D R A F T R E P O R T

Copy No. 1/2

Study Title

ANTI-HYPERLIPIDEMIC ACTIVITY

Test Facility

Radiant Research Services Pvt. Ltd

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COMPLIANCE STATEMENT

The Study Director hereby declares that the work was performed under his supervision and in accordance with the mutually agreed study plan and the in house procedures. It is assured that the reported results represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study. The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, documentation and reporting of the results.

_____

Study Director

Date: / /

CERTIFICATE OF AFFIRMATION AND CONFIDENTIALITY

The Management hereby attests to the originality, accuracy and authenticity of the study to the best of their knowledge. This report contains confidential and proprietary information of M/s. Radiant Research Services Pvt Ltd., which will not be disclosed to anyone without the expressed or written approval of authorized personnel.

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Management

Date: / /

DECLARATION

The Study **No# RR/160235/RD/PC-08/16-17**, entitled **"ANTI-HYPERLIPIDEMIC ACTIVITY"** has been inspected regularly according to the Standard Operating Procedures of the test facility's Quality Assurance Unit. The report was audited against the approved study plan and pertinent raw data and accurately reflects the raw data.

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Department Head

Date: / /

ABBREVIATION USED

Preclinical : PC

Clinical : CL

HCD : High Cholesterol Diet

TC : Total Cholesterol

TG : Triglycerides

HDL : High Density Lipoprotein

LDL : Low Density Lipoprotein

VLDL : Very Low Density Lipoprotein

1. STUDY DETAILS

1.1. Study title : Anti-hyperlipidemic activity

1.2. Study number : 160235

1.3. Test Substance : Obesidat

1.4. Sponsor : Guduchi, The ayurvedism

1.5. Test facility : Radiant Research Services Pvt. Ltd

No: 99/A, 8th Main, 3rd Phase,

Peenya Industrial Area,

Bangalore-560058.

1.6. Test schedule

Study Initiation Date : 01/09/2016

Experimental Start Date : 07/09/2016

Experimental Completion Date : 20/10/2016

Study Completion Date : 25/10/2016

1.7. Study Responsibilities

Study Director : Mr.Gopal

Study Co-ordinator : Mr. Dinesh

Study Personnel : Mr. Prakash

2. OBJECTIVE

The purpose of this study is to evaluate the effect of test substance on High cholesterol diet fed rats.

3. SUMMARY

In the present study, test substance in two dose levels were evaluated for anti-hyperlipidemic activity in wistar rats. Hyperlipidemia was induced by giving high cholesterol diet for six weeks. Rats on high cholesterol diet showed significant increase ($^{###}p<0.001$) in serum TC, TG, LDL, VLDL and decrease HDL levels. Treatment with test substance 45mg/kg and 90mg/kg showed significant decrease(**p<0.01) (***p<0.001) in serum TC, TG, LDL, VLDL values along with an increase HDL levels when compared to High cholesterol diet control groups. Thus test substance in two different dose levels is effective as an antihyperlipidemic agent when compared to High cholesterol diet control group.

4. GUIDELINES/REFERENCE

1. Khyati AS et al. Antihyperlipidemic activity of *Mangifera indica* leaf extract on rats fed with high cholesterol diet. Der Pharmacia Sinica, 2010: 1 (2): 156-161.
2. Pooja CO et al. Antioxidant and antihyperlipidemic activity of hibiscus sabdariffa linn leaves and calyces extracts in rats. Indian journal of experimental biology. 2009:47:276-282.
3. Shukla AK et al. Hypolipidemic Activity of *Lepidium Sativum* Linn. Seed in Rats. IOSR Journal of Pharmacy and Biological Sciences.2015:10(4):13-22.
4. Dipa AI et al. Anti-hyperlipidemic activity of aqueous extract of *terminalia chebula* & gaumutra in high cholesterol diet fed rats. An international journal of pharmaceutical sciences.2010:1(1):48-59.

5. AMENDMENT AND DEVIATION PROCEDURES

This study was conducted as per the standard protocol and approved study proposal, and we have not amended or deviated from the procedure.

6. TEST SUBSTANCE

Test substance/item	: Obesidat tablet
Common name	: --
Test substance code	: RR 160235
Batch No.	: G 002
Batch supplied by	: --
Batch produced on (Date)	: JUN-2016
Expiry date	: MAY-2019
Purity	: --
Physical appearance	: Solid tablet
Storage conditions	: Room Temperature

7. MATERIALS AND METHODS

7.1. Animal Husbandry

Animals were housed under temperature 22± 3^{o}C, relative humidity 50-70%, and 12 hour light and 12 hour dark cycle. Animals were housed in standard polypropylene cages with stainless steel top grill having facilities for pelleted food and drinking water in bottle. Sterile paddy husk was used as bedding material and changed every day. The diet and drinking water were free from any contaminant and provided *ad libitum*.

7.2. **Test System Details**

Animals : Rats

Strain : Wistar

Sex : Female

Source : In-house

Age : 4 - 6 weeks

7.3. **Feed Details**

Provided normal chow diet to group I, whereas, Group II -IV animals received High cholesterol diet throughout experimental period.

7.4. **Test items, Vehicle and Formulation details**

Test item : Obesidat tablet

Dose : 45mg/kg & 90mg/kg

Route/Frequency : *p.o.*, *q.d.*

Vehicle : 0.5% w/v Carboxyl methyl cellulose sodium.

Dose : Test 1 (45 mg/kg) and Test 2 (90 mg/kg)

7.5. **Formulation Preparation**

Formulation preparation was carried out by trituration method using mortar and pestle.

Formulation was prepared every day before dosing.

8. EXPERIMENTAL PROCEDURE

8.1. **Grouping:**

Female albino wistar rats weighing 175-200gms were divided into four groups, each group containing 6 animals and groups as mentioned below.

- **Group I:** Normal (vehicle control)
- **Group II:** High cholesterol diet control
- **Group III:** Test I (45 mg/kg along with High cholesterol diet)
- **Group IV:** Test II (90 mg/kg along with High cholesterol diet)

8.2. **Induction of hyperlipidemia:**

High cholesterol diet was prepared by mixing Cholesterol 2%, sodium cholate 1%, coconut oil 30% with standard powdered chow diet. The diet was placed in cage carefully and was administered daily for six weeks.

8.3. **Procedure**

Induction of high cholesterol diet to all the animals except group I (normal control) for six weeks. After two weeks of high cholesterol diet induction, group III and group IV received 45mg/kg & 90mg/kg of test substance daily for 30 days along with high cholesterol diet. Whereas group II served as HCD control and group I as vehicle control. Body weight of each animal was weighed weekly and feed consumption daily.

On 31st day, blood was collected by retro orbital route under isoflurane anesthesia after 12 hr fasting. Blood samples were centrifuged at 5000 rpm for 10 mins. Serum was separated and stored at -20^{0}c until biochemical evaluation were carried out. After collection of blood, animals were anesthetized with isoflurane and euthanized, visceral adipose tissues and liver tissues were removed, rinsed with phosphate buffered saline, weighed and stored for histological analysis.

The serum samples were analyzed by autoanalyzer using diagnostic kits for Total Cholesterol, Triglycerides, High Density Lipoprotein, Low Density Lipoprotein, and Very Low Density Lipoprotein. The liver sections were fixed and preserved in 10% neutral phosphate buffered formalin. Formalin-fixed liver tissues were embedded in paraffin using standard procedures. Sections were cut and stained with hematoxylin and eosin (H & E) were examined microscopically.

8.4. **Statistical analysis:** Results were expressed as mean± SEM, Data was analysed by one way ANOVA followed by Dunnett test against the positive control group. The $P<0.05$ value were considered as statistically significant.

9. RESULTS AND DISCUSSION

The present study has been undertaken to demonstrate the effect of test substances on rats fed with high cholesterol diet (HCD). Animals fed with HCD showed increase in body weight compared to those fed with normal chow diet and treated groups.

Treatment with test substance at doses of 45mg/kg b.wt and 90mg/kg b.wt controlled/regulated the HCD induced body weight gain significantly, when compared against control group (Table 1 & Fig 1). The average daily feed intake, which was similar at the start of the study by all the groups was reduced in treated groups following the treatment with test substance.

Different types of adipose tissues were isolated to measure the fat content to compare with treated and untreated groups (Table 3). The HCD group showed higher organ weight (liver & adipose tissues). Conversely, groups treated with test substances 45mg/kg and 90mg/kg, showed lower organ weight when compared to HCD control group (Fig 3).

Lipid profile was done for all animals from all groups (Table 4). It has been observed that rats fed with HCD consecutively for 6 weeks resulted in a marked increase in the level of lipids, characterized by elevated levels of total cholesterol, triglycerides, LDL, VLDL, and reduced levels of HDL when compared to normal control group, which received normal chow diet. An increased LDL level indicates hypercholesterolemia. However, test

substances 45mg/kg and 90mg/kg for 4 weeks reversed the hyperlipedimic effect produced by high cholesterol diet significantly (P<0.01). There was significant decrease (P<0.001) in TC, TG, LDL, VLDL levels and significant increase (p<0.01) in HDL level, when compared to HCD group (Fig 3).

Histopathology of liver section studied shows normal hepatocytes with no necrosis, fatty change or malignant cells seen (Fig 5a & Fig 5b). Liver section of HCD treated rats showed severe degree microvesicular and macrovesicular fatty change (Fig 5c & Fig 5d). Groups treated with test substances 45mg/kg and 90mg/kg, showed moderate degree microvesicular and macrovesicular fatty change (Fig 5e & Fig 5f and Fig 5g & Fig 5h).

10. CONCLUSION

Results of present study, treatment with test substances 45mg/kg and 90mg/kg significantly decreases the TC, TG, LDL, VLDL levels and significant increase in HDL level when compared to control group. Along with this, treatment with test substance also lowered in the food intake and further normalized the weight of different organs/tissues. These results were further confirmed by with the histopathological observations. Thus from above results can be concluded that test substance has significant anti-hyperlipidemic and weight regulation properties.

11. ARCHIVING

1. Test samples will be stored for 30 days after the final report submission
2. Raw data, documents, reports will be archived for 3 years

12. REPORT DISTRIBUTION

Sponsor: One signed final report (Copy no. 1/2) in original.

Archives: One signed final report (Copy no. 2/2) in original along with raw data file.

13. TABLES

Table 1: Effect of Test substance on Body weight

GROUPS	BODY WEIGHT (gms)						
	WEEK -0	WEEK -1	WEEK -2	WEEK -3	WEEK -4	WEEK -5	WEEK -6
I	184.8±1.83	191.3±1.02	199.3±1.20	207.2±1.30	217.2±1.60	227.8±1.17	236.0±1.86
II	185.2±1.87	198.0±2.25	207.7±1.84	217.5±1.89	228.7±1.99	239.0±2.34	247.8±2.60
III	185.3±1.52	194.2±1.47	201.8±1.72	210.7±2.35	220.2±1.35	230.5±1.28	239.7±2.47
IV	185.0±1.53	192.7±1.45	201.0±1.55	209.7±1.31	218.0±1.79	228.2±2.18	237.3±2.12

Values were expressed as mean±SEM,

Table 2: Effect of Test substance on feed consumption

GROUPS	FEED CONSUMPTION (gms)					
	WEEK -1	WEEK -2	WEEK -3	WEEK -4	WEEK -5	WEEK -6
I	96.7±1.67	98.3±2.11	98.5±3.04	98.3±3.07	98.3±2.11	98.3±2.47
II	125.0±1.29	122.5±1.12	120.5±1.89	119.2±1.54	119.2±2.39	116.7±2.47
III	124.2±1.54	122.2±1.30	120.3±1.33	119.0±1.53	119.2±2.39	116.3±2.19
IV	124.2±0.54	122.5±1.71	120.5±2.29	119.7±1.86	118.8±2.01	116.0±2.25

Values were expressed as mean±SEM,

Table 3: Effect of Test substance on organs weight

GROUPS	ORGAN WEIGHT (gms)				
	Liver	Epididymis	Subcutaneous adipose	Brown adipose	Renal adipose
I	4.9±0.17	1.2±0.09	0.6±0.05	0.3±0.03	0.2±0.00
II	7.2±0.27	2.5±0.25	2.3±0.07	0.6±0.03	0.4±0.01
III	6.6±0.27	1.6±0.10	0.8±0.05	0.5±0.01	0.3±0.01
IV	6.2±0.44	1.6±0.22	0.8±0.02	0.5±0.01	0.3±0.01

Values were expressed as mean±SEM,

Table 4: Effect of Test substance on lipid profile levels

GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	120.7±4.57	78.2±1.05	40.3±1.12	100.2±2.32	15.6±0.21
II	183.5±4.56$^{###}$	159.8±3.25$^{###}$	31.0±0.97$^{###}$	167.8±2.69$^{###}$	32.0±0.65$^{###}$
III	160.5±2.09**	140.8±2.37***	34.2±0.91	153.3±3.21**	28.2±0.47***
IV	155.7±3.70***	137.5±1.93***	35.7±0.56**	143.3±2.11***	27.5±0.39***

Values were expressed as mean±SEM, $^{###}$P<0.001 when compared to control, *P<0.05, **P<0.01, ***P<0.001 when compared to high cholesterol diet control.

Table 5: Histopathological analysis of liver from different groups

Group	Pathological observations
Group-I	**Fig-5a & Fig-5b:** Microscopy: Section studied shows normal hepatocytes arranged in lobular pattern around the central veins. These lobules are surrounded by normal portal triads containing portal venule, hepatic arteriole and bile ductule. The hepatic sinusoids are normal with normal connective tissue stroma. **No Necrosis, fatty change or malignant cells seen**
Group-II	**Fig-5c & Fig-5d:** Section studied shows normal hepatocytes arranged in lobular pattern around the central veins. These lobules are surrounded by normal portal triads containing portal venule, hepatic arteriole and bile ductule. The hepatic sinusoids show mild dilatation with normal connective tissue stroma. **Severe degree microvesicular and macrovesicular fatty change seen**
Group-III	**Fig-5e & Fig-5f:** Microscopy: Section studied shows normal hepatocytes arranged in lobular pattern around the central veins. These lobules are surrounded by normal portal triads containing portal venule, hepatic arteriole and bile ductule. The hepatic sinusoids show mild dilatation with normal connective tissue stroma. **Moderate degree microvesicular fatty change seen**
Group-IV	**Fig-5g & Fig-5h:** Section studied shows normal hepatocytes arranged in lobular pattern around the central veins. These lobules are surrounded by normal portal triads containing portal venule, hepatic arteriole and bile ductule. The hepatic sinusoids show mild dilatation with normal connective tissue stroma. **Moderate degree microvesicular and macrovesicular fatty change seen**

14. FIGURES

Fig 1: Effect of Test substance on body weight

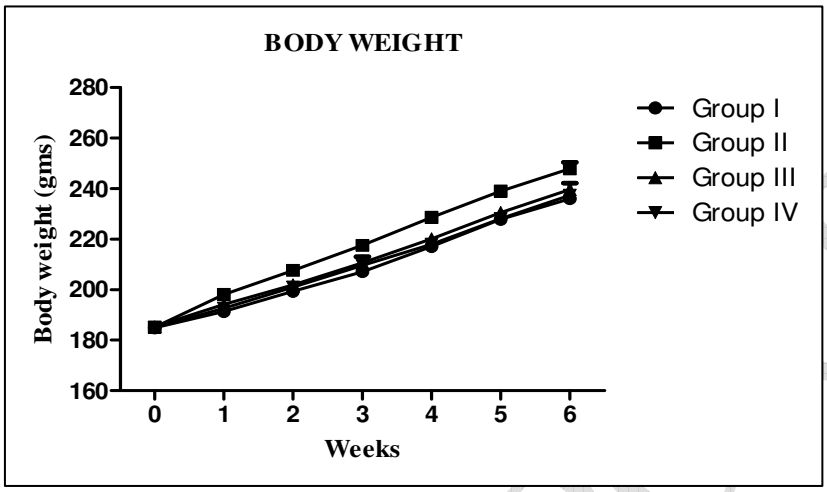

Fig 2: Effect of Test substance on feed consumption

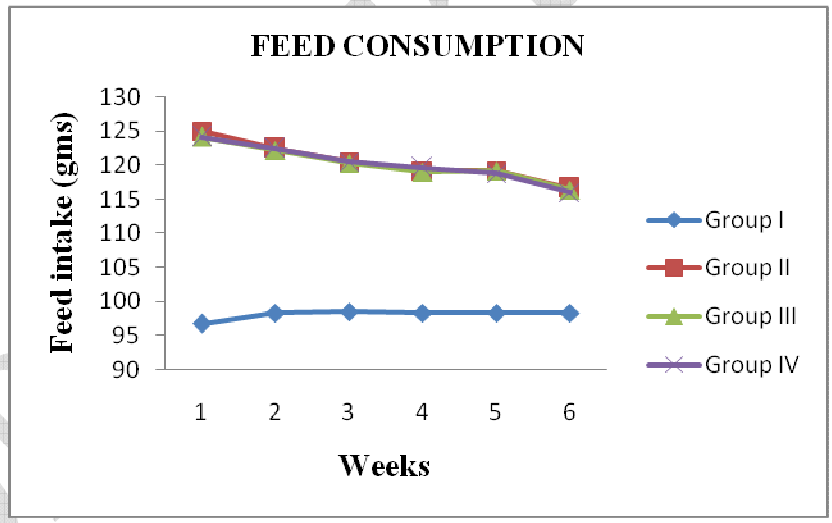

Fig 3: Effect of Test substance on organ weight

Fig 3a: Liver

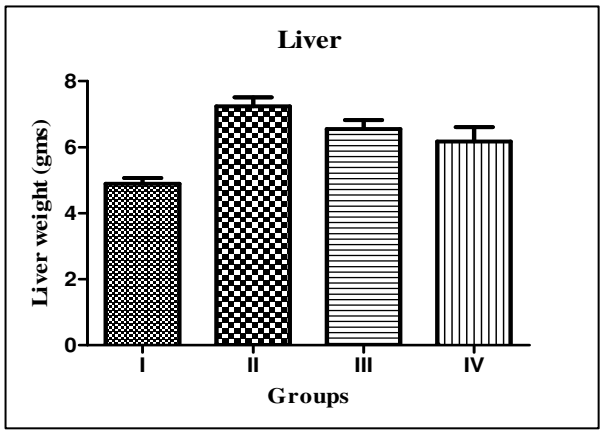

Fig 3b: Epididymal adipose

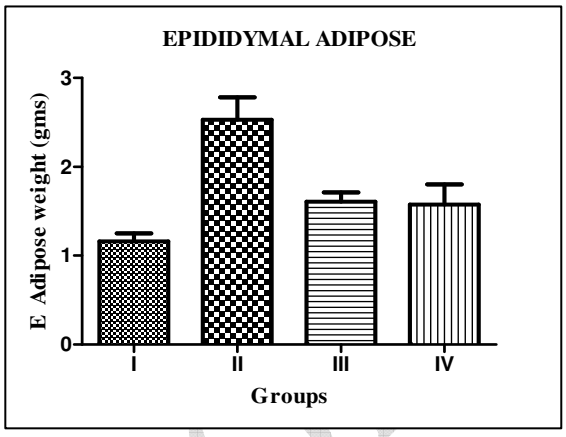

Fig3c: Subcutaneous adipose tissue

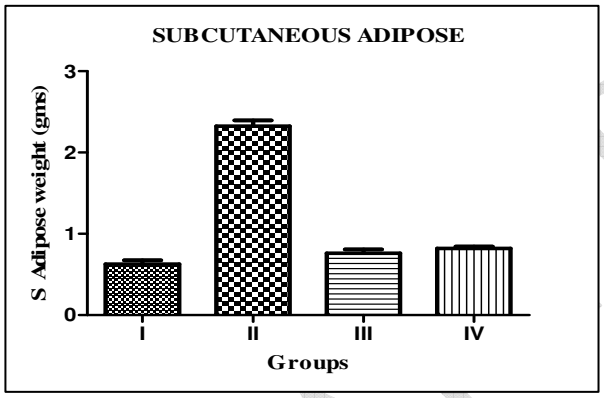

Fig 3d: Brown adipose tissue

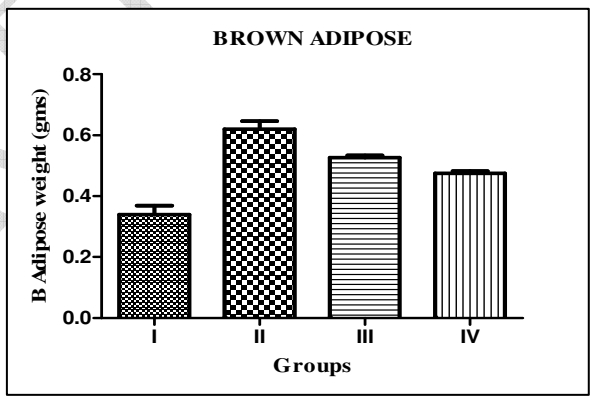

Fig 3e: Renal adipose tissue

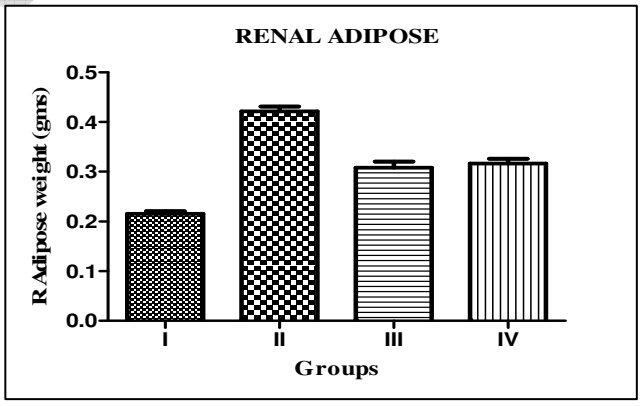

Fig 4: Effect of Test substance on lipid profile levels

Fig 4a: Total cholesterol level

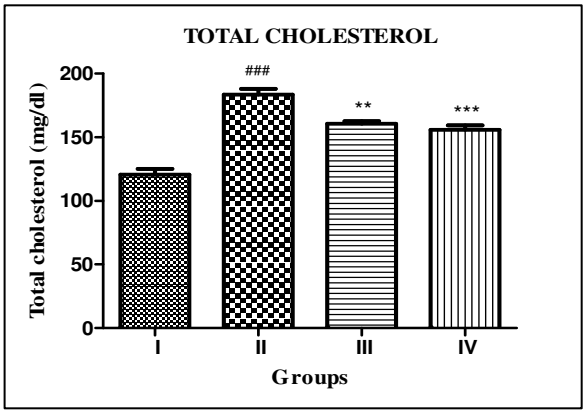

Fig 4b: Triglyceride level

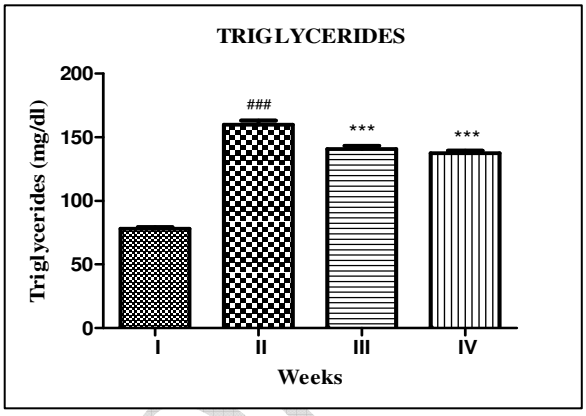

Fig 4c: HDL level

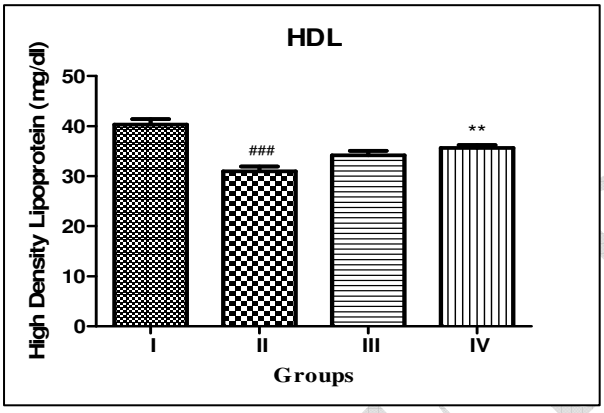

Fig 4d: LDL level

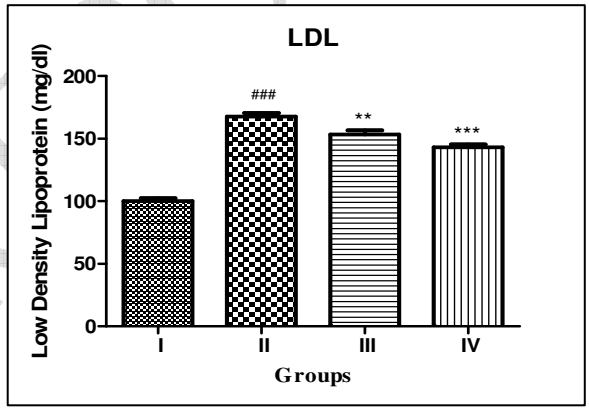

Fig 4e: VLDL level

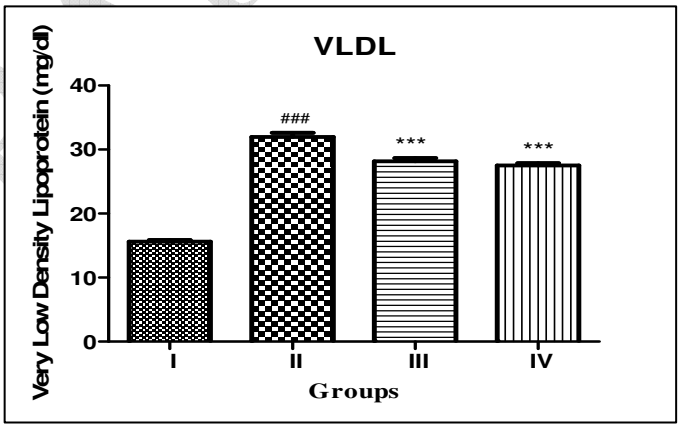

Fig 5: Histopathological results of liver

Group –I

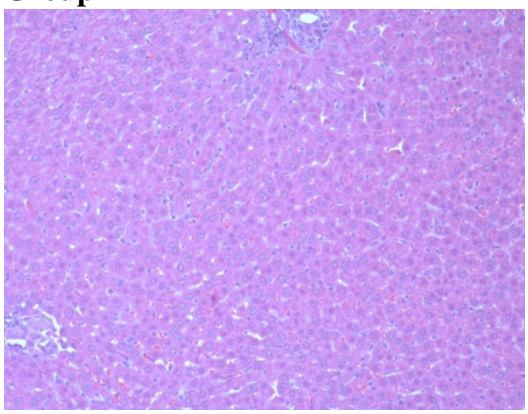

Fig-5a

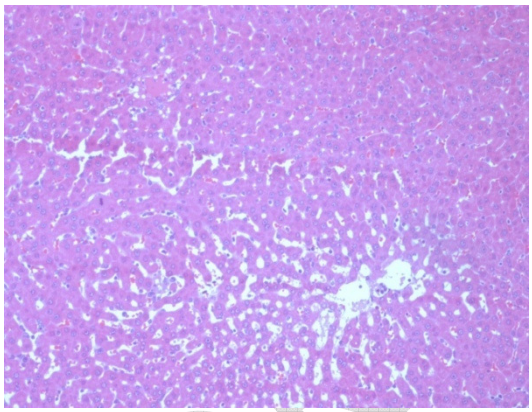

Fig-5b

Group –II

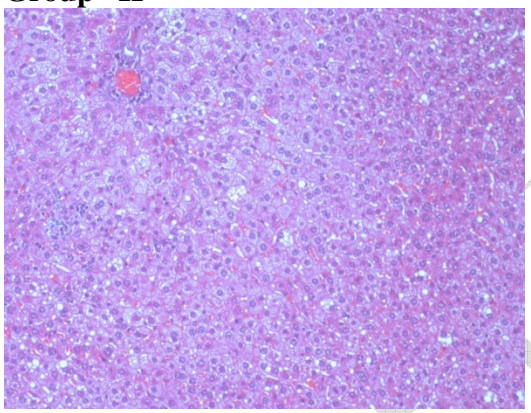

Fig-5c

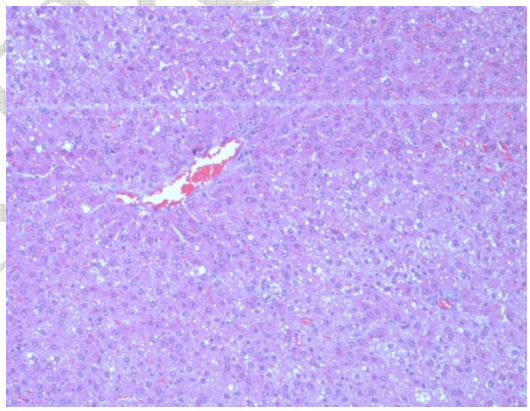

Fig-5d

Group –III

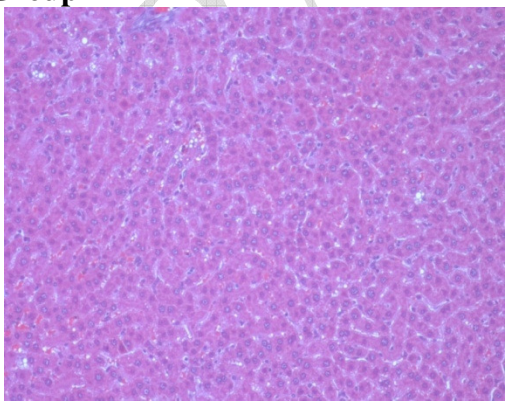

Fig-5e

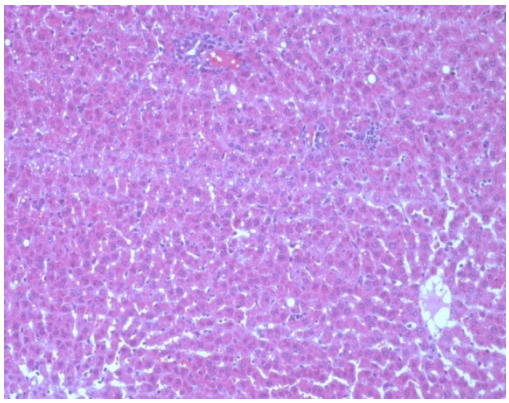

Fig-5f

Group –IV

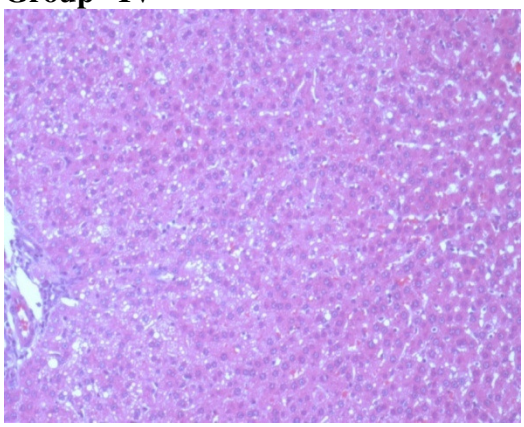

Fig-5g

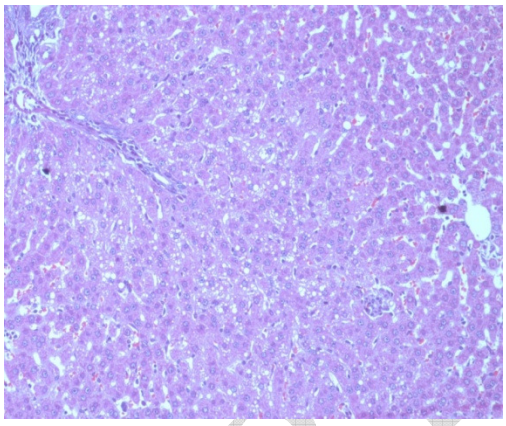

Fig-5h