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Antihyperlipidaemic Property of Methanol Leaf Extract of *Chrysophyllum albidum* in Albino Rats (Wistar Strain) Fed on High Fat Diet

Ibukun, Osebhahiemen*, Omoregie, S. Ehimwenma
Department of Biochemistry, Faculty of Basic medical Sciences, University of Medical Sciences, P.O. Box 536, Ondo, Ondo State, Nigeria
oojemekele@unimed.edu.ng
Malaria Research, Molecular Biology and Toxicology Unit, Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B 1154, Benin City, Nigeria.
ehiomoregie@yahoo.co.uk

ABSTRACT

Hyperlipidaemia is a well documented risk factor for cardiovascular disease, a major cause of mortality world-wide. *Chrysophyllum albidum* (white star apple) has several medicinal properties. Ingestion of the fruits has been found to lower lipid levels in the body, due to its high fibre content. Research works have shown that several parts of the plant are highly medicinal. The aim of this study was to evaluate the antihyperlipidaemic property of methanol leaf extract of *Chrysophyllum albidum* in Wistar rats fed with a high fat diet. Thirty male Wistar rats weighing between 130g-150g were distributed into five groups of six animals each. Group 1 received normal fat diet (NFD). Group 2 received high fat diet (HFD; Grower mash supplemented with 20% beef tallow). Group 3 received HFD and a standard lipid lowering drug, orlistat (20mg/kg body weight, p.o). Animals in group 4 and 5 were fed on HFD and received methanol leaf extract of *Chrysophyllum albidum,* 250mg/kg and 500mg/kg, p.o respectively. The administration was carried out for 56 days. On the 57th day, animals were sacrificed by cervical dislocation. Liver was harvested and blood samples were collected for biochemical analyses. Results showed that the administration of HFD increased the total cholesterol, LDL-Cholesterol, triglycerides levels and decreased the HDL- cholesterol levels significantly ($p < 0.05$). Simultaneous administration of *Chrysophyllum albidum* extract significantly ($p<0.05$) reduced the total cholesterol, LDL-Cholesterol, triglycerides levels and increased HDL- Cholesterol. Final body weights of animals that received *Chrysophyllum albidum* extract were significantly ($p<0.05$) lower than final body weights of animals in groups 2 (negative control). Results from Histopathology revealed inflammation of the liver of rats in group 2 (negative control). On the other hand, rats that received orlistat and *Chrysophyllum albidum* extract had normal liver architecture. It was concluded that *Chrysophyllum albidum* extract showed antihyperlipidemic effect in rats fed with beef tallow supplemented diet.

Indexing terms/Keywords

Antihyperlipidaemic, *Chrysophyllum albidum,* orlistat, beef tallow, cholesterol.

Academic Discipline and Sub-Disciplines

Biochemistry

SUBJECT CLASSIFICATION

Ethnomedicine, Pharmacology

TYPE (METHOD/APPROACH)

Experimental

INTRODUCTION

Synthetic drugs used in the management of hyperlipidaemia have several side effects such as abdominal pain, urticaria, photosensitivity, rhabdomyolysis, hence there is a need to search for alternative agents for management or treatment of the condition [1, 2]. Therapies with traditional medicine are perceived to be cheaper and safer than synthetic chemical drugs [3]. A number of medicinal plants have shown their beneficial effect on cardiovascular system by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects (Dwivedi, 2004). Examples of such plants are *Allium sativum*, *Euglena jambolana*, *Moringa olifera*, *Ocimum sanctum*, *T. arjuna* [4].

Chrysophyllum albidum, commonly called white star apple is a very useful medicinal plant common in the tropical and sub-tropical regions of the world [5]. In folklore medicine, *Chrysophyllum albidum* bark is employed for the treatment of yellow fever and malaria [6]. The leaf is used as an emollient and for the treatment of stomach ache and diarrhoea [7].Extracts of *Chrysophyllum albidum* have been reported to possess hepatoprotective, antiplasmodial and antibacterial activities [8, 9,10]. The ethanol extract of *Chrysophyllum albidum* seed cotyledon has been reported to possess Antihyperglycemic and hypolipidemic effect of in model of alloxan-induced diabetic rats [11].

There is lack of documented research on the antihyperlipidaemic potentials of leaves of *Chrysophyllum albidum*, therefore this present study is aimed at evaluating the antihyperlipidaemic properties of leaves of *Chrysophyllum albidum* in Wistar rats.

MATERIALS AND METHODS

Plants Collection and Authentication

Fresh leaves of *Chrysophyllum albidum* were collected from a private farm in Benin City and authenticated by a Botanist at the Department of Plant Biology and Biotechnology, University of Benin. Voucher specimen was deposited in the herbarium of the same department.

Experimental animals

Thirty male Wistar rats weighing between 130g-150g were used for the study. The animals were housed at the Animal House of Department of Biochemistry, University of Benin, Benin City at room temperature (25 ± 1^{o}C) with 12 hrs light and dark cycles. Water was given to the rats*ad libitum* and 20g of feed /day per animal was given.

Antihyperlipidaemic property study

The animals were distributed into five groups of six animals each and exposed to the following for 8 weeks:

- Group 1: Group 1: Normal-fat diet (NFD, Normal Control) + Vehicle (carboxy methyl cellulose, CMC (0.5%).
- Group 2: High-fat diet (HFD, Negative Control) + Vehicle (CMC)
- Group 3: HFD + Orlistat (positive control) 20mg/kg, p.o [12].
- Group 4: HFD + *Chrysophyllum albidum* (250mg/kg, p.o)
- Group 5: HFD + *Chrysophyllum albidum* (500mg/kg, p.o)

Where: Normal fat diet = laboratory rodent diet

High fat diet = laboratory rodent diet (80%) + beef tallow (20%, w/w) [13].

Measurement of Body Weight

The body weight (g) was recorded on day one and then weekly consecutively for eight weeks (56 days) using a digital weighing balance.

Biochemical Analyses and Histological analyses

After eight weeks (56 days) of administration, on 57th day, the animals were sacrificed by cervical dislocation, under anaesthesia. Blood samples were collected from abdominal aorta into sterile tubes and allowed to stand for 30 minutes at 20-25^{o}C. The clear serum was separated at 2,500 rpm for 10minutes using a table centrifuge and were used for biochemical analyses. The biochemical analyses (liver function tests, kidney function tests and lipid profile) were carried out using standard commercial kits. The liver function tests done were Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP). Urea and Creatinine levels in serum were used as indicators of kidney function.

Liver was harvested from each animal and used for histological analyses.

Statistical analysis

Data were analysed with one-way ANOVA followed by Tukey's post hoc test, with significance set to $p<0.05$. All analyses were performed using Graph pad InStat (3.0).

RESULTS

Figure 1 shows the mean body weight gain (BWG) of rats in various groups. Results showed that the BWG of rats in high fat diet control (rats fed on high fat diet alone, without treatment) was significantly higher ($p<0.05$) than BWG of rats treated with *Chrysophyllum albidum* leaf extract.

Plates 1-5 show Histological examination of the liver of experimental rats

Table 1 shows comparison of Composition of the normal fat diet (NFD) and high fat diet (HFD)

Table 2 shows the serum lipid profile, atherogenic index of experimental rats: total cholesterol, triglycerides, LDL-Cholesterol, VLDL-Cholesterol and atherogenic index were significantly higher ($p<0.05$) in high fat diet control than plant extract treated groups. However HDL-Cholesterol was significantly lower in high fat diet control than extract treated groups.

Table 3 shows effects of the plant extract on Serum Liver Function test enzymes; ALT, AST, ALP (U/l) in the experimental rats

Table 4 shows effect of the extract on Urea and Creatinine levels of the experimental rats

DISCUSSION

Dietary factors such as continuous ingestion of high amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis [14]. Several medicinal plants have shown their beneficial effect on cardiovascular system by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects [4]. Exploration of such medicinal plants is a major step in the discovery of new alternative agents to conventional lipid lowering drugs.

In this present study, the group of rats which fed on high fat diet (High fat diet control; group 2) showed an increase in mean body weight gain (BWG). These results agree with Milagro *et al*. [15] who reported that animals fed on the high fat diet showed higher body weight gain, compared with those on normal fat diet. Also, results revealed that BWG of the rats fed on high fat diet and concomitant *C. albidum* administration (groups 4 and 5) were significantly lower ($p<0.05$) than those of high fat diet control (Figure 1). Similar reduced BWG were observed in animals on high fat diet and orlistat-20mg/kg, a drug used for the treatment of obesity and hyperlipidaemia which acts by inhibiting pancreatic lipase, thereby impairing fat digestion and absorption.

Dyslipidemia has been characterized as a major risk factor for cardiovascular risk, including atherosclerosis [16, 17]. In the present study apart from reduction in weight, administration of *C. albidum* leaf extract at concentrations of 250mg/kg and 500mg/kg was observed to significantly lower ($p<0.05$) the levels of total cholesterol, triglycerides and LDL-cholesterol. *C. albidum* leaf extract at concentration of 500mg/kg increased the level of HDL-cholesterol level in comparison with rats in high fat diet control group. Similar results were obtained by Ghasi *et al*. [18], where treatment with crude extract of medicinal plant led to an increased serum HDL-Cholesterol level and decreased levels of total cholesterol, LDL-cholesterol and triglyceride. Thus, it can be suggested that leaves of *C. albidum* possess cardioprotective potential.

Furthermore, atherogenic index is regarded as a marker for various cardiovascular disorders; the higher the value, the higher the risk of developing cardiovascular disease and vice versa [19]. High fat diet intake resulted in increase in atherogenic index (Table 2). Treatment with 250mg/kg and 500mg/kg significantly ($p<0.05$) attenuated the atherogenic index.

High fat diet has been suggested to cause hepatocellular damage, as clearly seen by the marked elevation of serum liver function enzymes (AST and ALT) activities and histopathological results of liver in high fat diet control group. However, treatment with *C. albidum* led to a reduction in the enzyme levels, and restoration of normal hepatic architecture (plates 4 and 5) signifying the role of the extract in preventing liver dysfunction induced by high fat diet.

Also, the result of the present study also showed that extract of *C. albidum* has a significant improvement effect on the kidney functions as revealed by urea and creatinine concentrations (table 4).

In this study, results of effects of *C. albidum* extract on body weight gain, lipid profile, Liver function tests, kidney function tests and histology were comparable with those of orlistat, a standard antiobesity and hypolipidaemic drug.

CONCLUSION

Based on results from this present study, it can be concluded that methanol leaf extract of *C. albidum* is beneficial for weight management and blood lipid reduction.

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Table 1: Composition of the normal fat diet (NFD) and high fat diet (HFD)

	NFD	HFD
Protein	16%	12.8%
Fat	5%	24%
Carbohydrate	69%	55.2%
Vitamins and minerals	3%	2.4%
Fibre	7%	5.6%

Table 2: Serum lipid profile and Atherogenic index (AI) for the experimental groups

	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	AI
Group 1	147.78±5.69	96.13 ± 3.47	30.01±7.35	98.55±2.36	19.16±0.64	0.50±0.09
Group 2	199.59±8.53	123.33±4.23	101.11±2.13	73.81±3.29	24.67±0.85	1.71±0.06
Group 3	159.27±8.52	85.43±8.28	26.99±11.87	115.2±9.63	17.09±1.66	0.40±0.13
Group 4	120.75±18.13	71.39±8.55	41.85±1.84	57.25±2.65	14.45±1.62	1.10±0.09
Group 5	139.31±11.47	95.90±2.67	15.93±7.41	101.53±4.11	19.18±0.53	0.36±0.13

Where Groups 1, 2, 3, 4, 5 are Normal fat diet (NFD) control, High fat diet (HFD) control, Orlistat (20mg/kg), *C. albidum* (250mg/kg), *C.albidum* (500mg/kg) respectively

Values are expressed as Mean±SEM. (n=6). All groups are significantly different ($p < 0.05$) from HFD control.

Table 3: Serum Liver Function test enzymes; ALT, AST, ALP (U/l) in the experimental rats

	ALT	AST	ALP
Group 1	13.29±3.21	13.00±3.00	38.28±2.76
Group 2	30.34±2.40	38.50±2.50	39.66±4.14
Group 3	16.41±0.60	19.34±3.34	39.28±2.76
Group 4	13.58±1.90	19.16±2.84	39.66±4.14
Group 5	14.90±2.10	21.50±0.50	39.66±1.38

Values are expressed as Mean±SEM (n=6). For ALT and AST, all groups are significantly different ($p<0.05$) from HFD Control (group 2). For ALP, no significant difference ($p>0.05$) between other groups and HFD Control.

Table 4: Serum Urea and Creatinine levels in the experimental rats

	Urea (mg/dl)	Creatinine (mg/dl)
Group 1	12.49±0.04	0.77±0.26
Group 2	13.86±0.01	3.01±0.47
Group 3	12.61±0.06	0.89±0.13
Group 4	12.78±0.03	1.65±0.38
Group 5	12.65±0.05	1.53±0.26

Values are expressed as Mean±SEM. (n=6). All groups are significantly different ($p < 0.05$) from HFD control (Group 2).

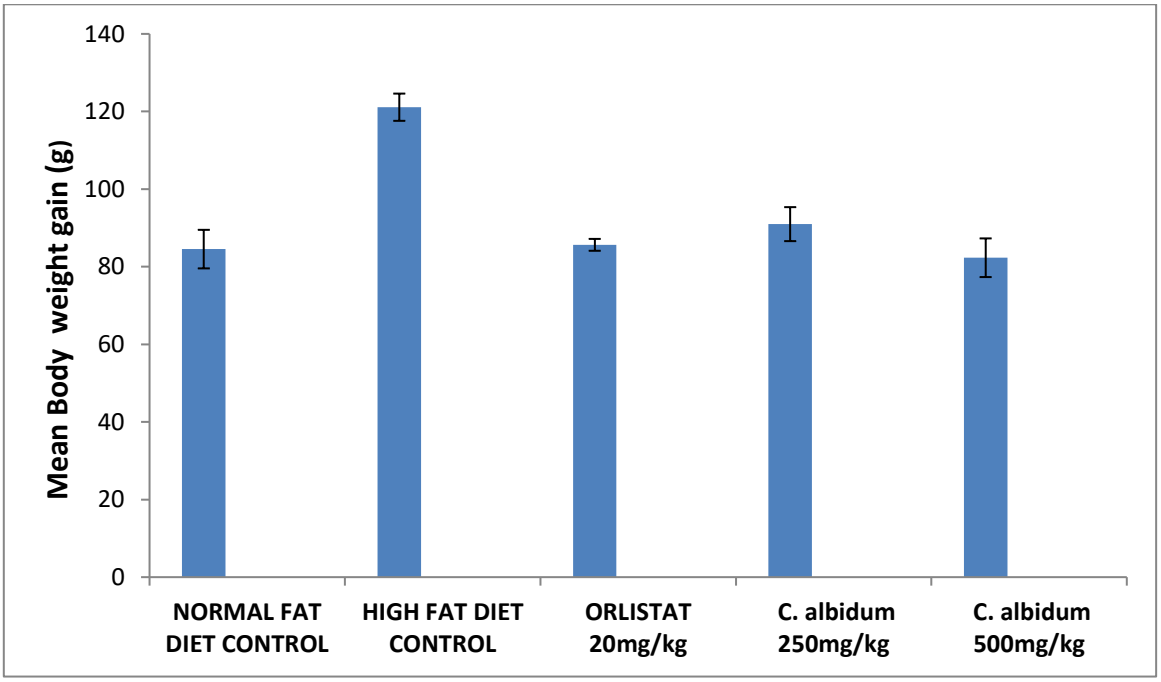

Figure 1: Mean Value of overall body weight gain for the experimental groups.

All groups have significantly ($p < 0.05$) lower mean body weight gain than the high fat diet control (negative control)

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Histological examination of the liver and kidney of experimental rats

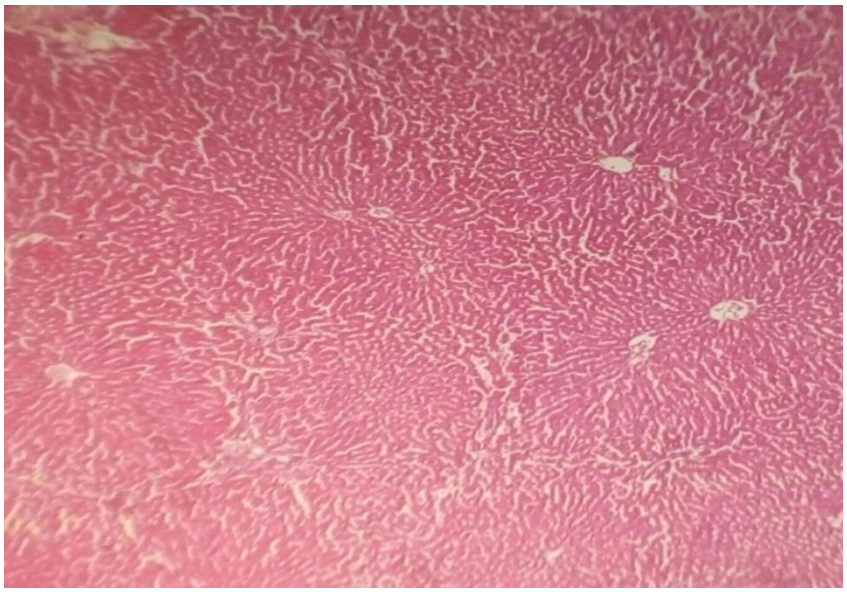

Plate 1: Normal histological structure of hepatic lobules in rat's liver fed on normal fat diet. Group 1 (H and E ×100)

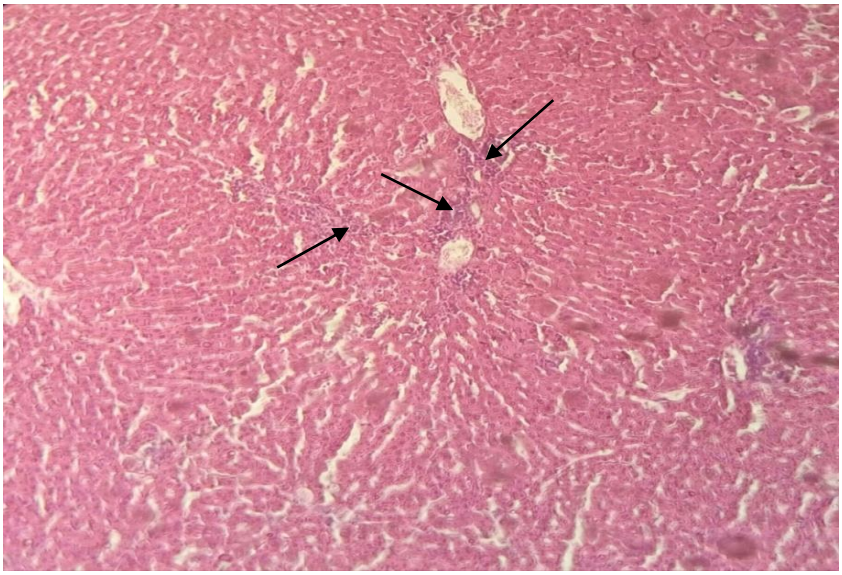

Plate 2: Liver of rat in group 2 (high fat diet control) showing mild focal centrilobular and lobular lymphocytic inflammation (arrows) (H and E ×100)

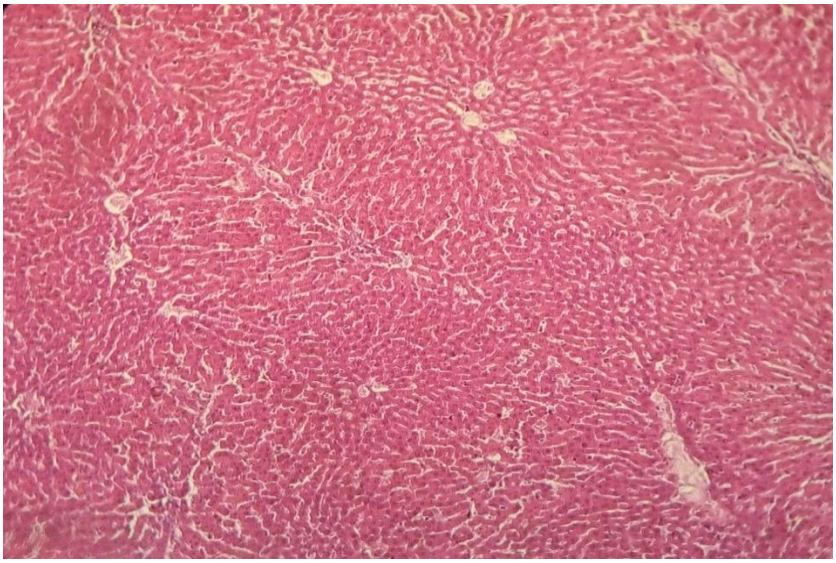

Plate 3: Liver of rat in group 3 (orlistat 20mg/kg) showing normal hepatic architecture (H and E ×100)

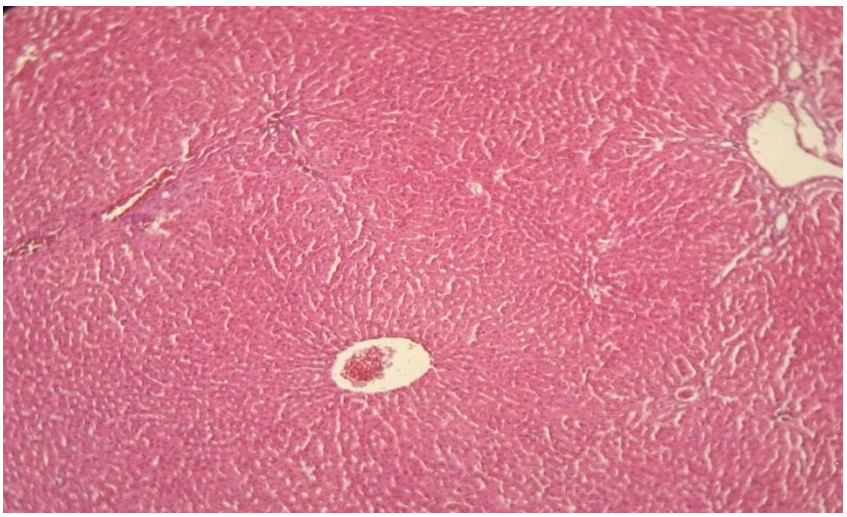

Plate 4: Liver of rat in group 4 (*C. albidum* 250mg/kg) showing normal hepatic architecture (H and E ×100)

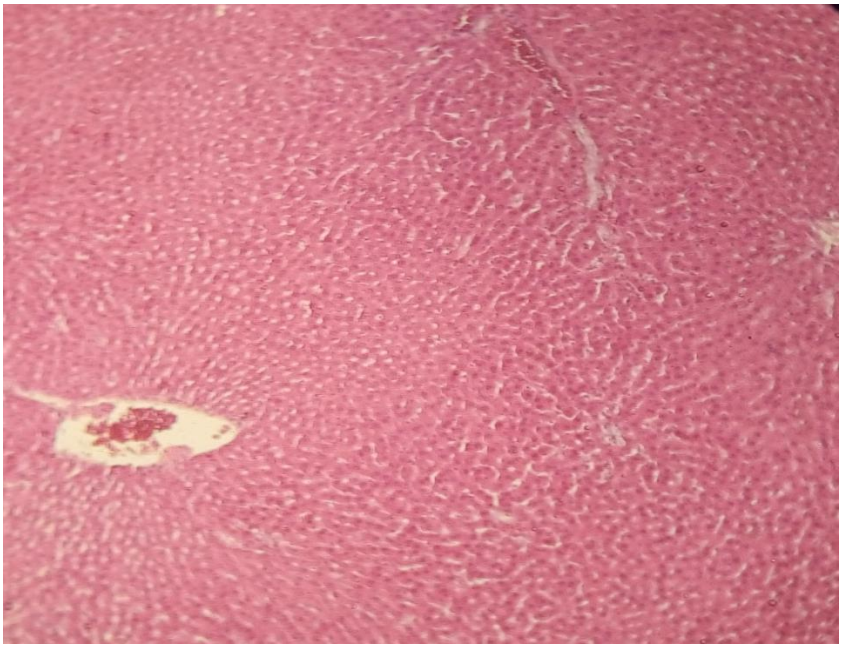

Plate 5: Liver of rat in group 5 (*C. albidum* 500mg/kg) showing normal hepatic architecture (H and E ×100)

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