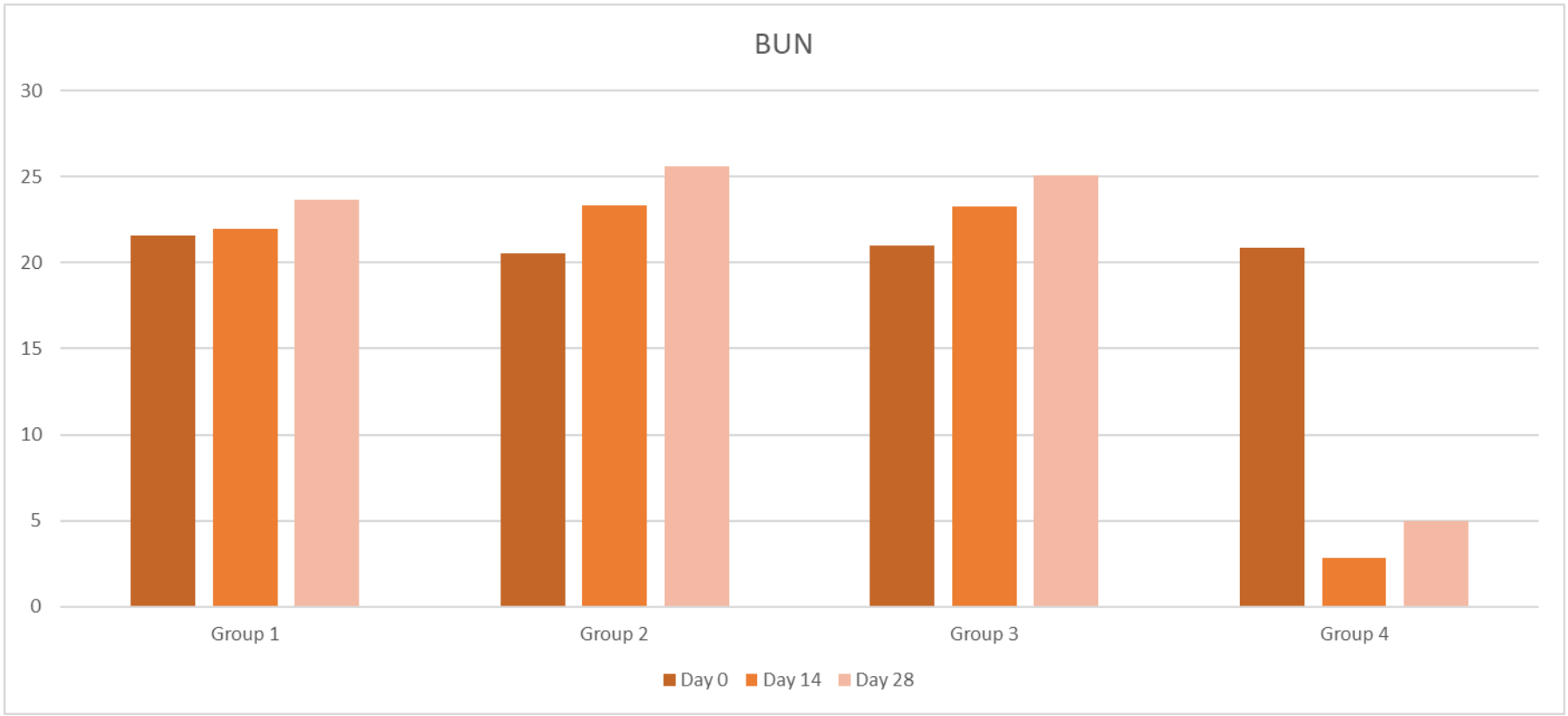

Fig.10 Mean serum BUN value at the different intervals

Table 22: Mean serum Creatinine level (mg/dl) in experimental rats

Gr.	Group and treatment	Intervals of study		
		0^{th} day	14^{th} Day	28^{th} Day
I	Group I (positive control)	0.77 ± 0.038	0.82 ± 0.031	0.83 ± 0.017
II	Group II (Negative control)	0.83 ± 0.031	0.83 ± 0.029	0.82 ± 0.025
III	Group III (Negative control)	0.82 ± 0.017	0.83 ± 0.039	0.83 ± 0.10
IV	Group IV (CpAE Treatment)	0.85 ± 0.015	0.84 ± 0.026	0.83 ± 0.026182
Stat		**NS**	**NS**	**NS**
CD				

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.6 Blood Urea Nitrogen (BUN)

The mean BUN mean concentration in group I, group II, group III, group IV on day 0, 14^{th},28^{th} ranged between 21.57 to 23.66, 20.55 to 25.58, 21.03 to 25.11, 20.88 to 23.72 mg/dl, respectively.

Non significant alterations in mean BUN levels was observed in all the experimental group animals with all the values were in normal physiological limits shown in table 23 and fig. 10.

Table 23 :Mean Blood Urea Nitrogen level (mg/dl) in experimental rats

Gr.	Group and treatment	Intervals of study		
		0^{th} day	14^{th} Day	28^{th} Day
I	Group I (positive control)	21.57 ± 1.12	22.003 ± 0.65	23.66 ± 0.68
II	Group II (Negative control)	20.55 ± 0.56	23.31 ± 0.44	25.58 ± 1.14
III	Group III (Statin)	21.03 ± 0.54	23.26 ± 0.75	25.11 ± 0.68
IV	Group IV (CpAE Treatment)	20.88 ± 0.57	23.50 ± 1.047	23.72 ± 1.51
Stat		**NS**	**NS**	**NS**
CD				

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.4.7 Serum Alanine Transaminase (ALT, IU/L) in experimental rats

The mean ALT concentration in group I, II, III and IV on day 0, 14^{th},28^{th} ranged between30.93 to 33.22 , 32.41 to 90.32, 30.19 to 66.55 , and 28.88 to 66.44 IU/L, respectively.

Statistically significant increase was observed in group II on day 14^{th} and 28^{th} as against its day 0 value. The elevation was observed to be significant in group II, III and IV on day 14th and 28th of the experiment whin compared with the ALT level in group I animals shown in table 24 and figure 11.

On day 14th the mean ALT level in group II, III and IV was statistically significant when compared with the day 14th value in group I animals.

Significant reduction in the mean ALT level was observed in treatment group animals i.e. group III and IV as against the values in group II on day 28th of the experiment indicating the positive effect of the treatment.

The values reported in the present investigation are in association with the findings of Nwangwa (2013)

Table 24: Mean Serum Alanine Transaminase (ALT, IU/L) in experimental rats

Groups of rats	Serum Alanine Transaminase (ALT, IU/L)		
	Intervals of study		
	0^{th} day	**14^{th} day**	**28^{th} day**
I	30.93 ± 1.35	33.22 ± 1.30^{b}	32.60 ± 1.71^{c}
II	32.41 ± 1.29	87.69 ± 1.78^{a}	90.32 ± 8.51^{a}
III	30.19 ± 1.61	66.55 ± 4.27^{a}	56.36 ± 2.12^{b}
IV	28.88 ± 1.48	66.44 ± 1.85^{a}	62.02 ± 0.88^{b}
Stat	NS	**S**	**S**
CD values		**CD (0.01) = 19.595** **CD (0.05) = 14.525**	**CD (0.01) = 20.321** **CD(0.05) = 15.062**

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

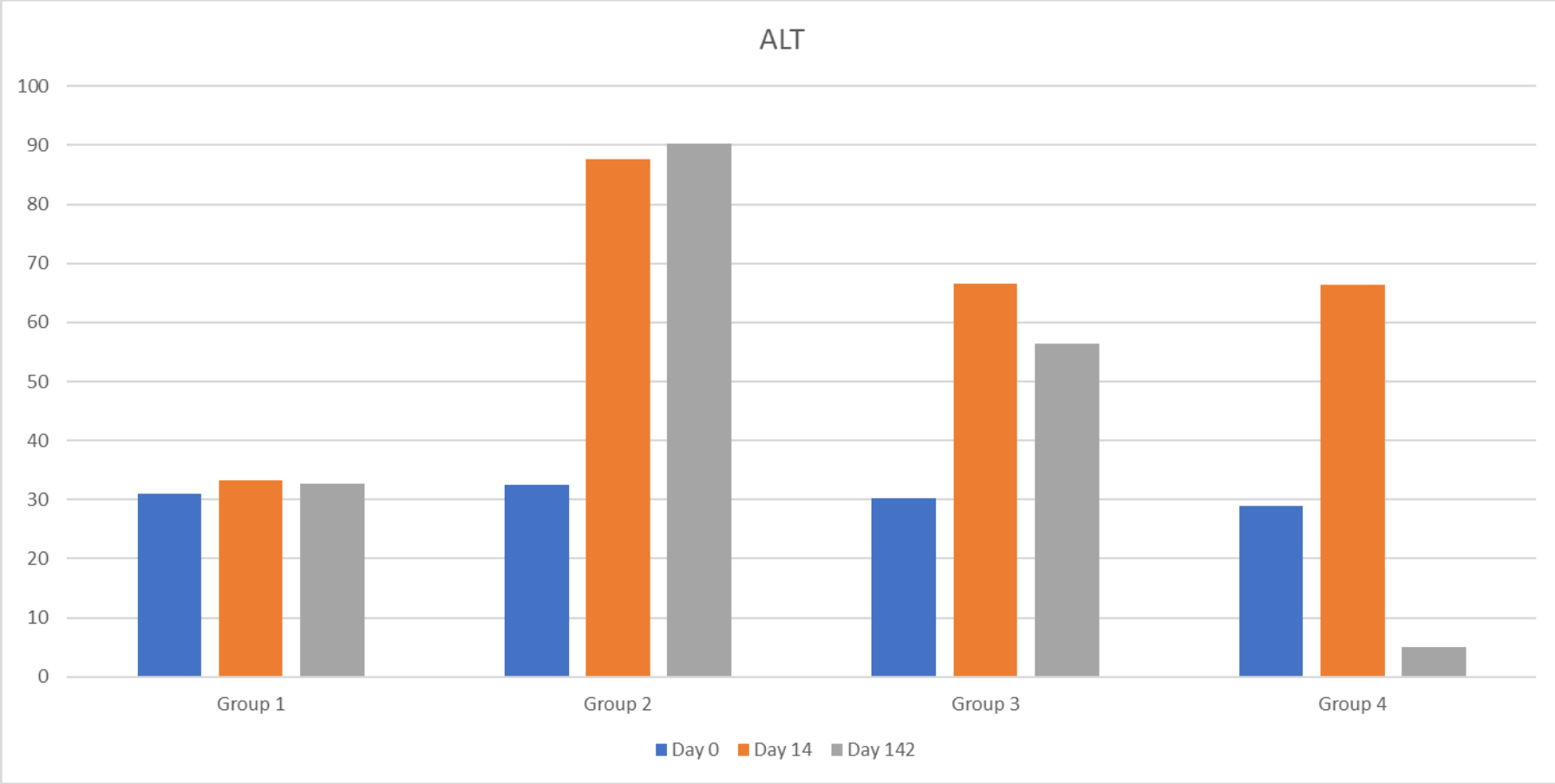

Fig.11 Mean ALT at the different intervals

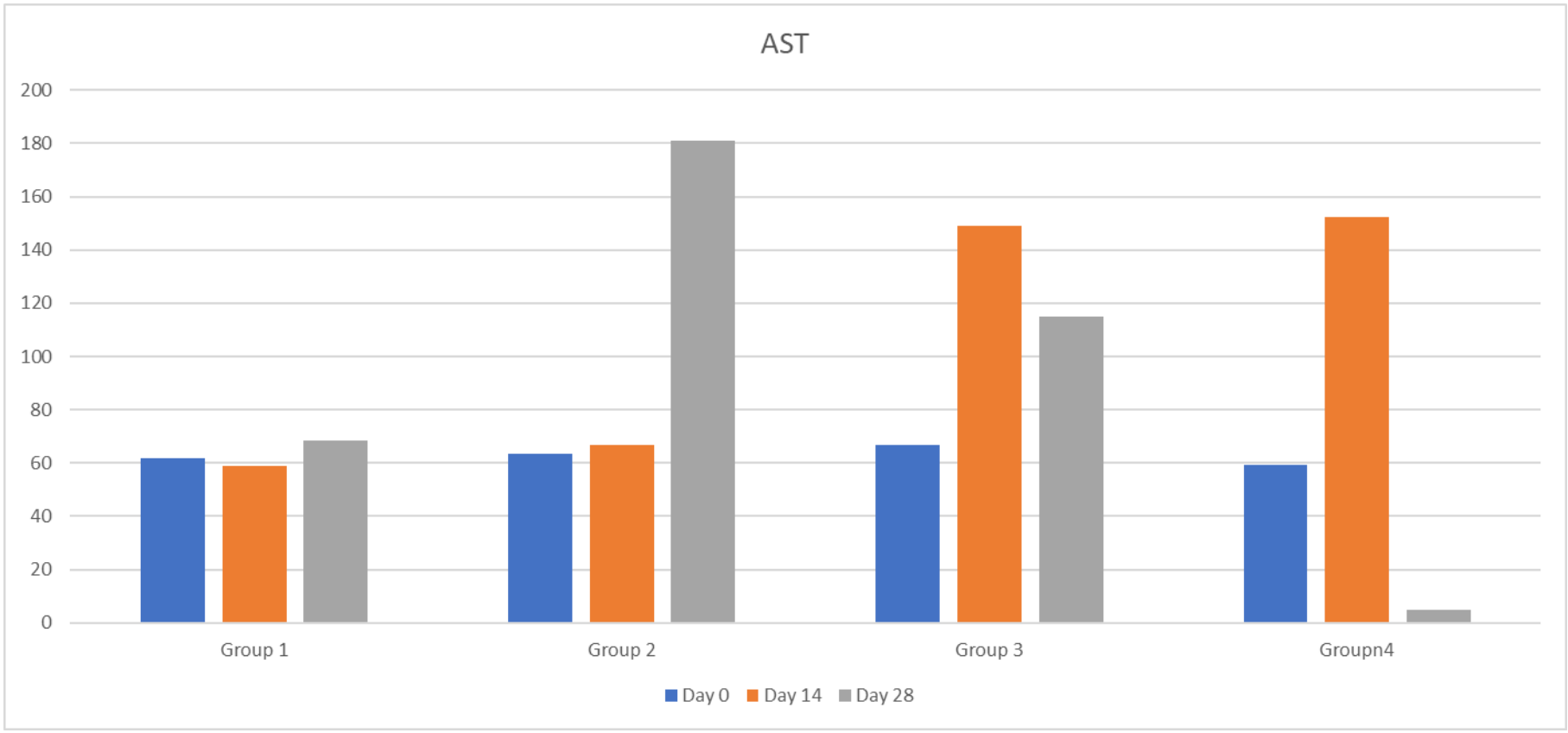

Fig.12 Mean AST at the different intervals

4.4.8 Serum Aspartate Transaminase AST (IU/L) in experimental rats.

The mean AST concentration in group I, II, III and IV on day 0, 14th,28th ranged between 59.01 to 68.32 , 63.49 to180 , 66 to148 , and 59.46 to 152.27 IU/L, respectively.

Statistically significant increase was observed in group III and IV on day 14th and 28th as against its day 0 value. The elevation was observed to be significant in group II, III and IV on day 14th and 28th of the experiment when compared with the AST level in group I animals shown in table 25 and figure 12.

The AST values in group I and II and group II and IV were at par with each other on day 14th of the experiment. However the values on day 28th were observed to be significantly higher in group II, III and Iv when compared with the day 28th value in group I animals.

Table 25: Mean Serum Aspartate Transaminase (Mean ± S.E. IU/L) in experimental rats

Groups of rats	Serum Aspartate Transaminase (Mean ± S.E. IU/L)		
	Intervals of study		
	0th day	14th day	28th day
I	61.71±2.90	59.01 ±2.64[b]	68.32 ±2.56[d]
II	63.49±2.04	66.82 ±3.34[b]	180.89 ±1.73[a]
III	66.78±3.13	148.90 ±1.71[a]	114.90 ±1.19[c]
IV	59.46±2.84	152.27 ±1.79[a]	130.64 ±0.88[b]
Stat	NS	S	S
CD values		CD(0.01) = 23.908 CD(0.05) = 17.721	CD(0.01) = 6.715 CD(0.05) = 4.978

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) ($p<0.05$)

4.6 Relative organ weight (Table 26; Fig 13)

4.6.1 Relative weight of Liver

The values of mean relative weight of liver in group I to IV were observed to be 3.95, 4.03, 3.66 and 3.77gm, respectively. No significant alteration in relative organ weight was observed.

4.6.2 Relative weight of Heart

The values of mean relative weight of heart in group I to IV were observed to be 0.45, 0.82, 0.33 and 0.40 gm respectively. no significant alteration in relative organ weight was observed.

4.6.3 Relative weight of Kidney

The values of mean relative weight of kidney in group I to IV were observed to be 0.46, 0.92, 0.91, 0.91 gm in group I, II, II and Iv animals respectively. no significant alteration in relative organ weight was observed.

4.6.4 Relative weight of spleen

The values of mean relative weight of spleen in group I to IV were observed to be 0.24, 0.28, 0.26 and 0.25, gm respectively. All the values were found to be does not vary significantly.

Table 26: Relative organ weight (G) in experimental rats

Gr.	Group and treatment	Day 28			
		Liver	Heart	Kidney	Spleen
I	Group I (positive control)	3.95 ± 0.17	0.45 ± 0.86	0.46 ± 0.86	0.24 ± 0.01
II	Group II (Negative control)	4.03 ± 0.22	0.82 ± 0.08	0.92 ± 0.016	0.28 ± 0.02
III	Group III (Statin)	3.66 ± 0.082	0.33 ± 0.047	0.91 ± 0.015	0.26 ± 0.02
IV	Group IV (CpAE Treatment)	3.77 ± 0.17	0.48 ± 0.04	0.91 ± 0.011	0.25 ± 0.01
Stat		NS	NS	NS	NS
CD					

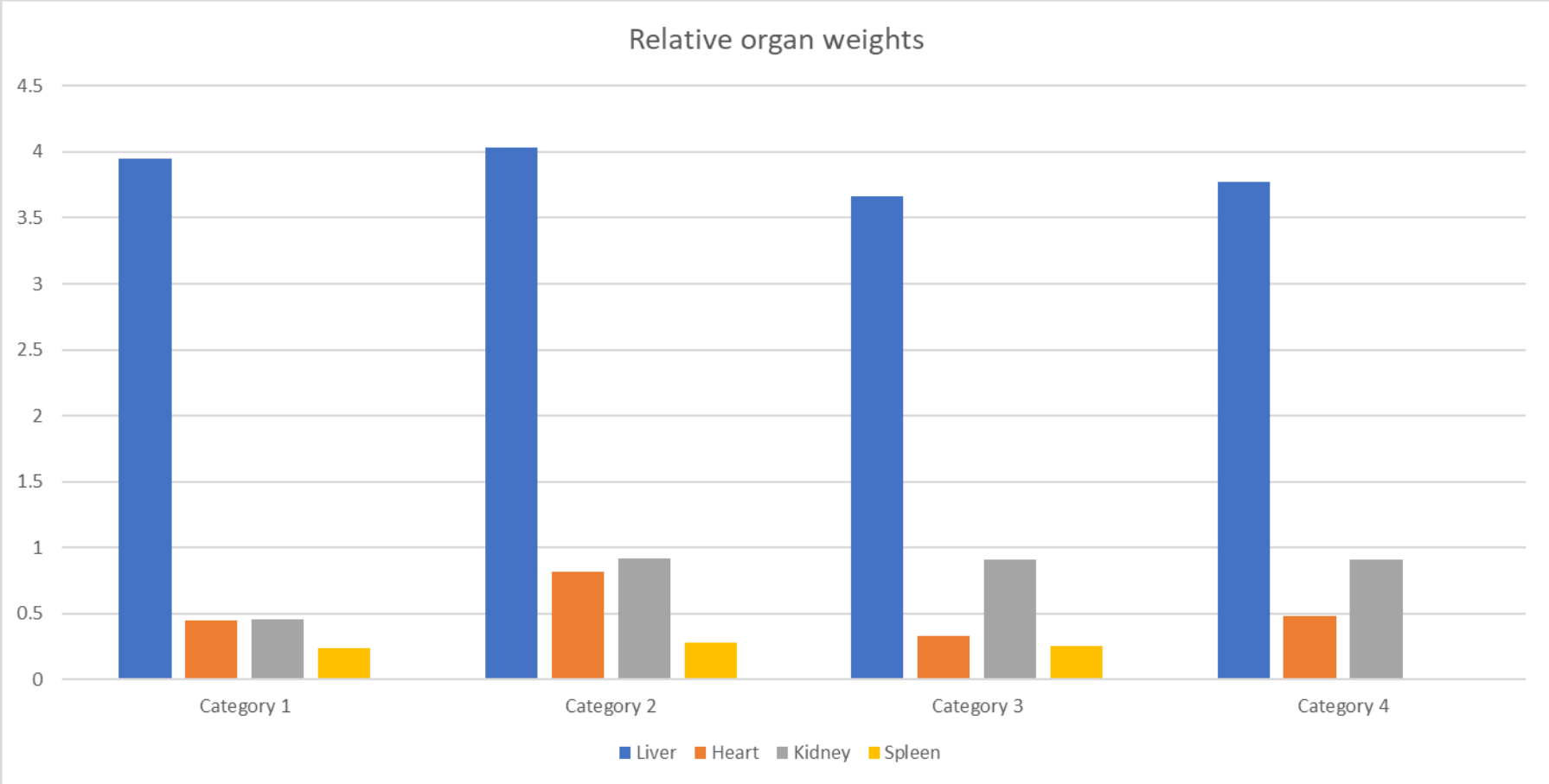

Fig.13 Relative organ weight of (grams)

Superscript a, b, c, d show significant difference within the column (between different groups of specific days) (p=0.05)

4.7.1 Gross pathological examination

At the end of the experiment the rats were sacrificed for the gross pathological changes for the evaluation of the organs abnormality if any. There was no any specified abnormality observed in the organs, but the liver from group II showed some enlarged liver as they were fed on high fat diet there may be accumulation of fats in the hepatic cells.

4.7.2 Histopathological examination

The organs Heart, Liver, kidneys and spleen of the rats used for the project were sacrificed for observing the histopatholigical alterations.

a) Heart : The sections of heart from group I did not show as much changes as compared to Group II. The sections of group II, study when subjected for histopathological evaluation observed minimal to mild congestion and mononuclear cell infilteration.

The sections of group III and IV and I did not show any remarkable histoarchitectural alteration. (Plate 7 to 10).

b) Liver: The histopathological examination of liver rats of group II, negative control revealed that mild to moderate, focal to multifocal congestion, dilatation of sinosidal spaces, circumscribed variable sized vacuoles indicating fatty changes and degenerative changes which indicated induction of ill effect of fatty diet. The liver samples of group III statins which were fed with fatty diet did not showed any appreciable, histomorphological alteration except minimal to mild congestion, and degenerative changes, however the liver samples of group IV showed minimal to mild focal congestion and degenerative changes and fatty changes. The rats fed with fatty diet and treated with papaya restored the histoarchitecture of liver at considerate level however it was not at that

level with the restoration achieved with treatment with statin and same was biochemical values. (Plate 11 to 14).

c) Kidney: On the histopathological evaluation of kidneys from the rats of group II at the end of study period showed mild to moderate degenerative changes, specially cystic degenerative changes focal to multifocal congestion and occasionally there was presence of hyaline casts in lumen of tubules. Also the mononuclear cell infilteration was observed in kidney sections. The kidney of rats from group III and IV did not revealed any specific changes except minimal to focal congestion and degenerative changes, indicating beneficial effects of treatment given. (Plate 15 to 18).

d) Spleen: On histopathological evaluation of spleen of rats of the experimental groups did not show any appreciable histopathological changes except minimal focal congestion in section of spleen from rats of group II. (Plate 19 to 22).

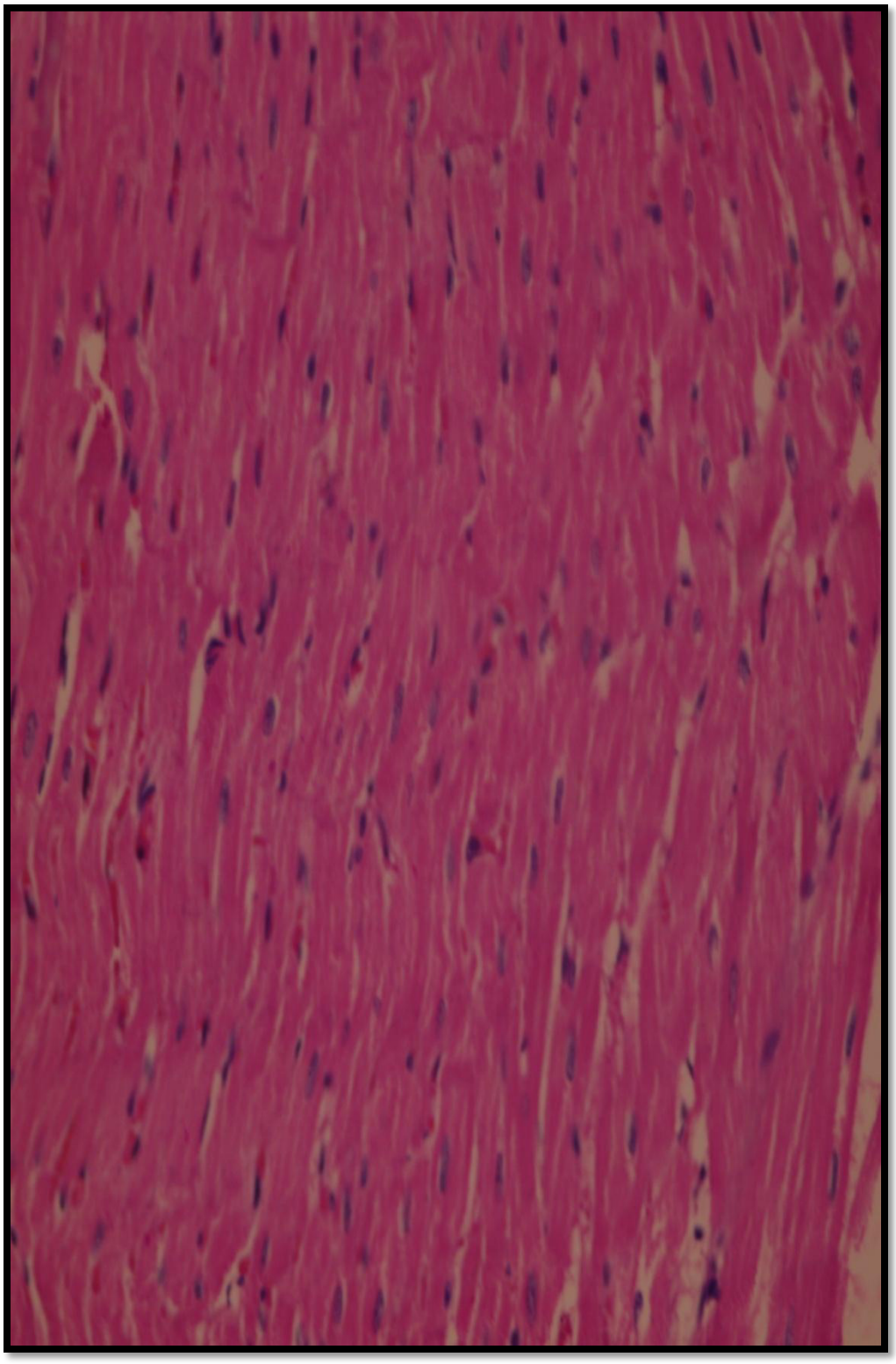

Plate 7. Group I heart section at 400X H and E stain

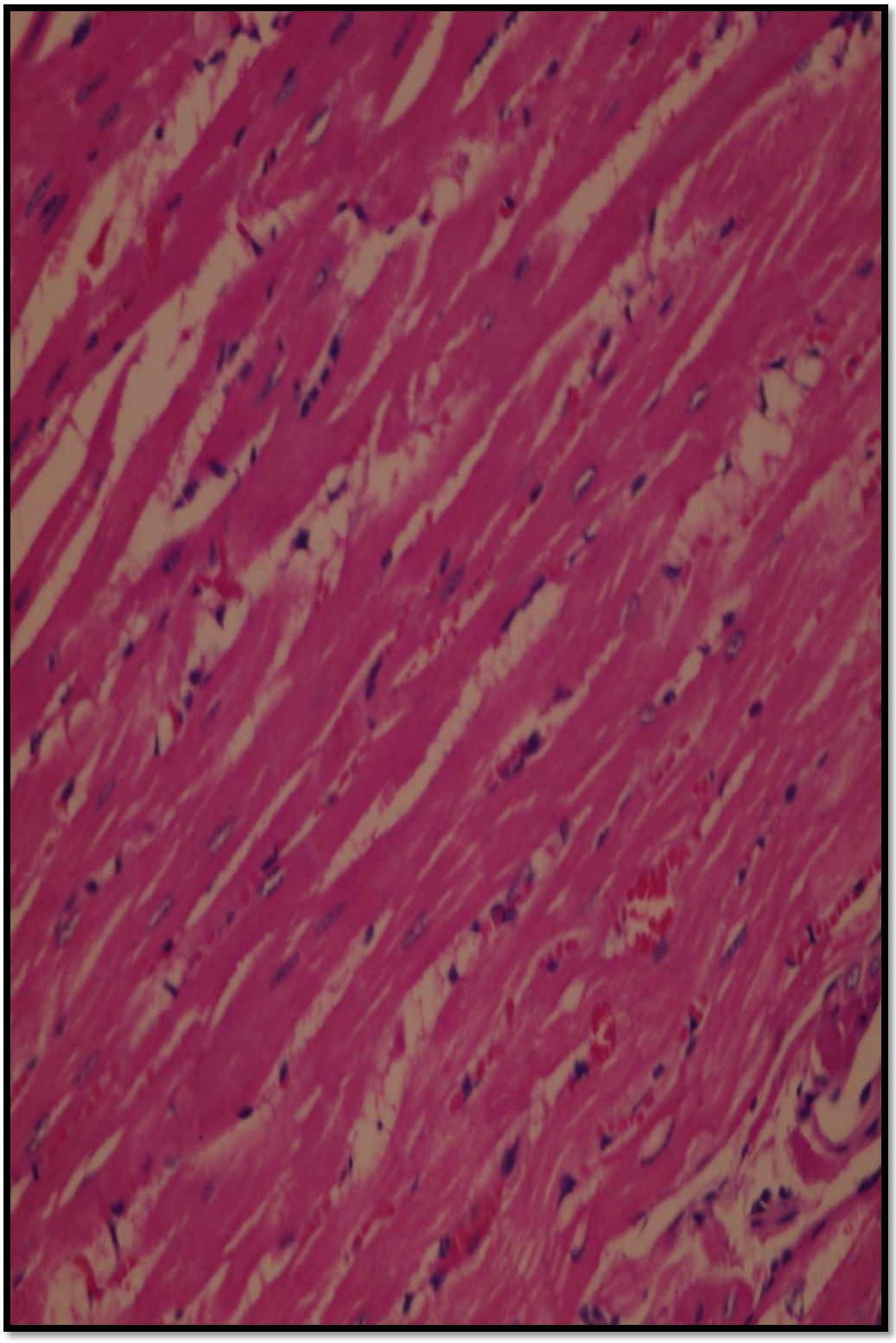

Plate 8. Group II heart section with fatty changes at 400X H and E stain

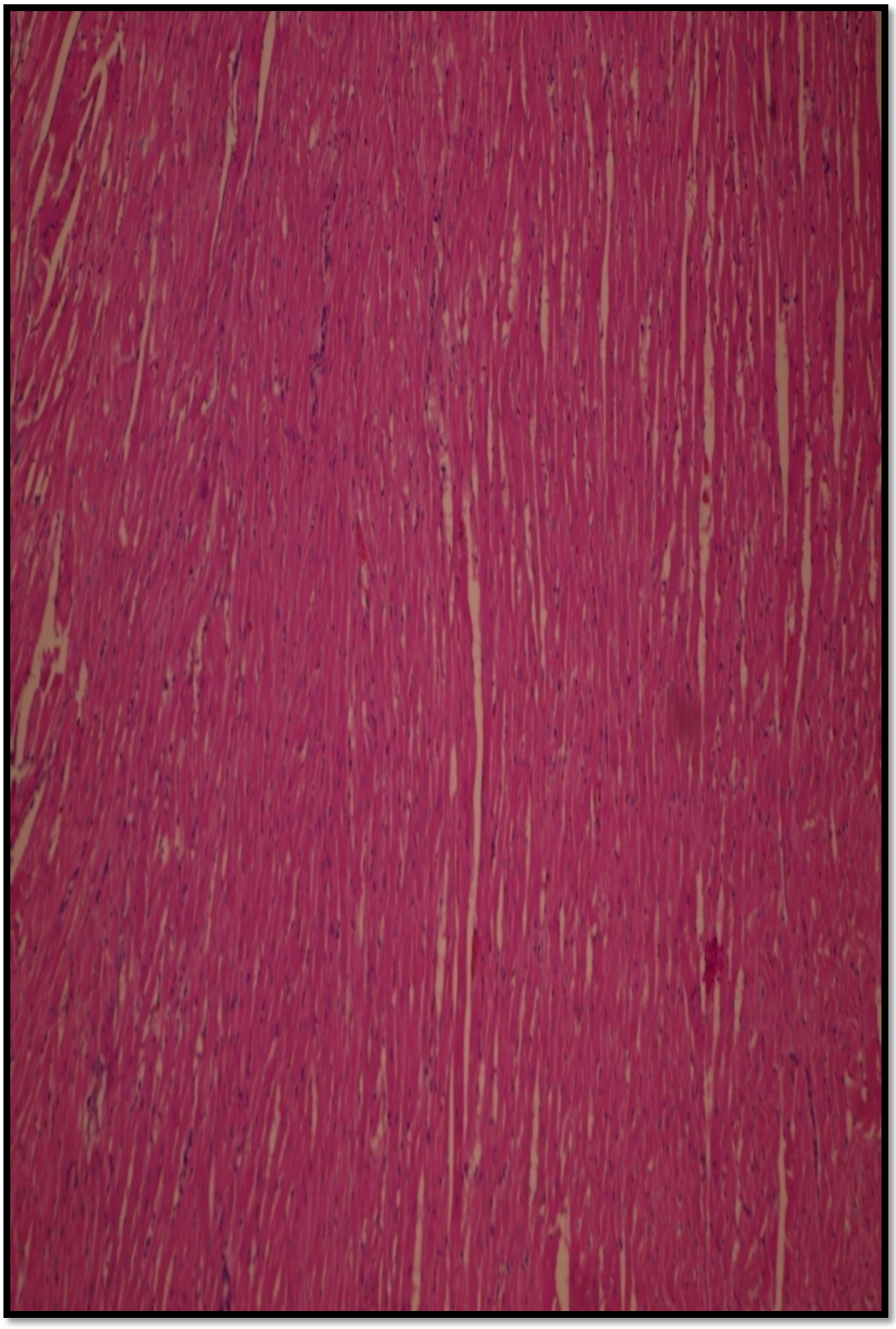

Plate 9. Group III heart section with mild fatty changes at 400 X H and E stain

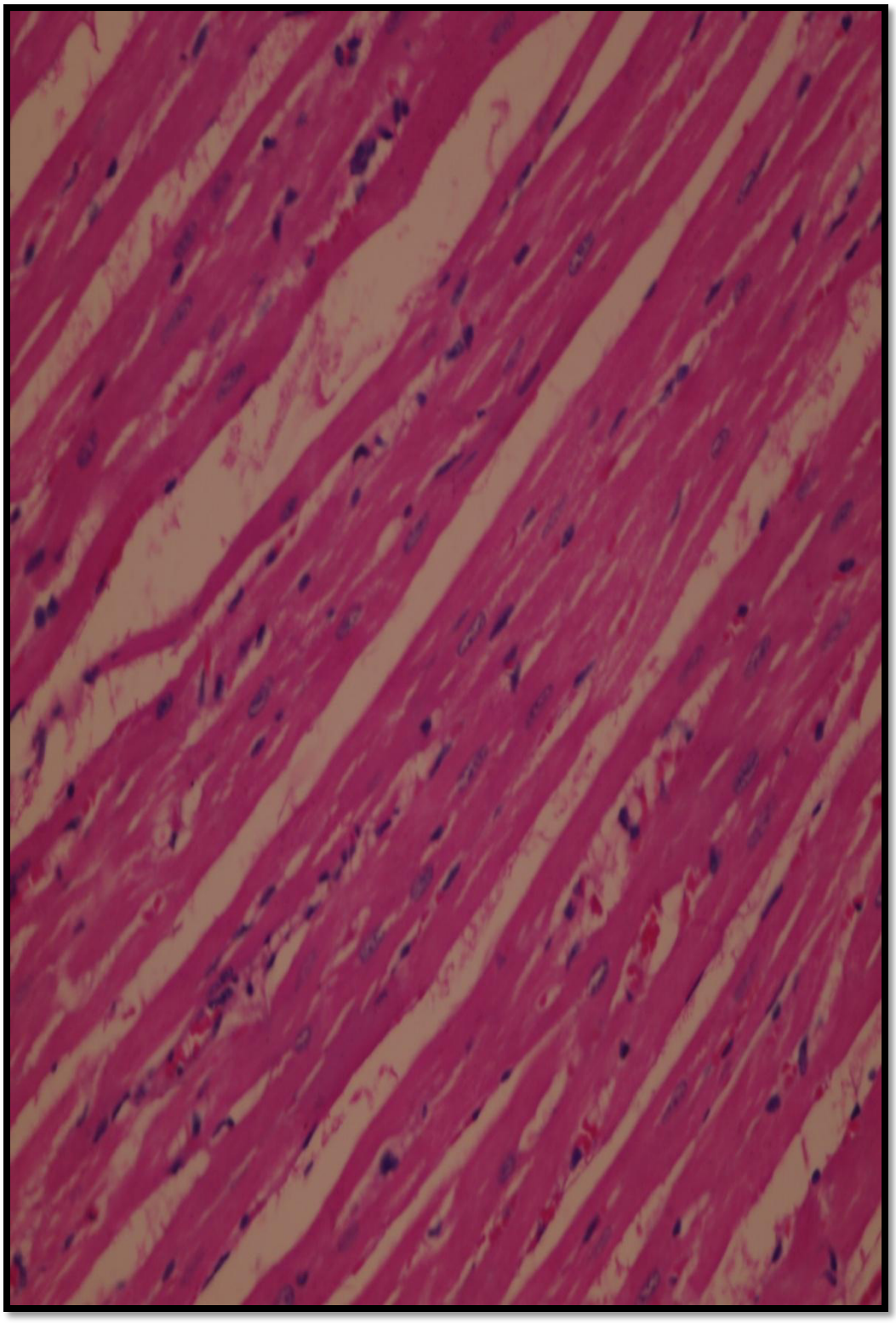

Plate 10. Group IV heart section with mild fatty changes at 400X H and E stain

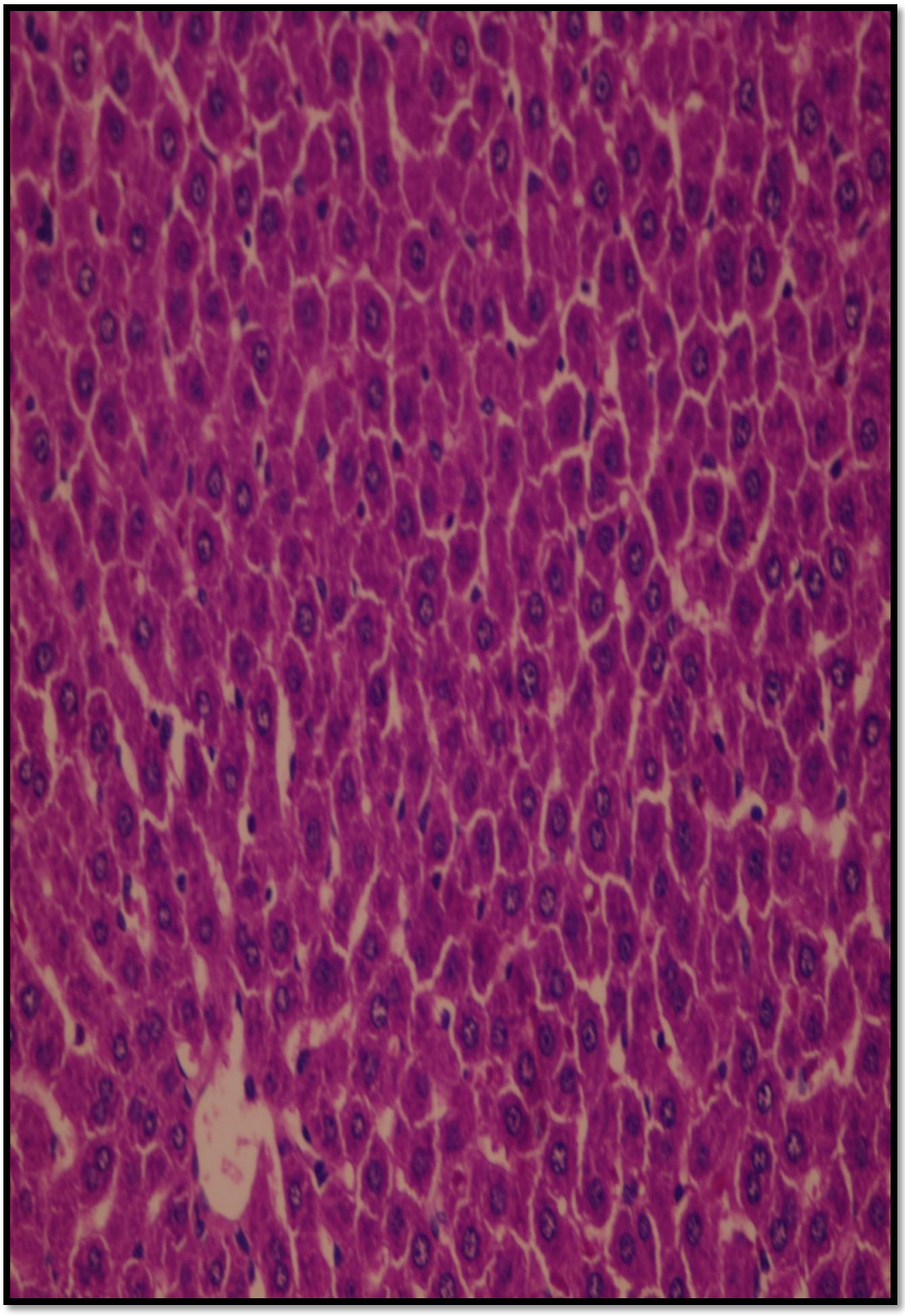

Plate 11. Group I liver section at 400X H and E stain

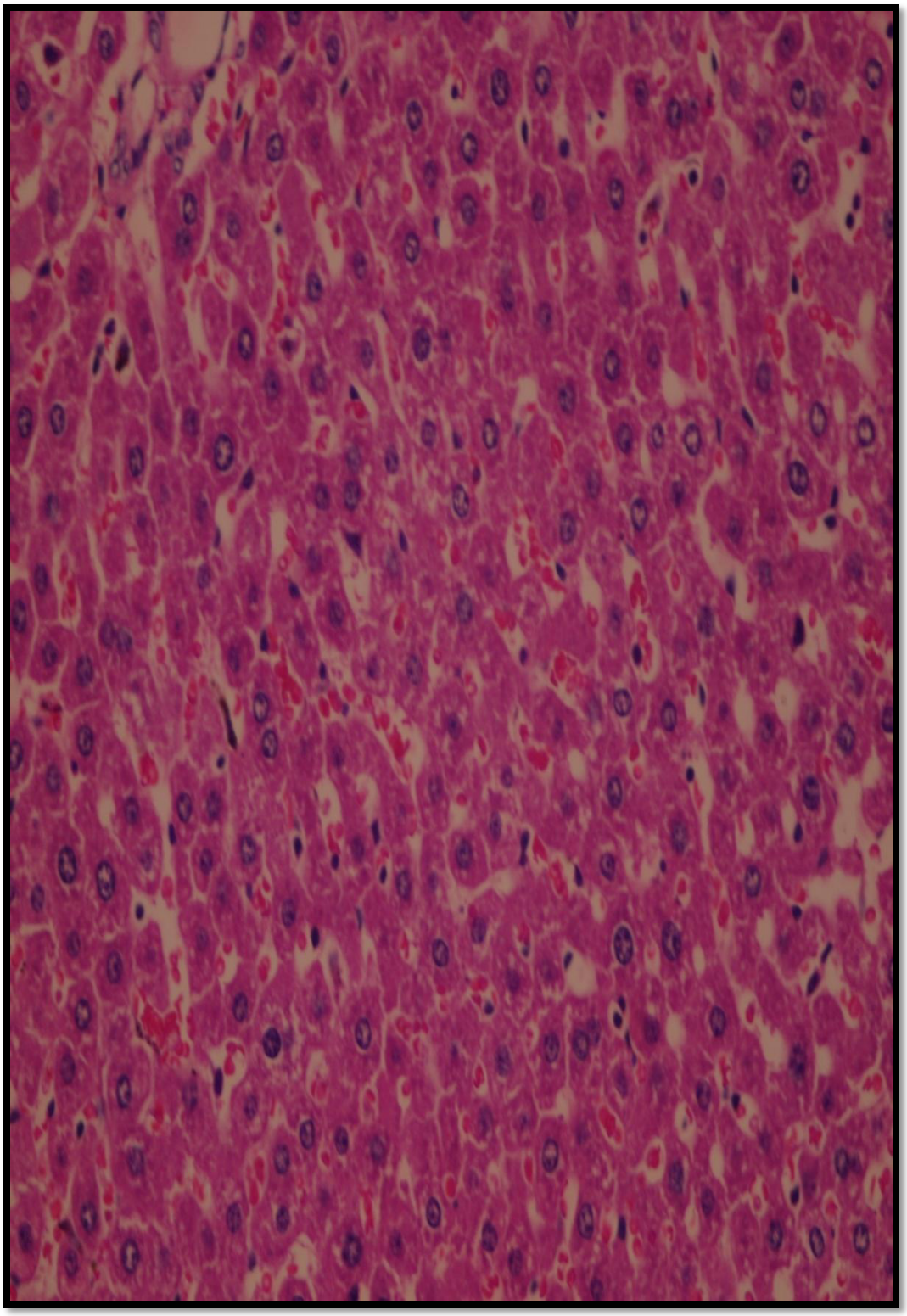

Plate 12. Group II liver section with fat deposition at 400X H and E stain

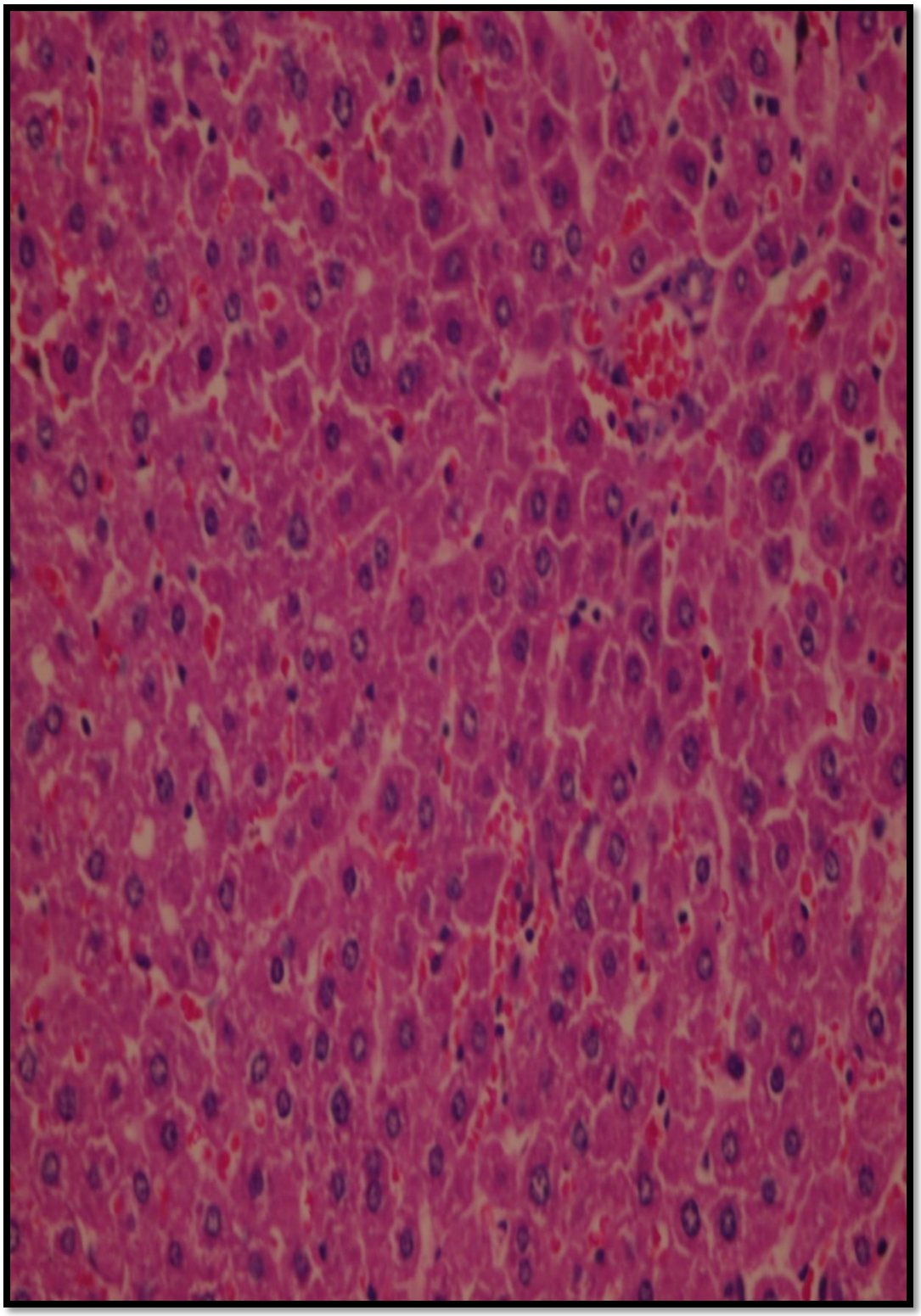

Plate 13. Group III liver section with slight fatty changes 400X H and E stain

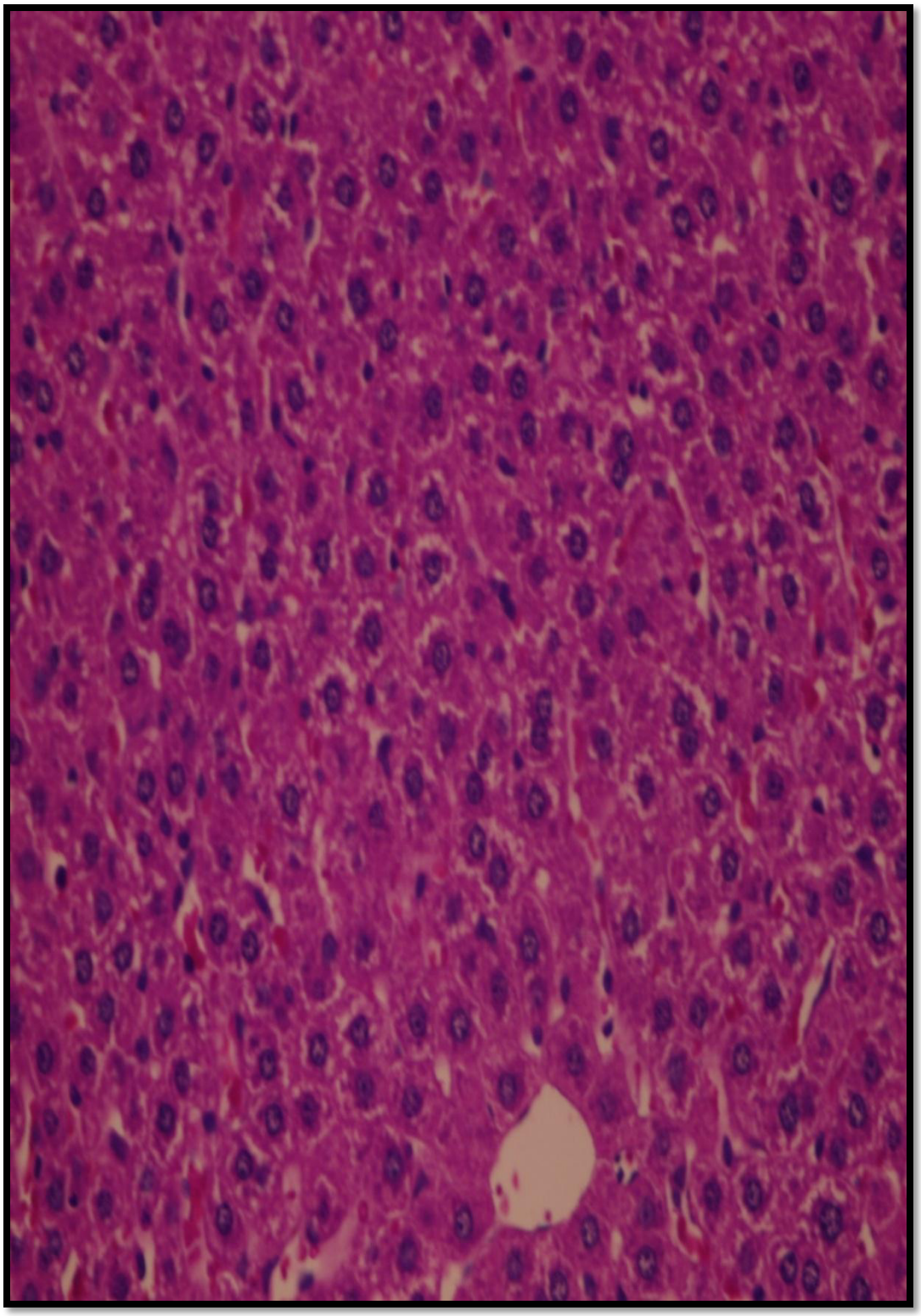

Plate 14. Group IV liver section with slight fatty changes at 400X H and E stain

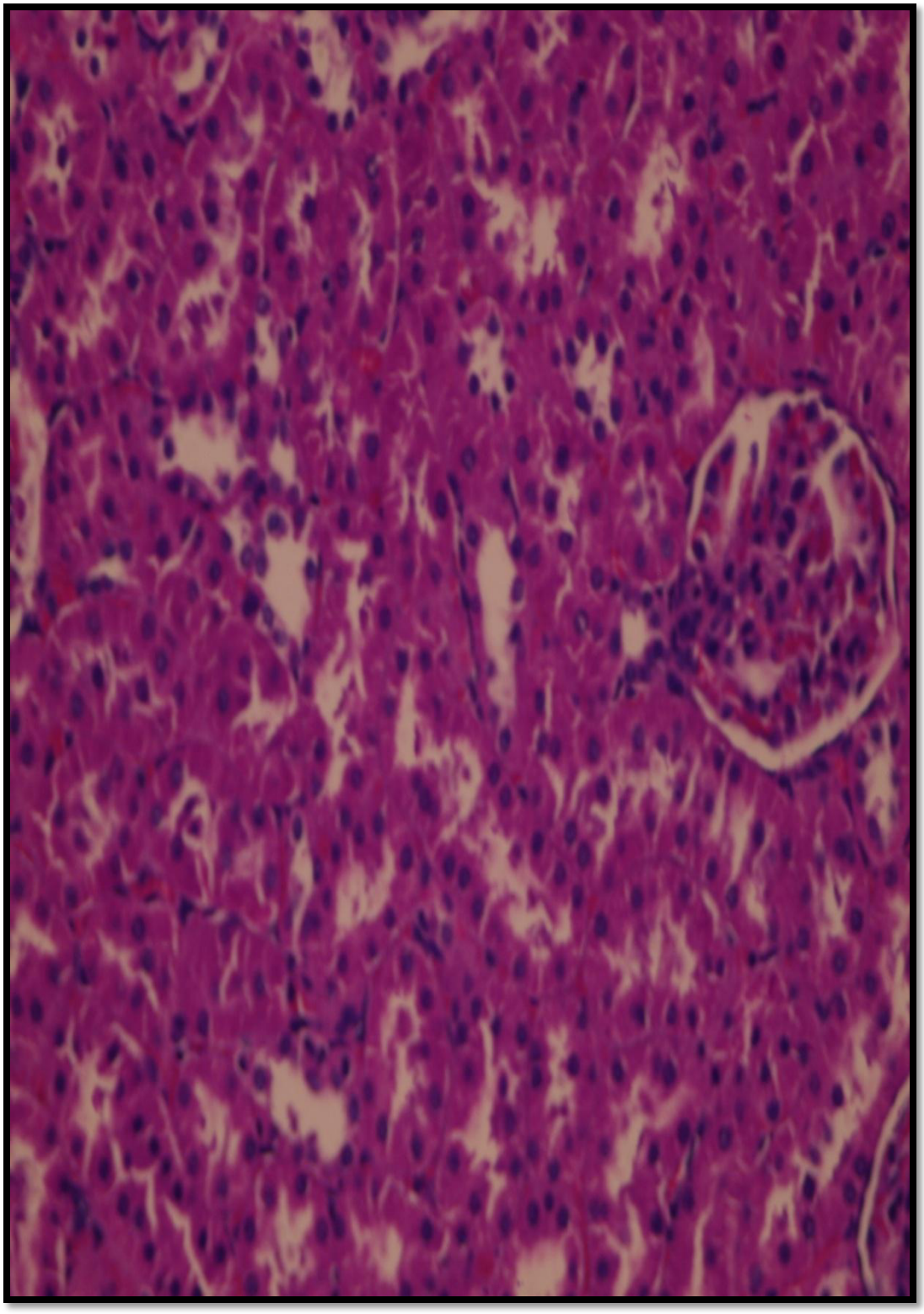

Plate 15. Group I Kidney section at 400X H and E stain

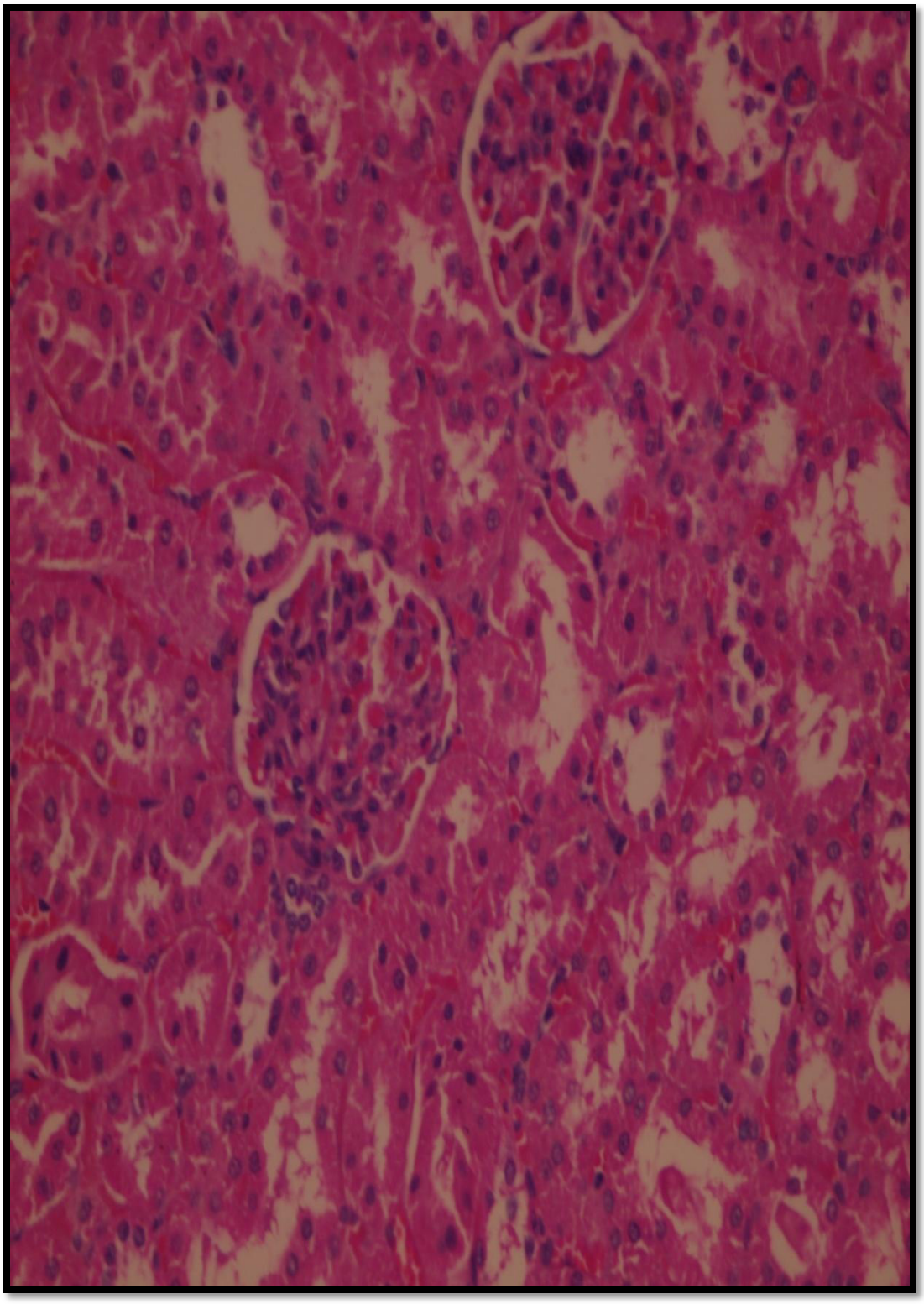

Plate 16. Group II kidney section with fatty changes in tubules at 400X H and E stain

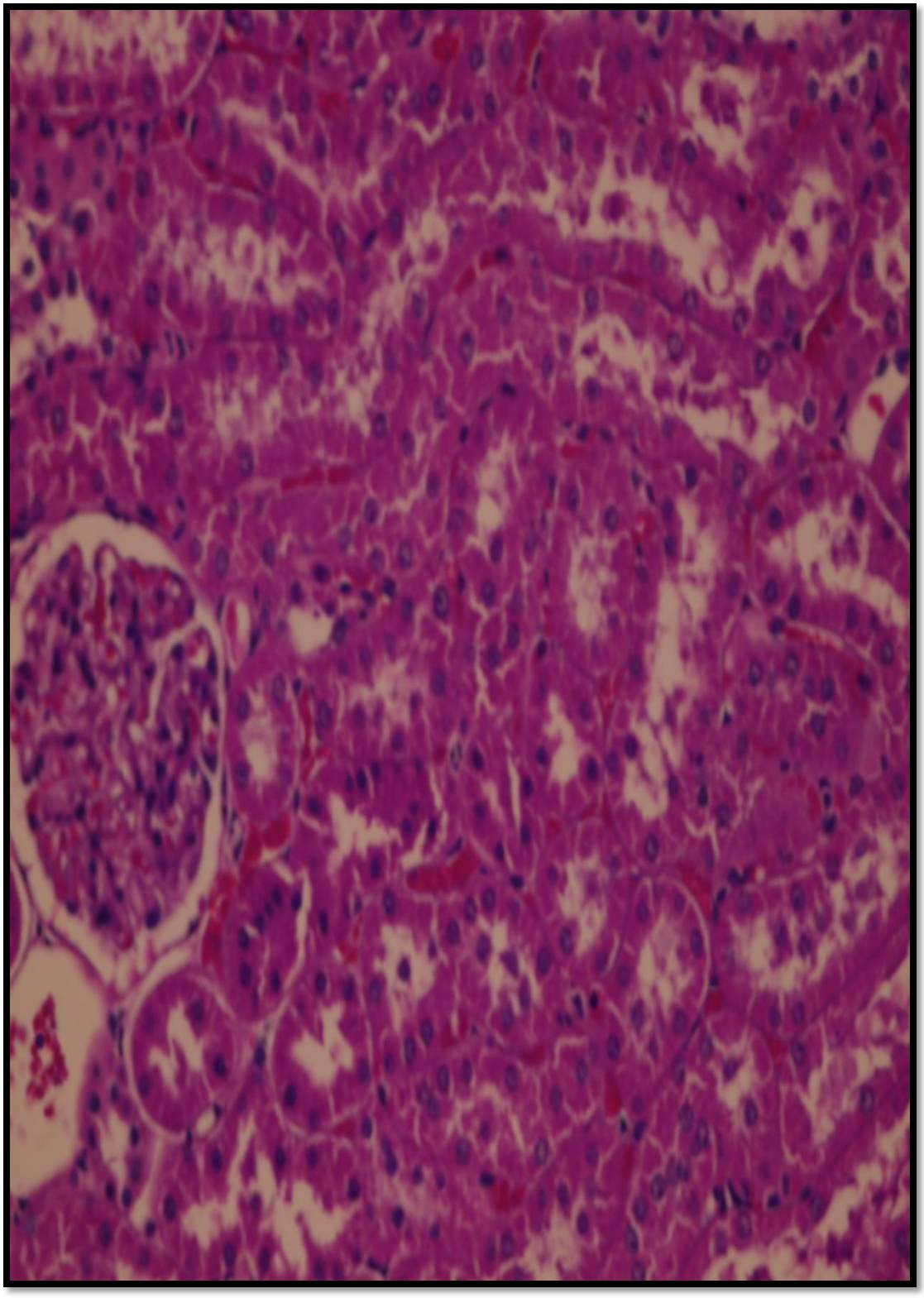

Plate 17. Group III kidney section with slight fatty changes at 400X H and Estain

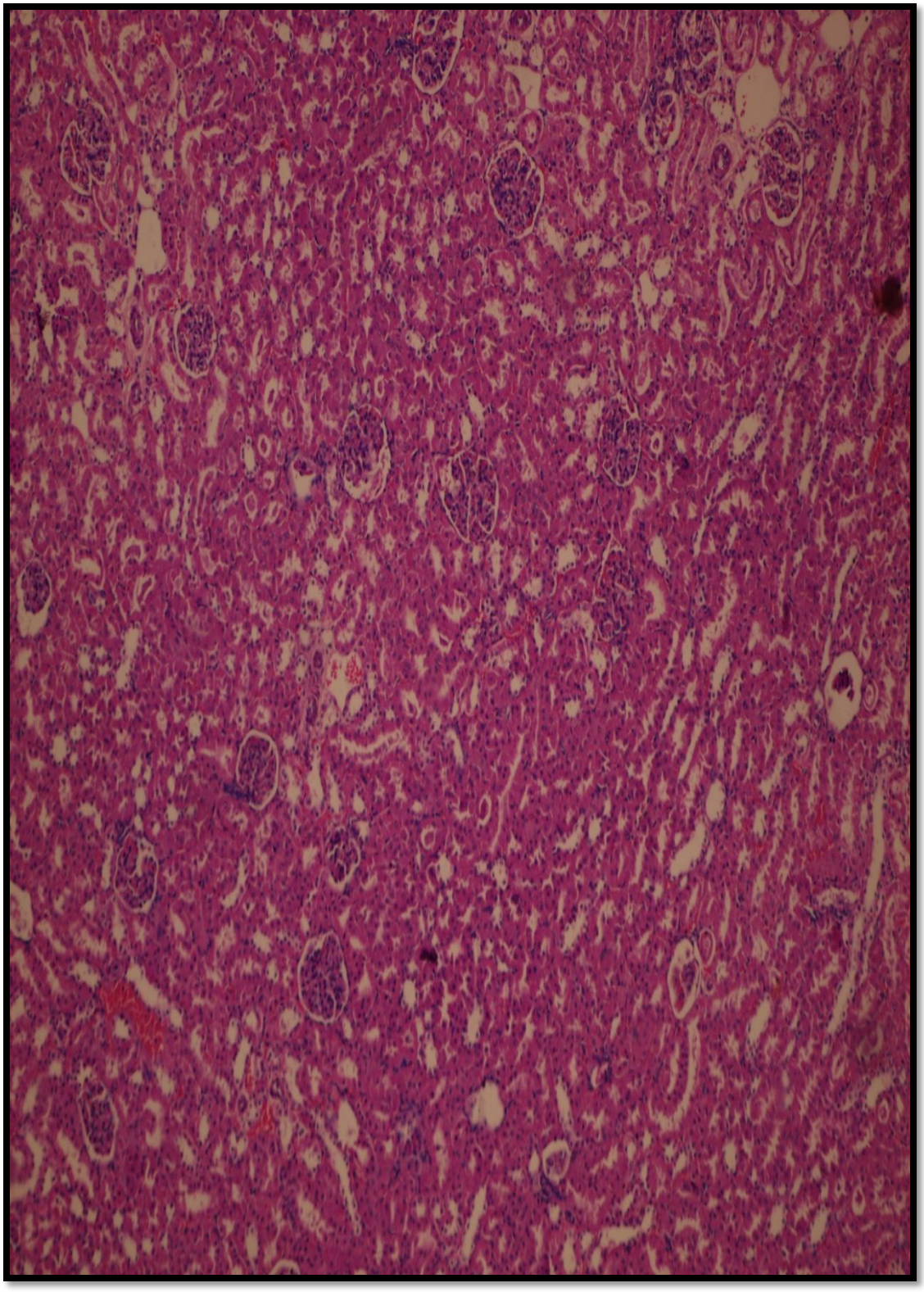

Plate 18. Group IV kidney section with slight fatty changes at 100X H and E stain

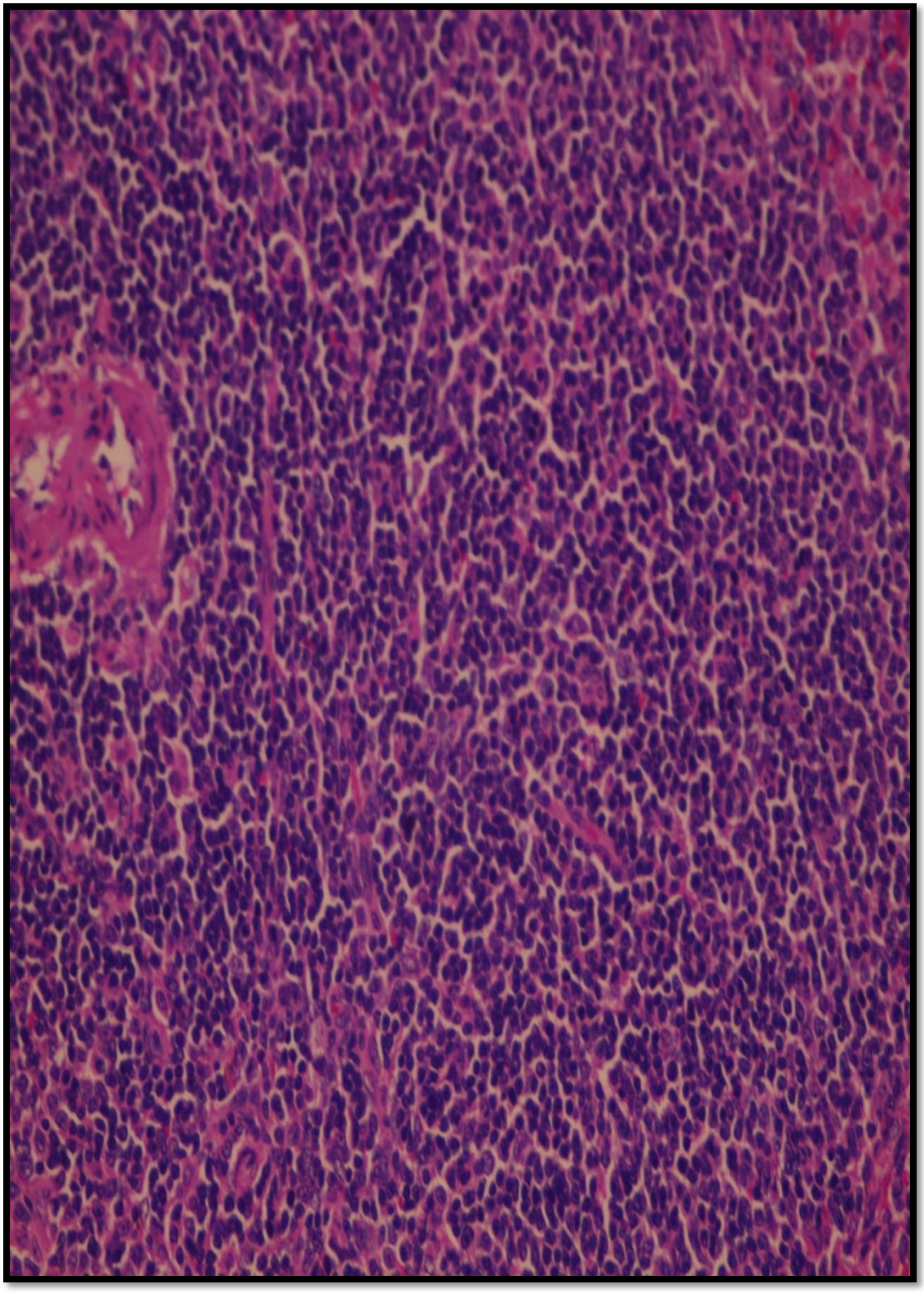

Plate 19. Group I spleen section at 400X H and E stain

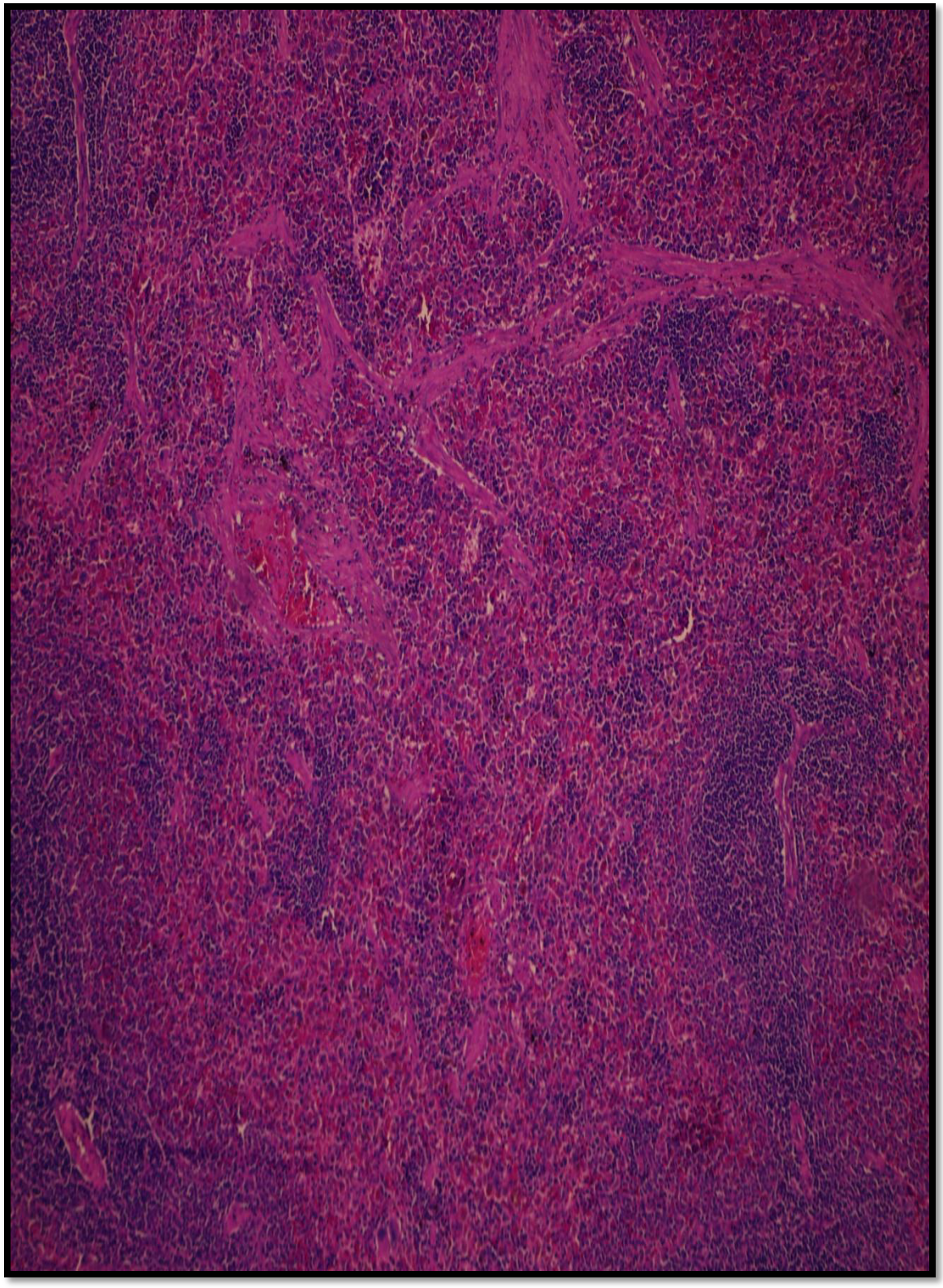

Plate 20. Group II spleen section at 100X H and E stain

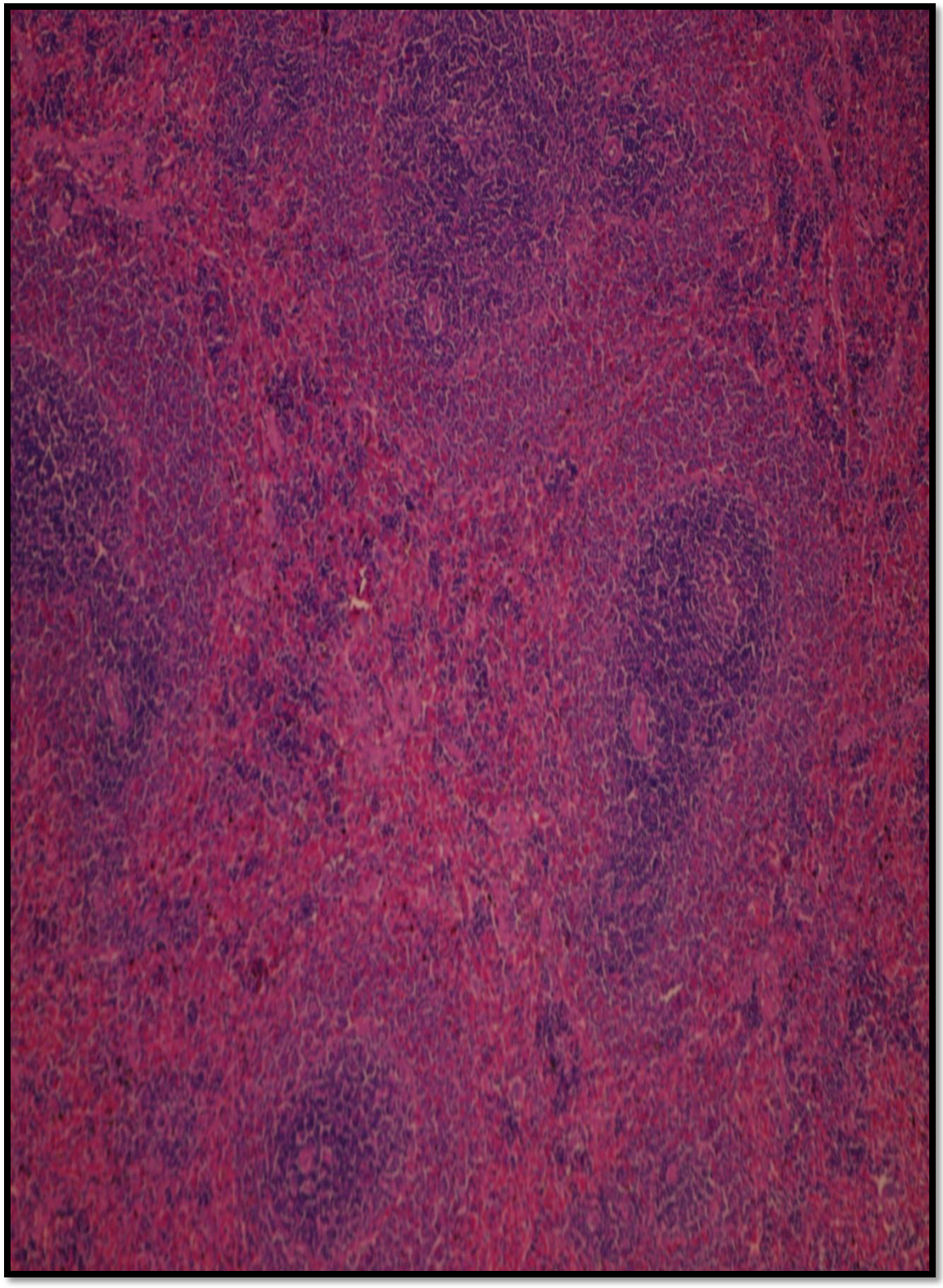

Plate 21. Group III spleen section at 100 X H and E

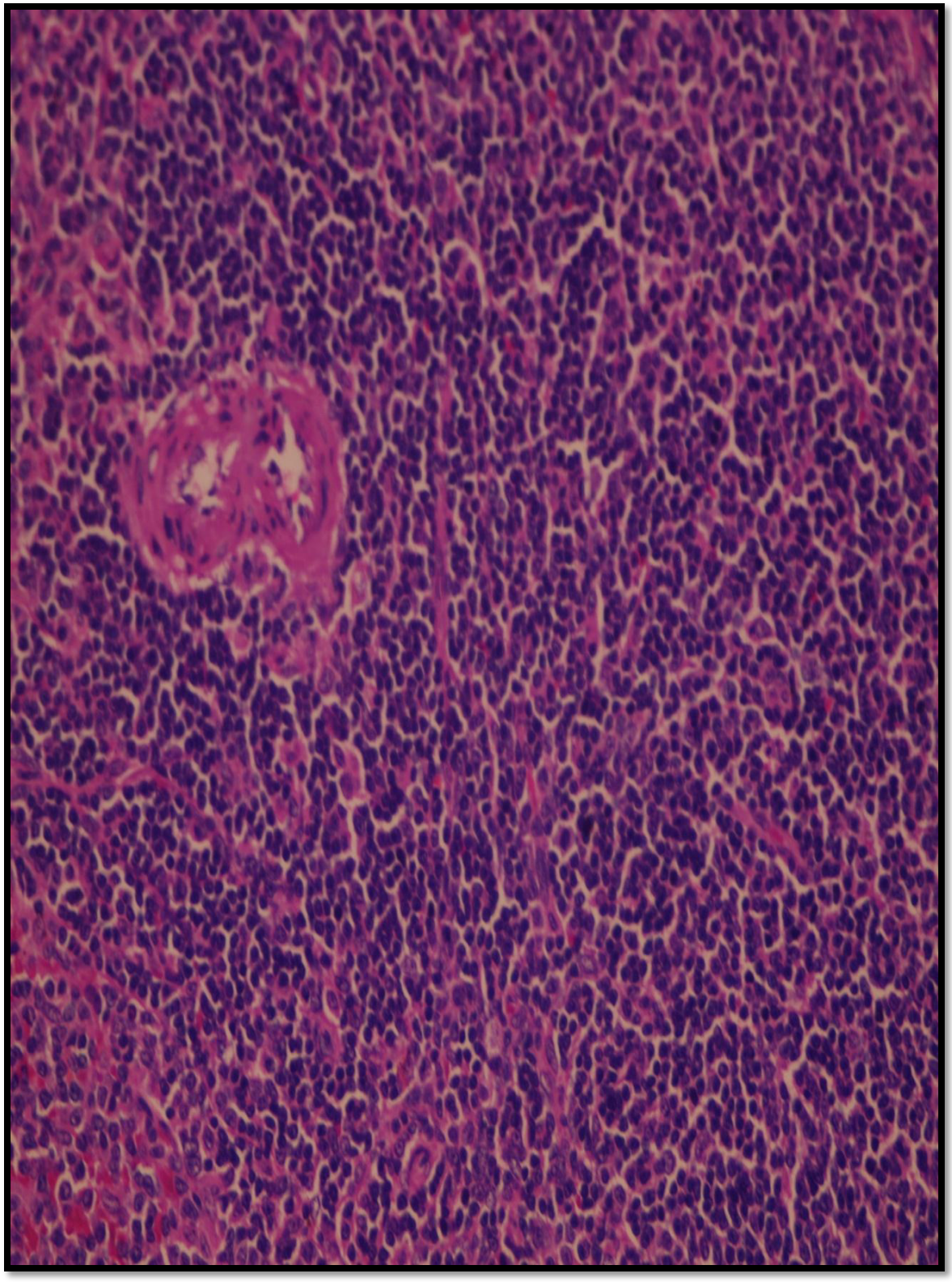

Plate 22. Group IV spleen section at 400X H and E stain

Summary and Conclusions

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was performed to study the detailed curative effect of *Carica papaya* aqeous extract against the atherosclerotic changes induced due to high fat diet in Wistar rats.

Total 32 Wistar rats were taken for the experiment and were divided into 4 groups, and in each groups there were eight Wistar rats and they were marked with picric acid as head, back, tail and unmarked, the groups were given names as group I, II, III, and IV.

Group I was healthy controlled, II was given high fat diet with no treatment, III was standard group given with statin drug 0.5 mg/kg body weight. tablet was mixed with distilled water as standard treatment and group IV was given aqueous seeds extract of *Carica papaya* at the dose rate of 60mg/ kg body weight.

Carica papaya fruit was collected from local market. Then the fruit was cut into 2 parts and seeds were separated from the fruit. The seeds were washed properly and gently thoroughly under tap water for two times and completely air dried at room temperature and the dried seeds were pulverized into fine powder using domestic mixer grinder, the seeds were then used for the aqueous extraction. The extract was weighed and stored in air tight and water-proof containers kept in a refrigerator at 4 ºC.

The effect of high fat diet and different treatments on the rats resulted in induction of impairment in the lipid profile which is indicative of atherosclerotic changes. Behavioral changes, Blood haematological parameters like haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC) were investigated. Biochemical parameters, serum alanine transaminase (ALT), serum aspartate transaminase (AST), creatinine (Cr), blood urea nitrogen (BUN) were investigated.

Along with blood haematological and biochemical parameters, lipid profile status of rats have also been studied.

Gross pathology, relative organ weight and histopathological changes in liver, kidney, heart and spleen were investigated in experimental rats.

During the experiment, rats from group I (healthy control) did not show any significant behavioral changes, rats from group II which were only fed with high fat diet with no treatment showed symptoms of dullness, restlessness, lethargic, and always lay back position depression.

There was significant decline in level of blood haemoglobin and total erythrocyte count in the group II. In group III, and IV showed the good results.

The group III and IV have showed elevation in TLC, TEC, neutrophil count. There was no much significant increased in serum ALT, AST, ALP, Cr and BUN level. However elevation in the lipid profile was found and the positive effects of the treatment i.e. reduction in the lipid profile in treatment groups was also found.

Conclusions :

From the observations reported in present investigation it can be concluded that use of high fat diet containing 1% cholesterol can induce hyperlipidemia.

An increase in heamaglobin and TEC level and decrease in cholesterol levels is observed in the treatment group indicates favourable effect of treatment with aqueous seeds extract of *Carica papaya*@ dose rate of 60mg/kg body weight orally.

The treatment with statin 0.5mg/kg oral dose alone showed restorative changes in the serum Chloesterol, High density lipo protein, Low density lipoprotein, Triglycerides however no significant alterations were observed in Creatinine and Blood Urea Nitrogen parameters.

No visible behavioural changes observed in healthy controlled group and other treatment group.

The histopthological changes in liver has shown the effect of treatment with statin with the dose of 0.5mg/kg body weight.

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Vitae

VITAE

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She has actively participated in different other extension activities carried out by the Department. During her B.V.Sc and M.V.Sc courses, she participated in different National Programs like National Service scheme (NSS). She also participated in different Conferences, Seminars, Symposia and Training Programme, Workshops from different disciplines like Veterinary Pathology,and Veterinary Pharmacology.

Thesis Abstract

प्रबंध सारांश

प्रबंधाचे शीर्षक	:	**विस्तार उंदरांचा हृदयावर पपईचा परिणामाचा अभ्यास**
विद्यार्थीचे नाव	:	क्षितिजा जगदीश मोरे
मार्गदर्शक	:	डॉ. एस. आर. राजुरकर प्राध्यापक व विभाग प्रमुख, औषधनिर्माण व विषशास्त्र विभाग पशुवैद्यक व पशुविज्ञान महाविद्यालय,परभणी
प्रदान करण्यात येणारी पदवी	:	एम. व्ही. एस. सी.
प्रदान करण्यात येणारे वर्ष	:	२०२१
मुख्य विषय	:	औषधनिर्माण व विषशास्त्र विभाग
प्रबंधाचे एकुण पाने	:	६२
सारांशातील एकुण शब्द	:	२०३
विद्यार्थ्यांची स्वाक्षरी	:	
विभाग प्रमुखाची स्वाक्षरी	:	

डॉ. एस. आर. राजुरकर
प्राध्यापक व विभाग प्रमुख,
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परभणी

सारांश

सदरील संशोधनात पपईच्या बियाणं पासून काढलेला अर्केच्या स्थूलथेवरील परीक्षण विस्तार जातीमधील उंदरांमध्ये करण्यात आले या अर्काचे पृथकारांण केल्यानंतर यात सॅपोनीन, फ्लेअवोनॉइड्स, अँथ्राक्विनोन, ग्लयकॉसिड्स, आढळले.

३२ उंदरावर्ती ५६ दिवसांचा प्रयोग केला त्यात ४ समूह केले प्रत्येक समूहात ८ उंदीर ठेवले त्यातील पहिल्या समूहाला प्रयोग शाळेतील रोजचे खाद्ये देण्यात आले व या समूहाला निरोगी समूह जाहीर केले २ रा ३ व ४ समूहाला अतिस्निग्ध पदार्थाचे खाद्ये दिले, या खाद्यामुळे कुठल्याही समधुहातील उंदरावर विशेष फरक जाणवला नाही तिसऱ्या समूहालाअटोरव्हास्टाटिन औषध तोंडाद्वारे दिले ०. ५ मिली ग्राम प्रति किलो वजनानुसार, चौथ्या समूहाला पपईच्या बियांपासून काढलेला अर्क ६० मिली ग्राम प्रति किलो वजन तोंडाद्वारे देण्यात आले.

उंदराच्या रक्तातील एच डी एल वाढले , व एल डी एल कमी झाले . सुक्षम दरशिये अभ्यासात यकृत मध्ये थोडे बदल आढळले आणि हृद्यांमध्ये काहीही आढळले नाही आणि कमूत्रपिंड ममुत्रपींडा मध्ये परिणाम दर्शवला नाही प्लिहा मध्ये विशेष परिणाम नाहीपपईचा पाण्यातील अर्काचे ६० मिली ग्रॅम प्रति किलो या मात्रेत स्थूलथेच्या विरोधात उपयुक्त परिणाम आढळले.

THESIS ABSTRACT

a)	Title of the thesis	:	**"EFFECTS OF *Carica papaya* ON CARDIAC PARAMETERS IN WISTAR RATS"**
b)	Full name of student	:	**More Kshitija Jagdish**
c)	Name and address of Major Advisor	:	**(Dr. S.R. Rajurkar)** Advisor/Guide Associate Professor Dept. of Vety. Pharmacology and Toxicology
d)	Degree to be awarded	:	M.V.Sc.
e)	Year of award of degree	:	2021
f)	Major subject	:	VETERINARY PHARMACOLOGY AND TOXICOLOGY
g)	Total number of pages in the thesis	:	62
h)	Number of words in the abstract	:	298
i)	Signature of Student	:	
j)	Signature, Name and address of forwarding authority (HOD/SH)	:	**Dr. S. R. Rajurkar** Professor and Head Dept. of Vety. Pharmacology &Toxicology.

ABSTRACT

The study was to examine if the *Carica papaya* seeds extract have hypolipidemic impact on high fat diet fed on Wistar rats over a 56 days period. Hot extraction method was used for preparation of extract. Aqueous extract of papaya has saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars. 4 groups of 32 Wistar rats were formed, 8 rats in each group. For 56 days, Group I was fed with standard pelleted feed and provided with safe drinking water, this was healthy control group. The treatment group, II, III, IV were fed on high fat diet in pellet form orally. During the experiment, rats from Group I did not show any behavioural changes, whereas rats from group II was only given high fat diet with no treatment showed decreased mobility and dullness. The rats in group III were given the medication atorvastatin @ 0.5mg/kg per orally and group IV rats were treated with seeds extract at a dose rate of 60mg/kg of body weight per oral route. In rats, there were no significant changes in blood parameters observed. There was drop in serum triglycerides, increase in HDL, cholesterol, reduction in LDL in treatment group of rats, but no significant changes in serum BUN and creatinine in rats which were fed on high fat diet. Liver of rats in group II showed enlargement on gross pathological examination. The rats in other 3 groups were found to have similar gross morphology. No changes were seen in kidneys, heart and spleen in any of the groups. Treatment with *Carica papaya* seeds extract at dose rate of 60mg/kg body weight was found beneficial in hypolipidemic and anti atherosclerotic effect on hyperlipidemia in Wistar rats.