THE EFFECT OF GARLIC POWDER ON BODY WEIGHT, LIVER WEIGHT AND LIPID PROFILE OF HYPERCHOLESTEROLEMIC INDUCED FEMALE WISTAR ALBINO RATS

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ABSTRACT

Background: Garlic (*Allium sativum L.)* is used as a spice and medicinal herb. Extensive clinical and scientific studies support the use of garlic for treatment of hypercholesterolemia; however, the potency of garlic product can vary substantially due to mode of preparation

Objectives: The study assessed effect of garlic powder on high cholesterol diet fed to female albino rats.

Material and method: Twenty four female albino rats weighing 80-130g were used for the study. These were divided into four groups. Each treatment (group) contained six rats and each treatment have 3 replicates of 2 rats per replicate. Group 1 was fed on normal diet (controls) and group 2 was fed on normal diet and garlic. Group 3 was fed on high cholesterol diet only while group 4 was fed on a high cholesterol diet and garlic. The serum lipids, lipoproteins and tissues histological appearances were determined using standard procedures. All statistical analysis was done using statistical packages for social sciences (SPSS) version 20.

Results: The serum total cholesterol, triglycerides, low density lipoprotein cholesterol (LDLC) was markedly reduced ($p< 0.05$) in the groups fed on high cholesterol diet + garlic as well as normal diet + garlic. Results showed significant decrease ($p<0.05$) in the mean weight of the liver in the group fed on high Cholesterol + garlic diet when compared with group feed on high cholesterol diet. The mean serum high density lipoprotein cholesterol (HDLC) was significantly higher ($p<0.05$) in these groups when compared to the control group. Histological findings showed pronounced atheromatous changes in the coronary artery of rats fed on high cholesterol diet.

Conclusion: The consumption of raw garlic has beneficial effect on plasma total cholesterol, triglyceride, HDL cholesterol and LDLC in rats fed on high cholesterol diet. Therefore, garlic can be used to reduce excess amount of cholesterol in the blood that can endanger health.

Key words: Garlic powder, Hyper-cholesterol, female albino rats.

INTRODUCTION

Elevated plasma level of cholesterol is linked to the development of atherosclerosis. This disease results when LDL-cholesterol are deposited in the wall of blood vessels [18]. Many studies have indicated that lowering plasma cholesterol may prevent, control and even reverse atherosclerosis and coronary heart disease [7]. The drugs used for lowering blood cholesterol generally have undesirable side effects. Hence, a harmless lipid-controlling mediation would be a welcome development. Medicinal and aromatic plants are the important renewable source of drugs. World Health Organization (WHO) also encourages the inclusion of medicinal plants in programs of developing countries because of the great potential these plants represent in combating various diseases. Recently, Sudan has produced certain drugs locally using important or native raw materials [17].

Garlic (*Allium sativum L.)* is used as a spice and medicinal herb. Most recent research works on garlic have used garlic in the form of medicinal tablets, raw, boiled, cooked and dried [20].Garlic contains 33 organosulfur compounds (OSC), several enzymes 17 amino acids (including all essential amino acids), and minerals such as phosphorus, calcium, iron, potassium, magnesium, selenium,, zinc and vitamin A, B, C and E [34].

Garlic has been used in folk medicine since ancient time for prophylaxis as well as cure of various diseases [4] [32]. Extensive clinical and scientific studies support the use of garlic for treatment of hypercholesterolemia; however, the potency of garlic product can vary substantially due to mode of preparation [12]. These studies include garlic tablets [29], garlic powder [24] [46], enteric coated garlic preparations i.e., Garlinase, Garlicin and Garique, essential oil and oil macerate [21], extract of frozen and raw garlic [42], water extractable fraction (WEF), petroleum extractable fraction (PEF), methanol

extractable fraction (MEF), aged garlic extract (AGE) [50], swallowing and chewing garlic. The hypercholesterolemic effect of the different preparations used was attributed to water soluble sulfur compounds, especially, S-allyl cysteine (SAC) and ajoene. However, fresh crushed garlic bulbs have been reported to contain high amount of allicin (3.7 mg/dl) [30] [21].

Garlic appears to enhance the synthesis of nitric oxides, which accounts for its anti-hypertensive and coagulant effects. With regard to the antioxidative activity of garlic, Rahman and Billington(36) have demonstrated that components of aged garlic extract inhibit the in vivo oxidation of LDL by chelating Cu^{2+}, scavenging superoxide ions, thus inhibiting the oxidation of protein and lipid moiety of human LDL-Cholesterol [13].

Allicin (diallythiosulfinate), a violent liquid is responsible for the pungent odor of garlic; representing approximately 70% of all thiosulfinate present in the crushed clove [8]. The component is not found in intact plants, but it is formed by action of the enzyme named allinase, derived from a non-proteinogenic amino acid s-allycysteines-oxide (aliin) at the time garlic is crushed [23]. In Garlic powder the conversion of alliin to allicin begins when water is added to the powder, being quickly degraded into diallyldisulfide (DADS), Vinylithiins and ajoenes[26]. Several in vitro studies have indicated that garlic and its component inhibits the key enzyme HMG-CoAreductase (3-hydroxi-3 methylglutaryl co enzyme A) which is associated with cholesterol and fatty acids synthesis [38][50].
Allicin opens transient receptor potential channel (TRAAI) and TRPVI(transient receptor potential vanilliod 1) that is responsible for the burning sensation of heat in raw garlic. Neurotransmitters are released from neurons in the spinal cord to generate pains signal. Active components of garlic may vary according to cultivar, harvest, and storage conditions. Also the amount of mineral content in its bulb depends on the content of the respective minerals in the soil where the bulb is grown. Allicin has been proposed as the active compound produced by garlic, which is responsible for its hypocholesterolemic effect. Since no garlic product on the market contains a detectable amount of allicin (<1ppm) [21], and also some of the bioactive compounds of garlic are affected by heat [18] [27]. The objective of the study is to determine the effect of garlic powder on body weight, liver weight, and the histological changes in the liver of hypercholesterolemic induced female wistar albino rats.

MATERIALS AND METHODS

PROCUREMENT OF FRESH GARLIC BULB AND PREPARATION

Garlic fresh bulb were purchased from Eke Ukwu Market in Owerri, Imo State. The garlic fresh bulbs were peeled, cut into pieces and oven dried at 60°c. The dried bulbs were grinded into powder.

PROCUREMENT OF THE RATS

Twenty four (24) Albino rats weighing (80-130g) were obtained from Imo State University Animal House Faculty of Medicine and Health Sciences. The rats were kept under standard laboratory condition of 12 hours light/dark cycle and housed in a cage. The animals were maintained on commercial feed (Top feed manufactured by Floor Mill Limited, Ibadan, Nigeria) and allowed to acclimatize for two weeks and was monitored during the two weeks and were found to be very active and healthy before trial commenced.

EXPERIMENTAL DESIGN

Rat Treatment: The rat were distributed to treatment using completely randomized design. Each treatment contained six rats and each treatment have 3 replicates of 2 rats per replicate. A total of 24 rats were used for the study. The rat was monitored daily and were given free access to water and feed.. The initial body weight was determined and individually weighed every one week for body weight gain.

Duration of Study: The study lasted for 44days (two weeks acclimatization period and 4 weeks treatment period) (A month and two weeks). Two hundred and fifty milligram (250mg) of garlic powder per kg body weight of the rats was included in the diet fed to the rats.

Treatments

1st Treatment – Normal commercial diet + water
2rd Treatment – Normal commercial diet + 250mg/kg body weight addition of garlic powder.
3rd Treatment – Normal commercial diet + 1% addition of cholesterol
4th Treatment – Normal commercial diet + 250mg/kg body weight addition of garlic + 1% addition of cholesterol.
Each replicate were feed with same quantity of the normal commercial feed.

Sample Collection

At the end of the experiments, the animals were fasted for twenty four (24) hours across treatment. The rats were anesthetized with 5mls of chloroform four-five mls of whole blood samples was collected with 5ml string through cardiac puncture into a plain glass tube, allowed to clot, retract and centrifuge at 4000rpm for 5minutes. The serum was separated into a plain container and stored at -80^{O}C before analyses.

The albino rat were sacrificed and the liver specimen kept under formalin condition. Blood metabolite assay were carried out which include the serum lipid profile of the different section of rats, the liver weight of the rats were assessed and histological changes in the liver across treatment were also assessed.

Specimen Storage and Handling during Testing
Samples were received frozen and stored at -80^{O}C until testing is performed. Upon completion of analysis, specimens are stored back at -80^{O}C.

HISTOLOGICAL METHOD
Tissue processing
Following fixation the gross anatomy was noted and represented section were taken and histologically processed by manual tissue processing method. Tissue processing involve the various stages between fixation and cutting of section [54].

Staining technique.
Paraffin section after they have been cut was attached to the slide and routine H&E staining method was used [54].

MICROSCOPY AND PHOTOMICROPHY
The sections were examined using Olmpus binocular microscope with an inbuilt lifting system. The sections were photomicrographed using a digit camera on olympus photomicroscope.

STATISTICAL ANALYSIS
All values were expressed as mean ± standard error of mean (SEM) of duplicate samples. The statistical analysis was carried out using ANOVAs i.e analysis of variance and separation of means using statistical package for social sciences version (SPSS) 20 at P<0.05

Determination of Cholesterol, triglyceride, high density lipoprotein and low density lipoprotein.
Method: Enzymatic colometric method (cholesterol oxidase)[53].

Equipment
Plain containers, test tubes, automatic micropipette, UV-1200 spectrophotometer, centrifuge, water bath.
Quality Control.
The analysis was quality controlled using commercially prepared Elitrol 1 (from Elitech Clinical System France).

RESULTS
Table 1 shows the effect of garlic powder on serum total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) in an induced hypercholesterolemic Wistar female albino rats. Significant difference (P< 0.05) in total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein were observed across the four groups.There was a significant increase (P< 0.05) in serum total cholesterol level in Group 3 (cholesterol fed untreated group) (2.72) when compared to the control group (group 1) (2.15), group 2 (2.05) and group 4 (2.36). Also, there was no significant (P> 0.05) difference in serum total cholesterol level in group 1, when compared to Group 2.

Table 1: The effect of garlic powder on serum total cholesterol, LDL-C, HDL-C and TG in induced hypercholesterolemic Wistar female albino rats.

parameters	group 1 (control)	group 2 (normal diet + garlic)	group 3 (normal diet + cholesterol)	group 4 (normal diet +garlic + cholesterol)	±SEM
Total Cholesterol mmol/L	2.15^{c}	2.05^{c}	2.72^{a}	2.36^{b}	0.10
Triglyceride mmol/L	1.00^{b}	0.84^{b}	1.43^{a}	1.07^{b}	0.07
HDL mmol/L	1.08^{b}	1.28^{a}	0.93^{b}	1.25^{a}	0.05
LDL mmol/L	0.87^{b}	0.60^{c}	1.26^{a}	1.18^{a}	0.09

***Note**: Mean values with different superscript along a row differs significantly (p<0.05), values were expressed in means ± SEM. Abbreviations: high density lipoprotein (HDL) low density lipoprotein (LDL), Standard error of mean (SEM).*

The result of the liver weight of rat across treatments were shown in Table 2. The result revealed that the liver weights of rat were significantly (P <0.05) higher in group 3 (6.37) as compared to the control group (5.29). There was a significant (P <0.05) decrease in the treated groups (groups 2 and 4) as compared to group 3. However, there is significant difference (P<0.05) between the treated groups (2

and 4). But significant (P <0.05) difference exist between the control group 1 and the cholesterol treated group 3 but not with those on diets 2 and 3.

Table 2 shows the effect of garlic powder on the liver weight of induce hypercholesterolemic Wistar female albino rats.

Table 2: The weight of rats' Liver (g)

Parameters	group 1 (control)	group 2 (normal diet + garlic)	group 3 (normal diet + cholesterol)	group 4 (normal diet+ cholesterol +garlic)	± SEM
Liver weight(g)	5.29^{bc}	4.84^{c}	6.37^{a}	5.57^{b}	0.16

Note: *Mean values with different superscript along a row differs significantly (p<0.05), values were expressed in means ± SEM (Standard error of mean).*

Effect of garlic powder on the weight increment of induced hypercholesterolemic Wistar female albino rats.

The results of weight of the rats across treatments (Table 3) showed that the initial body weights of the rat had no significant difference when compared among groups. The range for the final body weight of the four groups investigated lied between 100g and 140g (P<0.05). The result revealed that body weight of rat were significantly (p< 0.05) lower in the treated group (2) when compared with the control group (group 1). However, no significant (P>0.05) difference exist between group 3 and 4 when compared with the control group (group1).

Table 3 showed the effect of garlic powder on the weight increment of induced hypercholesterolemic Wistar female albino rats.

Table 3: The body weight of rats (g)

Parameters	group 1 (control)	group2 (normal diet + garlic)	group 3 (normal diet + cholesterol)	group 4 (normal diet +garlic + cholesterol)	±SEM
Initial body weight(g)	105^{a}	110^{a}	108^{a}	103^{a}	1.88
Final body weight(g)	130^{a}	100^{b}	140^{a}	123^{a}	5.55

Note: *Mean values with different superscript along a row differs significantly (p<0.05), values were expressed in means ± SEM (Standard error of the mean).*

Photomicrograph of Section of the Rat Liver.

Plate 1, 2, 3 and 4 below displayed the photomicrography of the sections of the rat liver used in the study. Plate 1: The photomicrograph (x 400) of the sections of the liver in the control group (group 1) showing the central vessel (CV) that are irregular in shape. The sinusoids (S) partly radiates from the vessels. The hepatocytes (H) appeared in cords and are multinucleated (N). The nuclei were open faced as can be observed in the normal liver sections. Plate 2: The photomicrography (x 400) of the section of the liver in the treated group (group 2) showing the central vessel that appear sclerotic in nature. The condition also extends into the wider dilated sinusoids. Some other sinusoids appeared like pockets traps in between the hepatocytes, here the hepatocytes appear enlarged and are interconnected like a net or lace material. Here it seems that each hepatocytes lodged a single nucleus and some of these appeared degenerating in nature. Plate 3: The photomicrograph (x 400) of the section of the liver in the cholesterol feed group (group 3) showing central vessels whose endothelium are intact, the vessel appeared sclerotic in nature and also there were infiltrations of the nuclei into the vessel lumen. The sinusoids radiates out from the vessel in the hexagonal pattern similar to those of the normal sections. Hepatocytes appeared in cords intervene by the sinusoids but are multi nucleated, the nuclei are open faced as seen in the normal section. There are slight lipid deposition on the section (liver) or accumulation of lipids by the organ. Plate 4: The

photomicrograph (x 400) of the section of the liver lobule in the treated group (group 4) showing the central vessel that the lumen were occupied by metastasized tissue probably from the hepatocytes which also occupies the large area of the organ. The sinusoids appeared by tiny trends to large of the hepatocytes. There were also metaplasia and hypertrophy of the nuclei such that each of the hepatocytes pledges many nuclei, some appeared in long chain. This may indicate hyper activity or functioning of the organ.

DISCUSSION

Commonly available garlic preparations in the form of garlic oil, garlic powder, pills and different extractions are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile [44, 15, 2]. Atherosclerosis with subsequent manifestations of cardiomyopathies is one of the major causes of morbidity and mortality in the world. Various studies indicate that high serum levels of cholesterol are strongly related to coronary atherosclerosis.

In the present study, the within-group analysis showed a significant increase in the serum total cholesterol level in the groups fed on high cholesterol diet, reduced plasma triglyceride (TC), was observed in rats fed on high cholesterol diet + garlic and normal diet + garlic. These reductions were statistically significant although rats in group 2 (normal diet + garlic) had more significant reductions, this could be attributed to the lowering effect on blood pressure and improved lipid profile [44, 15]. Others have also demonstrated the hypolipidemic effect of aged garlic extract when added to diets of rats fed cholesterol [6, 35]. Reported mechanisms include direct reduction of cholesterol production by the liver by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase[51] and increased bile acid excretion [43].

The results showed that serum triglycerides increased significantly in rats feed high cholesterol diet, however, reduced serum triglycerides were obtained in the group fed on high cholesterol diet + garlic and normal diet + garlic. These results are in line with Ali [3], who suggested that administration of garlic to rats is effective in decreasing total cholesterol and triglycerides significantly.

As evident from the study, garlic also decreases plasma triglyceride level in the rats fed on garlic containing diets, most likely through the stimulation of lipase. Available reports from a similar study indicated that garlic is a potential stimulant of lipase [47]. The results of this study

suggest that garlic has a hypolipidaemic effect.

In the study, the group that had garlic incorporated in their diet (groups, 2 and 4) had a significant increase in serum HDL cholesterol. These findings agree with Aouadi[5], who reported that feeding a supplement with 10% fresh crushed garlic and 2% cholesterol to rats resulted in a significant reduction in LDL-cholesterol levels. However, HDL-cholesterol increased significantly. In reverse cholesterol transport by HDL, cholesterol ester transferred to VLDL in a process facilitated by apo D which is known as cholesteryl ester transfer protein (CETP). Therefore, the inhibition of apo D has been proposed as a strategy to increase HDL-cholesterol level [39].

The result of the study showed that within-group analysis there were no significant difference in serum LDL in group 3 when compared with the animals in group 4 (normal diet + garlic + cholesterol). However, conversely, the serum LDL was significantly decreased in the group of animals whose diet had garlic incorporated (group 2) when compared to the animals on the normal diet (group 1). This, however, supports the ability of garlic to reduce LDL concentration. These results agreed with Shela[41] who reported that adding aqueous extract of raw garlic with 1% cholesterol to rabbits, decreased plasma total cholesterol and LDL-cholesterol significantly in the groups treated with garlic. This finding contradicted with the result of Abbas [1] who reported that chewing of garlic (crushed garlic) did not affect plasma LDL-cholesterol levels. This reduction in LDL-cholesterol level may be due to allicin which reduces the production and release of LDL-cholesterol by the liver and promotes LDL receptors activity in the liver cells, which helps the liver to clear the circulating LDL-cholesterol [22].

Despite attempts made by researchers, few choices are available to reverse or even retard the progression of non-alcoholic fatty liver disease [31]. To date, weight loss is a high priority to improving liver injury induced by non-alcoholic fatty liver disease [10]. The role of natural food items which can be used daily has been searched for. Garlic has been drawing experimental and clinical attention for a long time due to its promising lipid-lowering effects. The result of the study showed that at sacrifice, there were statistically significant increases in the mean weights of the liver in the animals fed on high cholesterol diet only when compared with the groups on the normal diet, normal diet + garlic and high cholesterol diet + garlic. The increase in relative liver weight of rat fed high cholesterol diet was due to accumulation of fat, causing hypertrophy of hepatocytes, as described by Kumar [28] who indicated similar findings in fatty change in the liver. This finding was in accordance to D'souza[11] who also observed a highly significant increase in relative liver weight in male Sprague-Dawley rats receiving high-fat (60% calories from fat) and corticosterone group. It could also be

speculated that the increase in the wet tissue weight in this organs, was probably due to the absence of garlic in the diet coupled with the high cholesterol content.

The significant decrease in relative mean weight of liver in group 2 animals as compared to group 3 was due to the protection provided by garlic administration. In these groups, there was less deposition of fat in the liver and decreased the size of hepatocytes. These findings were in accordance to Rajasree[37] who also observed a significant reduction in liver weight in alcohol-fed rats after a simultaneous feeding of garlic protein as compared to alcohol-fed controls. However, a study on rats fed on palm kernel oil for twelve weeks showed a similar finding [14].

Although no significant difference was observed in the final weight of animal between group 3 and 1, but group 3 had the highest mean weight (140 ± 17.32), this increase was significant when compared to group 2. The most probable reason for a lack of significant in the mean weight could be attributed to the garlic inclusion. This increase in body weight gain is in agreement with Elkayam[15], who reported obesity, hypercholesterolemia, and hyperglycaemia as leading detrimental outcomes of lack of physical activity and high caloric diet in daily routine. There was a significant ($p<0.05$) decrease in the final weight (body weight gain) of the group feed with garlic. The reduction in body weight in the group fed on normal diet + garlic may be suggesting that consumption of raw garlic could reduce body weight.

The histopathological findings of high cholesterol treated group 3 animals were in agreement with findings of El Kot[16]. The liver showing central vessels whose endothelium are intact, the vessel appeared sclerotic in nature and also there were infiltrations of the nuclei into the vessel lumen. Hepatocytes appeared in cords intervene by the sinusoids but are multinucleated, which was similar to the findings of Zuniga [52] who also showed hepatocytes were filled with multilocular fat droplets of varying sizes due to lithogenic diet in mice. Some hepatocytes showed shrinkage of nuclei, with disruption of chromatin material (Plate 3). These findings were most likely due to an influx of excessive saturated dietary fat, which could not be oxidized or leave the organ as enough Apo proteins were not available, leading to excess deposition of triglycerides in hepatocytes, same as described by Tiniakos[45]. Excess fats also caused damage to cell membranes by lipid peroxidation, leading to dilatation of veins.

The observations of the present study in group 4 (high cholesterol diet with garlic) showed that protective effect of garlic reverted hepatic lobular architecture. There were also metaplasia and hypertrophy of the nuclei such that each of the hepatocytes pledges many nuclei, some appeared in a long chain. This showed hyperactivity or functioning of the organ, which was in agreement with Sharma [40] who also showed that after using low dose aged garlic extract in sodium nitrate-treated liver, some hepatocytes showed vacuolized cytoplasm and central vein appeared congested. The anti-oxidative activities of garlic could be related to its contents of cysteine-
containing bioactive compounds, diallylsulfur compounds, and flavonoids, which are known to exert antioxidant effects.

CONCLUSION

In conclusion the result of this study showed that garlic powder supplemented diet decreased the levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, the liver weight in an induced hypercholesterolemic Wistar female albino rats. The study showed a significant increase in the level of high-density lipoprotein-cholesterol thus suggest that garlic preparation could prevent diet-induced hypercholesterolemia, fat accumulation in hepatocytes. It will also preserve the normal morphology of the liver and may have a protective role against atherosclerosis. The study result also showed that garlic changed the morphology of rats that were not induced with hypercholesterolemia, this may suggest that garlic has a detrimental health complication to the subject that are healthy.

1. It is recommended that further studies should be conducted to ascertain the safe level of garlic consumption in healthy subjects. This will guide health professionals in their recommendation.

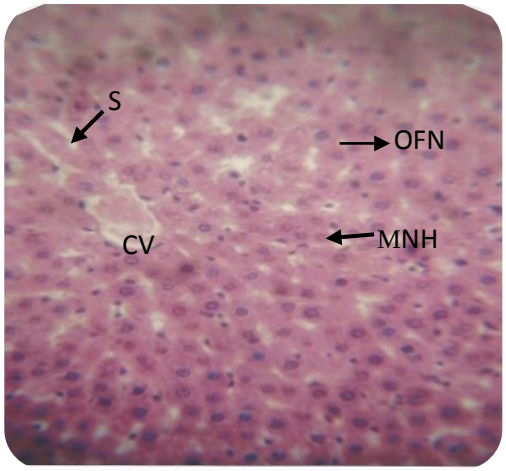

Plate 1: 4µm thick section of rat liver showing central vessel (CV), Open faced nuclei (OFN), Multi-nucleated hepatocytes (mNH), Sinusoids (S) (Photomicrograph x 400)

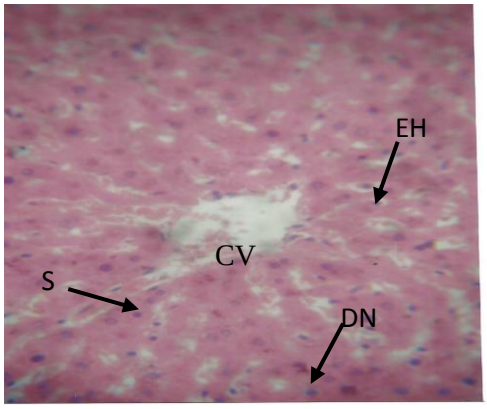

Plate 2: 4µm thick section of rat liver showing sclerotic central vessel (CV), degenerating nuclei (DN),enlarged hepatocytes (EH), Dilated Sinusoids (S) (Photomicrograph x 400)

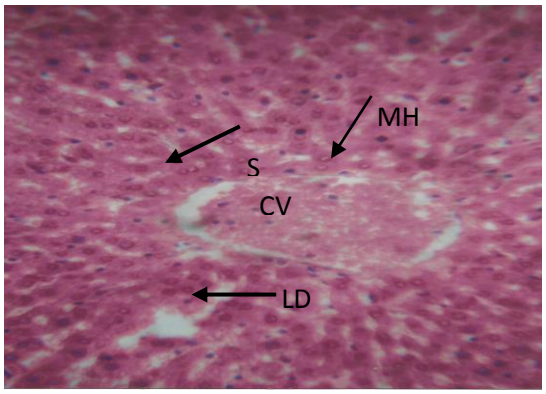

Plate 3: 4µm thick section of rat liver showing sclerotic central vessel (CV), Multinucleated hepatocytes(MH), Sinusoids (S), Lipid deposit (LD) (Photomicrograph x 400)

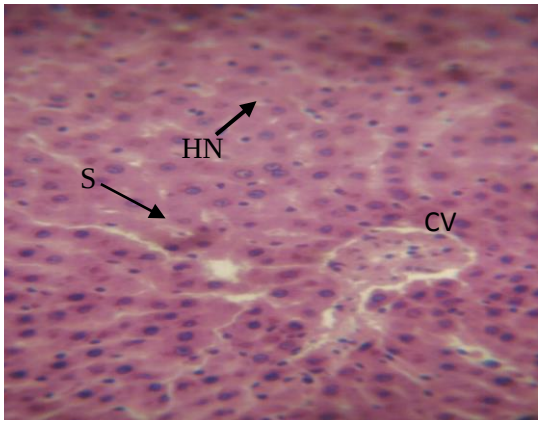

Plate 4: 4µm thick section of rat liver showing sclerotic central vessel (CV), Sinusoids (S), Hypertrophy of the nuclei (HN) (Photomicrograph x 400)

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