Investigation of Antihyperlidemic activity of *Bixa Orellana* shells extract against Triton-X induced hyperlipidemia in *Rattus Nurvagicus*

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ABSTRACT

Vascular diseases including Atherosclerosis, Carotid Artery Disease, Carotid Artery Stenosis and peripheral arterial disease are one of the major contributors to the global burden of disease. Present study has been conducted to perform antihyperlidemic activity of *Bixa Orellana* shells extract against Triton-X induced hyperlipidemia in Rattus Nurvagicus. Hyperlipidemia in experimental rats was evidenced by an enhancement in the levels of serum cholesterol, triglycerides (TGs), low density lipoprotein (LDL), very LDL (VLDL) and decrease in high density lipoprotein (HDL). The characterization of *Bixa Orellana* shells extract was performed by the help of standard biochemical analysis. The alcoholic extract of *Bixa Orellana* shells extract (BOE 250 and 500 mg/kg body weight) was administered daily for 7 days in the experimental animals for the assessment of decrease in the hyperlipidemia. Hyperlipidemia was induced in experimental animals three days before the commencement of study by administering intraperitoneal injection of Triton X 100 (100mg/kg) in physiological saline solution after overnight fasting of 18 hrs. The Antihyperlipidemic activity was assessed using various biochemical parameters like, AST, ALT, ALP, Albumin, Total Protein, Total Cholesterol (TC), Triglyceride (TG), High density lipoprotein-Cholesterol (HDL-C), Low density lipoprotein-Cholesterol (LDL-C), and Very low density lipoprotein (LDL-C). However, the results demonstrated that the treatment with *Bixa Orellana* shells extract significantly ($P<0.05$ - $P<0.005$) and dose-dependently prevented lipid-induced increase in TC, TG, LDL-C, LDL-C, SGPT, and SGOT. The study revealed that *Bixa Orellana* shells possess high antioxidant activities. Histopathological study further decreased the inflammatory cells infiltration.

Key Words: Hyperlipidemia, Atherosclerosis, *Bixa Orellana*, Triglyceride, inflammatory.

Introduction

Hyperlipidemia is a medical condition characterized by elevated blood cholesterol and lipoprotein values. Plasma lipids include cholesterol levels, triglycerides (TGs), phosphate lipid and esters of cholesterol . Plasma lipoproteins, on the other hand, consist of VLDL, low-density lipoprotein (LDL), and decreased high-density lipoprotein (HDL). Lipoproteins are macromolecules that combine lipids and proteins. Their structure allows the lipids to mix easily with other watery bodily fluids. They are classified as non-polar lipids, lipids that are polar, and particular proteins. Non-polar lipids include cholesteryl esters and triglycerides, whereas polar lipids comprise unesterified cholesterol and phospholipids. The particular proteins are also referred to as apolipoproteins. Apolipoproteins are amphiphilic proteins that can bind to both lipids and plasma. Lipoproteins are also classed based on their densities. There are HDL and non-HDL such as chylomicrons (CM), VLDL, LDL, and intermediate-density lipoproteins (IDL) (1)

Atherosclerosis has been present for more than 4,000 years (2). The relationship with lipids is much younger. In 1665, Robert Boyle (1627-1691), as in “the law of”, discovered a fat transport system in animals. François Poulletier de la Salle (1719-1788) first identified solid cholesterol in gallstones in 1769 (3). Some ten years before, he isolated crystals from cholesterol for the first time. As his work was never published, attribution and dating are known only roughly, quoted by Pierre-Joseph Macquer (1718-1784) and Felix Vicq-d'Azyr (1748-1794) (5,6)

According to the Center for Disease Control and Prevention (CDC), 73.5 million or 31.7% of adults in the United States have high levels of LDL-C and are at twice the risk for heart disease than people with normal levels. Only 48.1% are receiving treatment to lower LDL-C levels. Recent data suggests that the classic disorder, familial hypercholesterolemia has a prevalence of estimate of 1/300,000 as homozygous and 1/250 as a heterozygote. In certain populations such as the French Canadians, Lebanese, and Afrikaners it could be as high as 1/100 (7,8,9,10)

Dyslipidemias involve clinically increased levels of cholesterol and/or triglycerides that may be accompanied with decreased HDL levels. Many of these patients have excessive hepatic VLDL production associated with elevated levels of triglycerides (TG), decreased levels of HDL cholesterol, and variable increased levels in LDL cholesterol. Excessive hepatic VLDL production can be driven by genetic factors, high carbohydrate diets, excessive alcohol use, obesity, insulin resistance, and nephrotic syndrome (11.). LDL levels have been causally associated with the risk of atherosclerotic cardiovascular disease (ASCVD) through absolute magnitude and cumulative duration of LDL-C exposure (12.). There is no current evidence for an established protective role for HDL against atherosclerosis (13,14).

Bixa orellana L. belongs to the Bixaceae family, also known as achiote, is a shrub native to tropical American countries (15) (16). Its cultivation has expanded to the Caribbean, African, and Asian regions. The traditional use of B. orellana in alleviating inflammation was reported. Infusion and decoction of B. orellana leaves have been used to treat sore throat, fever, bronchitis, conjunctivitis, gastric ulcer, and rheumatism. The fruit pup is used on burn-injured skin to prevent formation of blisters and sores. The seeds have been used for treating bronchitis and healing wounds in addition to be antibiotic and expectorant (17) (18). Bixa

orellana L. (achiote) is a commercially important plant grown for its natural dye annatto, which is derived from the arils of seeds. Annatto, which contains the antioxidant bixin, is used in food, cosmetic, and textile industries as a natural colorant. (19)

Material & Method

Plant Material Collection and Authentication: The shells of Bixa orellana were collected from local market of Ghaziabad, Uttar Pradesh, India in the January month of 2024. The plant fruit material was authenticated by Botanist Dr. K.C Bhatt, Principal Scientist at [ICAR-(National Bureau of Plant Genetic Resources)] (NBPGR), New Delhi, [Voucher No. (AC - 35/2024)].

Chemicals and Reagents:

All the analytical grade chemicals and reagents were used for the study. The chemicals were procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India. Organic solvents like ethanol, methanol (high-pressure liquid chromatography grade, Merck), Triron-X, 5% Tween, formalin, eosin, paraffin, chloroform, ethyl acetate, sulphuric acid, potassium permanganate and trichloroacetic acid were used. The drug used as standard was Atorvastatin (Sigma Chemicals, USA (BCBF6608V) Made by china). All the enzymatic kits and kits used for estimation of cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were procured from from Span Diagnostics Ltd. Surat (76LS200-60). Electronic Balance (ShimadzuAUX 220), Autoanalyzer (ShimadzuAUX 220), Research Centrifuge (Almicro Micromeasures &amp) were used during the study.

Preparation of Extract for Animal Study: The plant materials (shells) for 7 days were dried in sun before being dried at 50 °C in hot air oven (JSR, Korea). A mechanical grinder was used to powder the dried plant materials. As solvents, water that has been distilled, seventy percent ethanol, and seventy per cent methanol were utilised in the extraction procedure. The powdered sample (10 g) was placed in conical bottles containing distilled water (30%) with ethanol (70%), and methanol (70%), and shaken for three days at room temperature in orbital shaker. Using filter paper of Whatman No. 1, extracts were filtered, and resulting filtrates were then concentrated by utilising a rotary evaporator set to 45 °C. Lastly, extract containing extracts stocks (10 mg/mL) were made with NaCl (0.1 N). Stock extracts were diluted for quantitative measurement and qualitative screening of phytochemicals (20).

Preparation of TLC Sample: A fruit sample of 10 g was coarsely powdered and extracted in soxhlet extractor with 300 ml methanol and ethanol till sample discoloured. The extract was strained and condensed till dryness by help of a rotary vacuum evaporator under decreased pressure below 40 1°C. The yield as a percentage was observed to be 12.69% w/w. A preliminary TLC plate were sliced into smaller pieces and activated for 30 minutes at 110°C. Using the capillary, a single sample spot was put to the plate. Then plate was created in compartment with the required solvent mixtures for bixin and amino acids. The ratios of sample distances travelled relative to solvent distances were recognised as coloured dots on the TLC plates. Every plate was later put within a closed jar containing iodine vapour, which enabled to more precisely determine every location for greater visualization (21).

Table 1. Solvent system for TLC of *Bixa orellana* extract

Sr. No.	*Bixa orellana* Extract for identification	Solvent system (Ratio)
1.	Bixin	ethyl acetate: n-hexane (2: 8, 3:7, 4:6, 5:5)
2.	Amino acid (Leucine, Glycine, Proline)	formic acid: ethyl acetate: chloroform (1:4:5)

Animals: Wistar albino rat weighing 150-220 gm were used. The animal were housed at 22±3ºC with 12:12 hours light and dark cycle. They have free access to food and water ad libitum. The animals were acclimatized for a period of 7 days before the study. Animal studies were carried out in the laboratory of Institute for Industrial Research and Toxicology. All the experimental study was conducted in accordance with CPCSEA and IAEC guideline. The experimental protocol was approved by the Institute's Animal Ethics Committee with the approval Ref No MCSCOP/IAEC/2024/01/MPL09.

Phytochemical constituent's determination

The following phytochemicals such as tannins, alkaloids, flavonoids, saponins, steroids, terpenoids, carbohydrate, and proteins were determined by the methods described by Mikail and Venkitachalapathi kalaiselvi (22)
(23)

EXPERIMENTAL PROCEDURE

The 25 arbitrarily divided Rattus norvegicus in five groups, each with 5. During the group II to V, the infusion into the buffered saline of Triton x 100 (100 mg/kg) in single intraperitoneal injection in Rattus norvegicus rats after an overnight fast for 18 hours was initiated. The first group received traditional diet, liquid, and 5 percent Tween 80 orally. Triton-soluble-x-100mg/kg body weight has been administered in group II-V species. The second group got a 5% tween 80(po) regular dose for seven days after 72 hours of tritone injection. The third was issued for seven days, using 10mg/kg standardized Atorvastatin. During 5 percent tween 80 po for 7 days following stimulating hyperlipidemia, the normal doses of Carrum carvi hydroalcoholic extraction (CHE) 200 and 400 mg/kg bd. Wt. were administered to IV and V. 10 hours before blood screening, food was removed

Groupings: The animals were divided into five groups as shown in table.

Table 2: Design of experiment

S.No.	Groups	Treatment	Dose	Animals
1.	I (Normal)	5%Tween	As per b.wt	5
2.	II (Disease)	Triton-x	100mg\kg b.wt in 5%Tween	5
3.	III (Standard)	Atorvastatin + Triton-x	10mg\kg b.wt p.o+100mg\kg b.wt	5
4.	IV	CHE + Triton-x	200mg\kg	5

	(Extract at low dose)		b.wt+100mg\kg b.wt	
5.	V (Extract at high dose)	CHE + Triton-x	400mg\kg b.wt p.o+100mg\kg b.wt	5

Blood was collected from retro orbital plexus of the animals under anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 min to get serum. After separation of serum from blood, the parameters of lipid profile which was observed.

RESULTS

Primary phytochemical screening of *B. orellana* fruit extracts

Table 3: Primary phytochemical screening of *B. orellana* fruit extract with different constituents

S.NO	CONSTITUENTS	OBSERVATIONS
1	Steroids	++
2	Alkaloids	++
3	Phenolic compounds	++
4	Flavanoids	++
5	Gums and mucilages	++
6	Tannins	++
7	Carbohydrates	++
8	Saponins	+++
9	Glycosides	++
10	Proteins	++
11	phytosterols	++
Key: +++ =High, ++ =Moderate, + =Normal concentration, - = Absent		

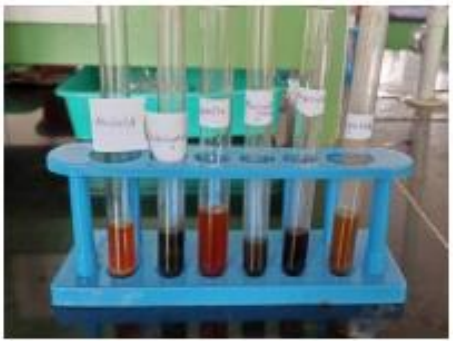

Figure 1: Different solvent *Bixa orellana* extracts

5.2. Standardization of extract of plant by Thin Layer Chromatography

2. **Thin Layer Chromatography for plant extracts standardisation**

The occurrence of diverse chemical compounds is clearly specified by many marks on plant extract TLC plates as indicated in the picture. The retention factors (Rf) were computed. As noted in, various combinations of n-hexane and ethyl acetate solvent system for bixin and Chloroform, ethyl acetate, and formic acid for amino acids (Proline, Leucine, and Glycine) yielded several Rf values for the plant extract *Bixa orellana*. As shown in **Table 4.**

Table 4. Several R_f values evaluated for numerous solvents ratios for *B. orellana* extract.

Extract of *Bixa orellana* for identifying	**Solvents used**	**Ratio**	**R_f values**
Bixin	n-hexane: ethyl acetate	8:2, 7:3, 6:4, 5:5	0.3, 0.7; 0.2, 0.5, 0.6; 0.3, 0.7, 0.8; 0.4, 0.7
Amino acid (Leucine, Glycine, Proline)	chloroform: ethyl acetate: formic acid	5:4:1	0.6; 0.3; 0.2

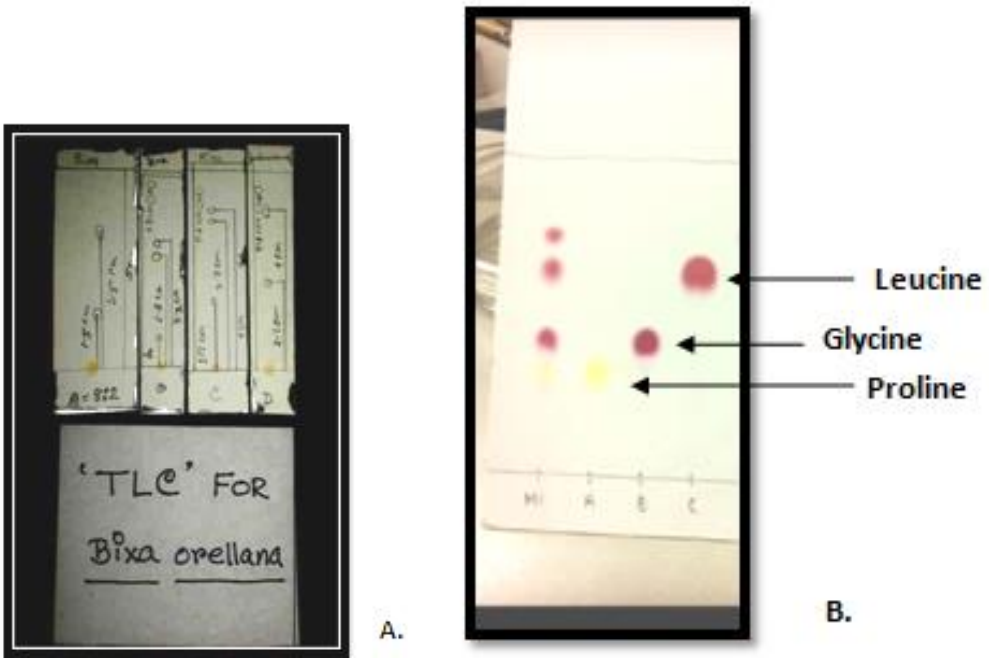

Figure 2. Identification on TLC plate: A. Bixin B. Amino acids (Proline, Glycine and Leucine) by extract of *Bixa orellana*.

5.3. Gross anatomy of liver from control and experimental groups of animals.

At the end of the experiment, Table 5 the administration of Triton-X 100 has resulted in increase of body weight gain compared to control group, while a significant change in the liver weight was observed in different intervention group. The animals fed with standard drug had lower body weight (BW), adipose tissue weight (ATW) and the same liver weight (LW) than those fed with the control diet. However, an increased LW/BW ratio of the animals fed with lower drug dose was observed. The mean liver weight in control and both doses of Bixa orellana at 250 and 500 mg/kg dose showed dose dependent decrease in body weight of standard and BOE treated animals. Figure 3 shows the gross anatomy of liver from control and experimental groups of animals. Figure 3A shows the normal anatomical structure of Group I- Normal control. Marked area by circle (figure 3B, C) indicates the damaged area of the liver in Triton-X treated-group II and BOE (250 mg/kg) treated group IV. BOE treated group V (500 mg/kg) reveals the normal structure of liver which was comparable to the lovastatin treated Group III having the normal anatomy of liver in figure 3D, E.

Table 5: - Body weight (BW), adipose tissue weight (ATW) from abdominal fat, liver weight (LW), liver- and body weight ratio, and adipose tissue- and body-weight ratio in rats fed standard group, control, toxic and extract dose group.

S.No.	BW (g)	ATW (g)	LW (g)	LW/BW (x100)	ATW/BW (x100)
Normal Control	220.68±25.60	6.62±1.80	7.12±0.52	3.19±0.25	2.58±0.72
Triton-X	288.23±35.62	9.21±1.90	12.11±0.65	4.20±0.28	3.19±0.87
Triton-X + Lovastatin (10mg/kg)	215.52±24.15	5.52±2.12	6.32±0.23	2.9±0.45	2.56±0.75
Triton-X + BOE (250mg/kg)	250.13±22.5	7.11±1.50	10.09±0.41	4.03±0.48	2.84±0.58
Triton-X + BOE (500mg/kg)	245.11±15.3	5.09±1.21	8.05±0.32	3.28±12	2.07±01

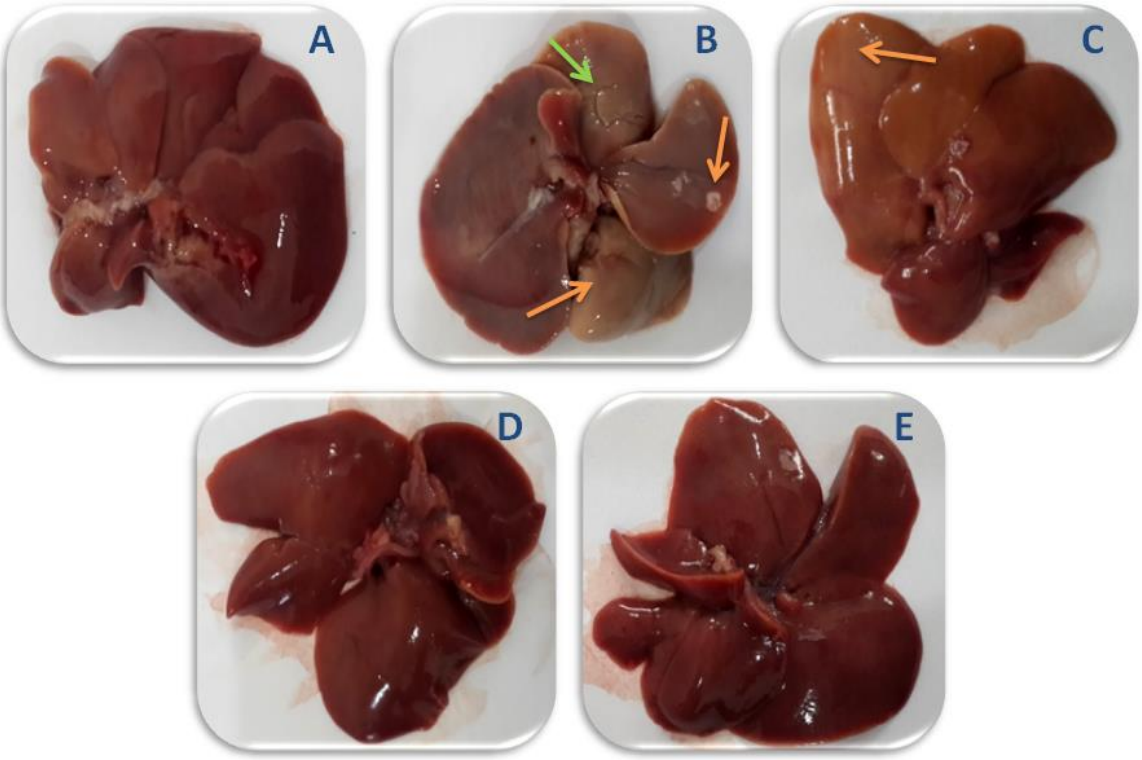

Figure 3: Gross anatomy of liver from control and experimental groups of animals, arrow mark indicated that damage of liver cell.

5.4. Effect of *B. orellana* treatment on experimental dyslipidemia

In the present study Table 6 revealed that, alcoholic extract of *Bixa orellana* significantly decreases the levels of serum TG, serum TC, LDL and VLDL and increase HDL levels. After the intervention of Triton X, there was a significant increase in the serum lipid parameters. These finding indicate the successful development of hyperlipidemia model.

The intervention group with high dose extract for seven days showed a significant decrease in the total cholesterol value that was similar to the lovastatin treated group, however a reduction was also observe in low dose group but that was not so significant when compared to lovastatin treated group but was significant when compared with disease control group. The disease control group i.e. the group that continued throughout experiment on triton X showed a significant higher value of total cholesterol and tri-glyceride so this result again confirmed the successfulness of hyperlipidemia model.

Meanwhile, no change was observed in the lipid values of control group throughout the experiment. The Tri-glyceride an important marker for the hyperlipidemia was also estimated in all groups. The higher values of TG in Disease control group in compared to control showed the hyperlipidemia effect of Triton -X intervention, A decrease in the TG and total cholesterol level in higher dose level group indicate that the *Bixa orellana* has a hyperlipidemia effect that can be explored for its therapeutic use.

5.4.1 Total Cholesterol

The total cholesterol values were raised significantly after the intervention of Triton X. The value was found significantly reduced in standard drug group ($p<0.005$) and high dose treated group ($p< 0.005$). The values of total cholesterol were also decreased in low dose group but were not found significantly reduced when compared with standard drug group**.**

5.4.2 Triglyceride

The Triglyceride values were found significantly raised after the intervention of triton X i.e. in disease control group. The standard drug treatment reduces the values of TG but was found significantly higher than the control group. The *Bixa orellana* treatment at high dose also reduces the values of TG in serum but still was significantly higher when compared with control. Though the result indicates that the treatment with high dose level have similar effect like standard drug**.**

5.4.3 High density lipoprotein-Cholesterol

The HDL values were found to be significantly lowered after the intervention of triton X these finding are comparable with the findings of total cholesterol in the present study, The *Bixa orellana* intervention at high doses have raised the HDL-C values more than the alone treatment of standard drug i.e. Atorvastatin. One of the interesting finding of *Bixa orellana* treatment that it raised the HDL-C level.

5.4.4 Low density lipoprotein-Cholesterol

These finding indicates the same type of pattern as found in case of HDL, the lower value of LDL-C in high dose intervention group than the standard drug was found to be a very interesting finding as these results indicate the positive role of *Bixa orellana* in lipid metabolism, this point is later discussed in brief in conclusion section of this study.

5.4.5 Very low-density lipoprotein (VLDL)

The values of VLDL were found to be higher in disease control group means that the Triton X, it not only altered the lipid metabolism but also indicates the changes in liver as VLDL is mainly produced in liver.

This is also an interesting finding of study that the TC and TG got towards the lower side after the intervention of *Bixa orellana* but the high level of VLDL had made some clues in liver metabolism that has to be addressed and needed further to investigation of *Bixa orellana.*

Table 6: Effect of *Bixa orellana* on Serum TC, TG, HDL-C, LDL-C, and VLDL Levels in Triton X-100 Induced Hyperlipidemia in Rats

S.No.	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)
Normal Control	78.5±8.5	112.4±11.7	162.6±13.79	47.4±5.2	2.54±0.12
Triton-X	335.4±5.1	2361.5±11.5	801.1±13.02	36.3±3.9	18.9±0.57
Triton-X + Lovastatin (10mg/kg)	305.3±7.7	2002.1±11.7	724.8±11.17	39.7±8.9	7.66±0.35
Triton-X + BOE (250mg/kg)	272.2 ± 10.1*	2013.2±11.3*	701.1 ± 3.6*	44.7±9.3*	14.74 ± 0.35*
Triton-X + BOE (500mg/kg)	270.1± 6.25*	1999.0 ± 9.5*	575.4± 12.1*	58.5±6.8*	12.1 ± 0.36*

*Values are expressed as mean ± SEM (n=5 animals in each group). *P<0.005 as compared with normal control group, #P<0.005 as compared with triton-X treated group, SEM: Standard error of mean, *Bixa orellana* hydroalcoholic extract.

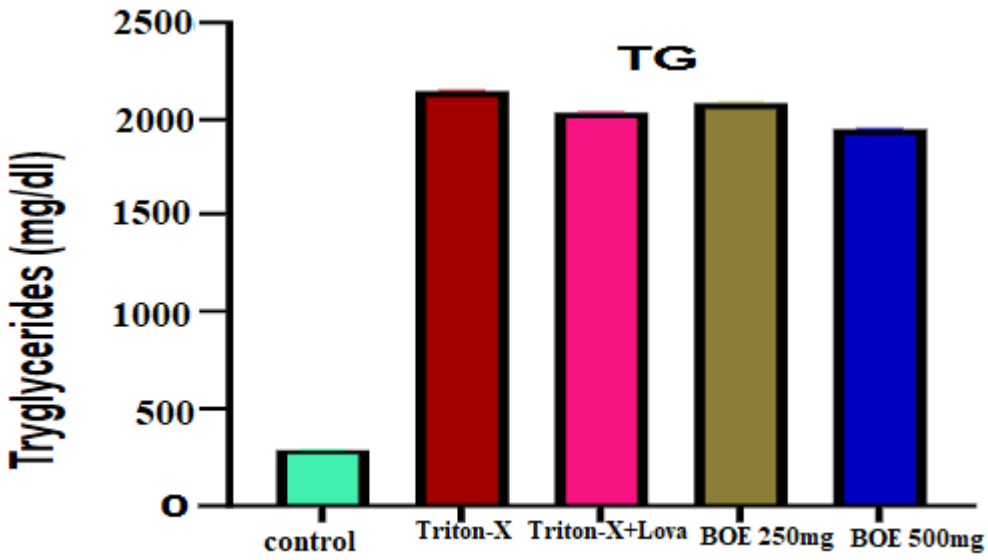

Figure 4: Effect of *Bixa orellana* shells extract on plasma Triglycerides levels in triton-X induced hyperlipidemic rats when compared with control group. Values are mean ± SEM of six rats **$p \leq 0.005$.

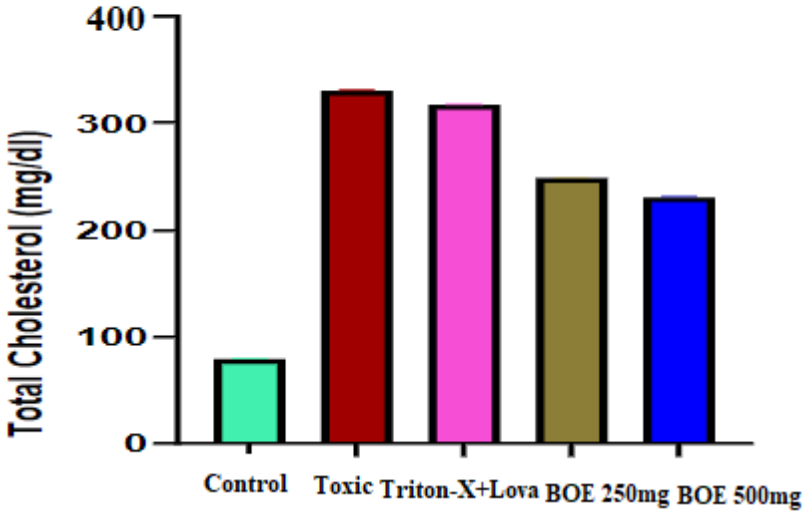

Figure 5: Effect of *Bixa orellana* shells extract on plasma Total Cholesterol levels in triton-X induced hyperlipidemic rats when compared with control group. Values are mean ± SEM of six rats **$p \leq 0.005$.

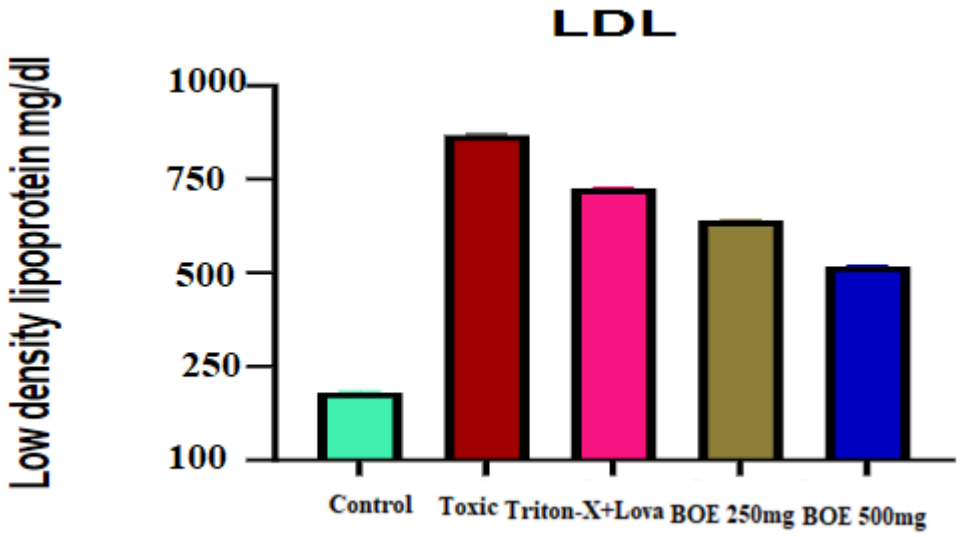

Figure 6: Effect of *Bixa orellana* shells extract on plasma LDL-C levels in triton-X induced hyperlipidemic rats when compared with control group. Values are mean ± SEM of six rats **$p \leq 0.005$.

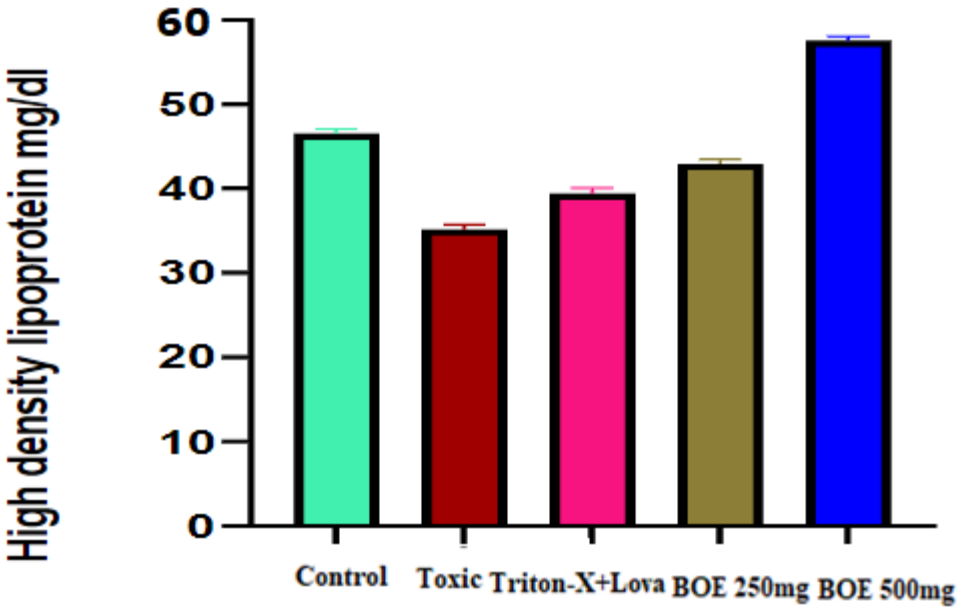

Figure 7: Effect of *Bixa orellana* shells extract on plasma HDL-C levels in triton-X induced hyperlipidemic rats when compared with control group. Values are mean ± SEM of six rats **$p \leq 0.005$.

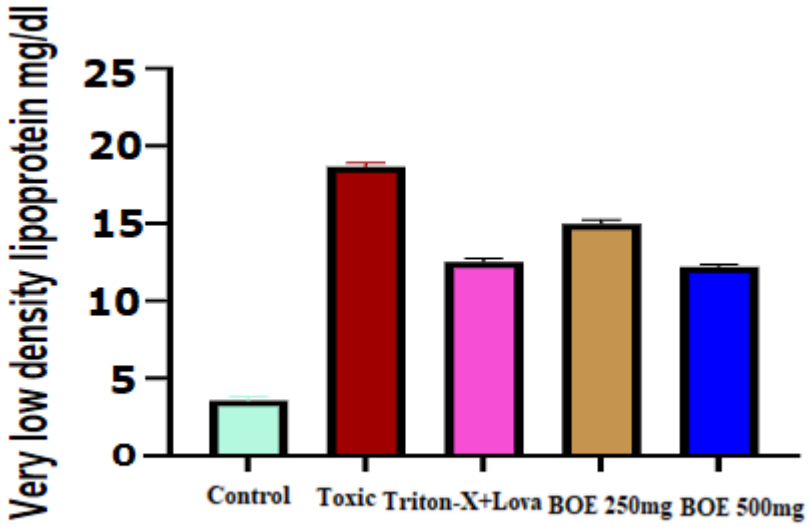

Fig 8: Effect of *Bixa orellana* shells extract on plasma VLDL-C levels in triton-X induced hyperlipidemic rats when compared with control group. Values are mean ± SEM of six rats **$p \leq 0.005$.

The data are expressed as mean ± SEM. n = 8-10 rats per group. *$p < 0.05$, compared with control group (ANOVA followed by Tukey's test).

5.5. BIOCHEMICAL PARAMETERS

The Liver enzymes were estimated in all groups to access the liver function in all groups, the AST, ALT, ALP enzymes, Albumin and total protein were estimated and found that Triton X not only altered the lipid metabolism but also distorted the liver enzyme levels. The values of AST, ALT, ALP, Albumin and total protein were found significantly raised in disease control group when compared with control group.

The values were found to normalize after the intervention of standard, and BOE extract group while a significant reduction in the values of AST, ALT, ALP, ALB and Total Protein were observed in low dose group as shown in table and figure.

Table 7:

S.No.	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (mg/dl)	Total Protein (g/dl)
Normal Control	119.5 ± 4.2	48.1±5.2	27.87±6.72	501.78±59.72	48.51±8.90
Triton-X	192.5 ± 5.2*	78.5±6.2	66.62±6.74	515.28±73.88	51.49±5.02

Triton-X + Lovastatin (10mg/kg)	155.8 ± 7.1*	67.2±7.2	45.21±7.41	451.28±71.33	35.01±6.50
Triton-X + BOE (250mg/kg)	161±5.4	64.7±7.4	46.96±5.92	463.05±66.84	35.00±6.52
Triton-X + BOE (500mg/kg)	201.8±5.7	66.9±5.8	47.84±5.62	466.02±60.45	29.02±4.35

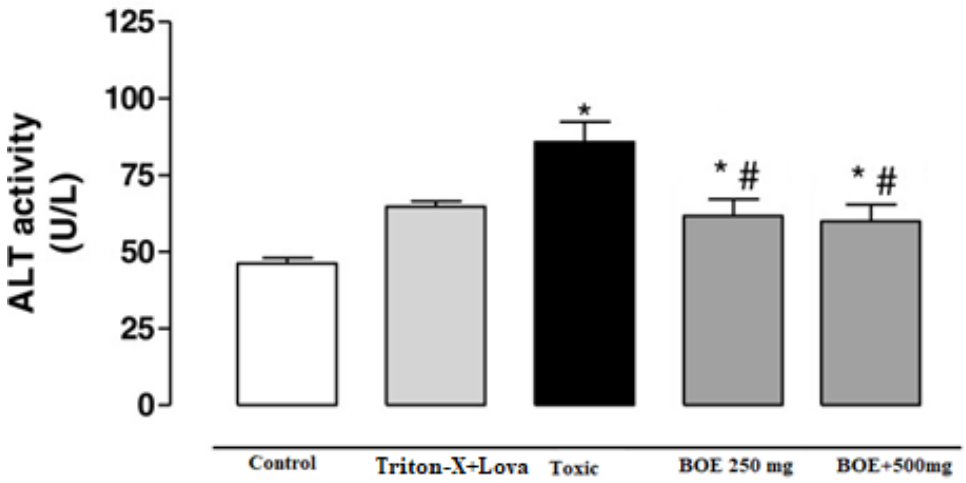

Figure 9: Effect of *Bixa orellana* shells extract on the activity of alanine transaminase (ALT) in the serum of control and ethanol induced hepatotoxicity in rats.
Values are expressed as mean ± S.E.M. of 5 rats in each group
P values: #<0.001 compared with respective control group I
P values: *<0.05, **<0.01, ***<0.001 compared with group II (Ethanol).

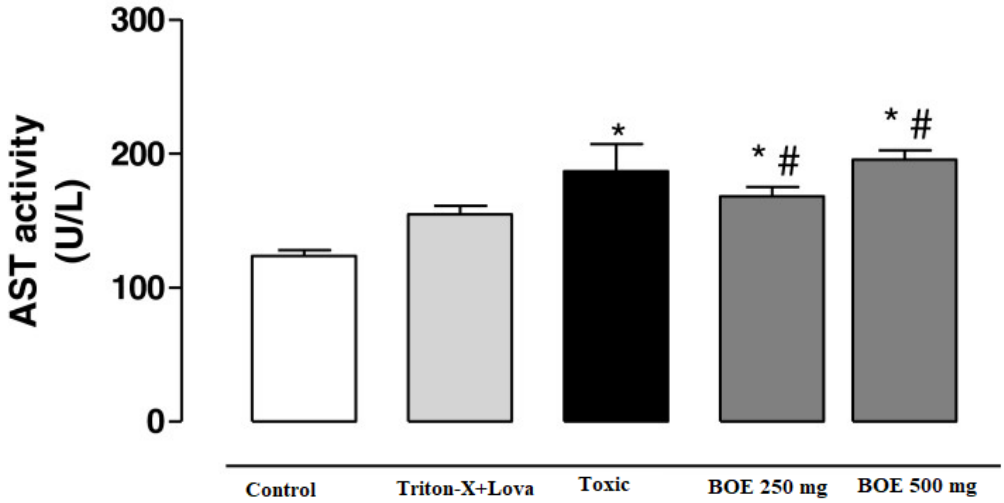

Figure 10: Effect of *Bixa orellana* shells extract on the activity of aspartate transaminase (AST) in the serum of control and ethanol induced hepatotoxicity in rats.

Values are expressed as mean ± S.E.M. of 5 rats in each group
P values: #<0.001 compared with respective control group I
P values: *<0.05, **<0.01, ***<0.001 compared with group II (Ethanol).

5.4 ANTIOXIDANT PARAMETERS

The Levels of GSH, SOD, catalase in serum and MDA was estimated in all groups and the results are shown in table __, figure___.

Table 8: Effect of *Carum Carvi* on Tissue SOD, GSH, CAT and MDA in Triton X-100-Induced Hyperlipidemia in Rats

S.No.	SOD (U/mg)	GSH nmol/mg protein.	CATALASE (µmol /L)	MDA (nmol MDA/mg protein)
Normal Control	19.33±0.05	68.36±1.34	54.65±1.63	1.21±0.01
Triton-X	12.24±0.15	25.38±2.45	16.75±0.51	9.73±0.01
Triton-X+ Lovastatin (10mg/kg)	15.49±0.9	68.68±1.67	53.13±0.84	2.38±0.01
CCHE (250mg/kg)	17.38±0.11	66.49±1.35	42.26±0.76	2.64±0.01#
CCHE (500mg/kg)	18.01±0.11	67.59±1.49	43.54±0.74	1.32±.01##

All values were expressed as mean± SEM, n=5, analyzed by ANOVA followed by Dunnett's t test *p<0.005; when compared with the vehicle control; # P<0.005; when compared with the Triton X-100 group

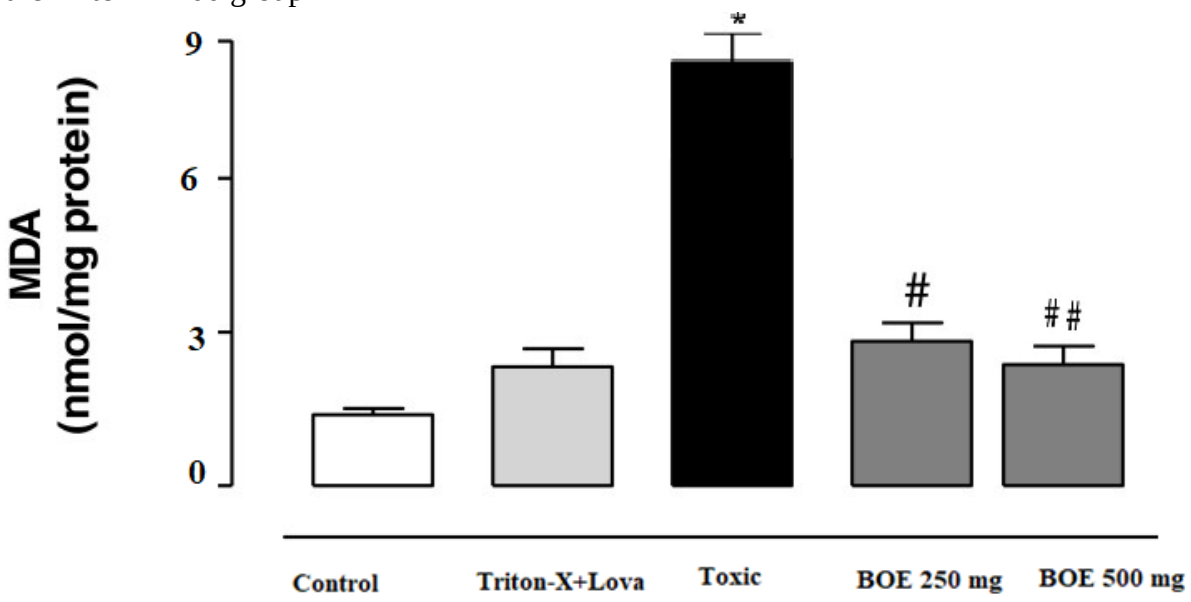

Figure 11: Effect of *Bixa orellana* shells extract on the activity of Malondialdehyde (MDA) in the serum of control and ethanol induced hepatotoxicity in rats.

Values are expressed as mean ± S.E.M. of 5 rats in each group
P values: #<0.001 compared with respective control group I
P values: *<0.05, **<0.01, ***<0.001 compared with group II (Ethanol).

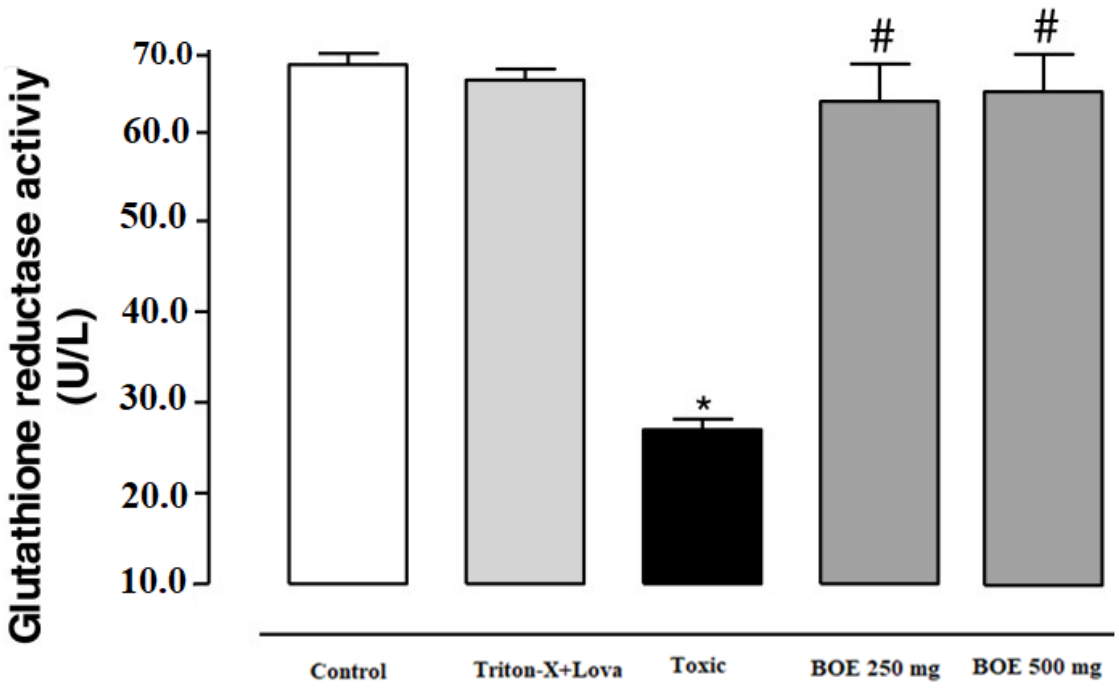

Figure 12: Effect of *Bixa orellana* shells extract on the activity of Glutathione reductase (GSH) in the serum of control and ethanol induced hepatotoxicity in rats.
Values are expressed as mean ± S.E.M. of 5 rats in each group
P values: #<0.001 compared with respective control group I
P values: *<0.05, **<0.01, ***<0.001 compared with group II (Ethanol).

Effects of Bixa orellana extract on oxidative stress parameters and on NO production

The SOD activity of the toxic group was significantly ($P < 0.01$) decreased in serum, and in liver compared with the normal control group. Blood SOD activity was 15.49±0.9 ($P < 0.01$), 17.38±0.11 ($P < 0.01$), and 18.01±0.11 ($P < 0.01$), IU/L in rats treated with Lovastatin and alcoholic extract of *Bixa orellana* at 250, and 500 mg/kg body weight, respectively. (Table 8).

Catalase activity was significantly ($P < 0.01$) decreased in serum, and in the liver in the toxic compared with the normal control. The alcoholic extract of *Bixa orellana* at different doses significantly increased the catalase activity in the serum and in liver, but with a more remarkable increase in the blood 43.54±0.74; $P < 0.01$) at 500 mg/kg body weight compared with the control group (Table 8).

Serum GSH levels were 68.36±1.34, 25.38±2.45 ($P < 0.01$), 68.68±1.67, 66.49±1.35 ($P < 0.05$), and 67.59±1.49 µmol/L, respectively, in Normal Control, toxic, and rats treated with lovastatin and alcoholic extract of *Bixa orellana* at 250, 500 mg/kg body weight (Table 8).
The concentration of MDA significantly increased ($P < 0.01$) in serum, and in liver of toxic rats compared to normal control rats. The alcoholic extract of *Bixa Orellana* in different doses caused a significant decrease ($P < 0.05$) in the level of MDA in serum, and in the liver compared with the negative controls (Table 8).

HISTOPATHOLOGICAL ASSESSMENT

Histopathological studies of the liver of normal control treated animals showed normal histology (Figures 13A). In animals treated with Triton-X, inflammation, necrosis and hydropic degeneration of hepatic cells was observed (Figure 13B). Liver section of rats treated with triton-X and lovastatin showing normal morphology of hepatocyte (figure 13E). The group that was pretreated with BOE shell extract showed that severe hepatic lesions induced by triton-X were partially prevented (Figure 13D and C), which were in agreement with the results of the serum aminotransferases activities and lipid peroxidation.

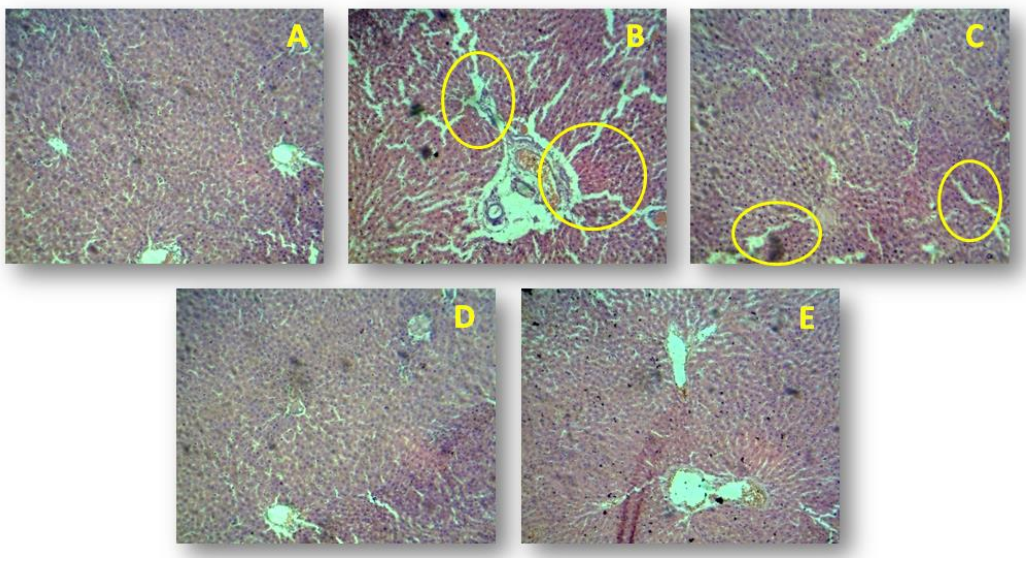

Figure 13. Histopathology of liver from control and experimental groups of animals, circle mark indicated that damage of liver cells.

DISCUSSION AND CONCLUSION:

After the intervention of Triton X there was a significant increase in the serum lipid parameters. These finding indicate the successful development of hyperlipidemia model.The intervention group with high dose extract for seven days showed a significant decrease in the total cholesterol value that was similar to the Atorvastatin treated group, however a reduction was also observe in low dose group but that was not so significant when compared to Atorvastatin treated group but was significant when compared with disease control group. The disease control group i.e. the group that continued throughout experiment on triton X showed a significant higher value of total cholesterol and tri-glyceride so this result again

confirmed the successfulness of hyperlipidemia model. No changes were observed in the lipid values of control group throughout the experiment.

HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) is the key enzyme of cholesterol biosynthesis). NADPH (nicotinamide adenine dinucleotide phosphate) is necessary for fatty acids and cholesterol synthesis. Caraway cholesterol lowering activity could be related to its role in reducing the activity of HMG-CoA reductase and reducing concentration of NADPH[20]. Bioactive metabolites of caraway are carvone, carvocol, limonene, thymol, carveol, linalool, estragol and anethole. These agents possess antioxidant activity which could play an important role in reducing body weight[21]. Caraway increases secretion of hormones, affecting the sense of satiety such as leptin and cholecystokinin (CCK) which in turn results in delayed gastric emptying, loss of appetite and decrease in body fat [22]. It also stimulates apoptosis in preadipocytes, as well as lipolysis in fat droplets [23]

The Tri-glyceride an important marker for the hyperlipidemia was also estimated in all groups. The higher values of TG in Disease control group as compared to control showed the hyperlipidemia effect of Triton -X intervention, decrease in the TG and total cholesterol level in higher dose level group indicated that the *Bixa orellana shells* has a hyperlipidemia effect that can be explored for its therapeutic use.

The anti-oxidant parameter result indicates that a significant raise in the levels of MDA was observed in triton x treated group alone when compared with control, however a significant reduction was observed in low and high dose group was observed when compared with Atorvastatin control group. The levels of both SOD and catalase were found to be lowered after the triton x alone intervention. The levels of SOD retain to normal control in atorvastatin alone and in high dose while a significant improvement was observed in low dose group when compared to disease control group.

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