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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *LAWSONIA ALBA* LEAVES ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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Carbon tetrachloride, hepatoprotective activity, *Lawsonia alba*, silymarin

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ABSTRACT

The objective of the present investigation was to study hepatoprotective activity of aqueous, alcoholic extracts, fractions and simultaneous administration of aqueous and alcoholic extracts of *Lawsonia alba* leaves using carbon tetrachloride induced liver damage in rats. The liver damage in rats was produced by intraperitonial administration carbon tetrachloride (1.5ml/kg body weight in olive oil 1:7) in either sex wistar rats (150-200gm) once daily for seven days and the extent of damage was studied by assessing biochemical parameters such as SGPT, SGOT, ALP and TBIL in serum. Histopathological changes in liver were studied and liver weights were also recorded. The aqueous, alcoholic extracts and fractions of *Lawsonia alba* leaves (300mg/kg body weight) were orally administered to the experimental animals and its effect on biochemical parameters and histopathological examinations were compared with silymarin(100mg/kg body weight) treated animals. The results indicated that biochemical changes produced by carbon tetrachloride were restored to normal by aqueous, alcoholic extracts and fractions of *Lawsonia alba* leaves.

INTRODUCTION

Lawsonia alba (Lythraceae) commonly known as “Henna”(mehendi) is a shrub frequently cultivated in India, the middle east and along the African coast of Mediterranean sea. (1)Besides its use in cosmetics for staining hands and as hair dye the leaves are used as prophylactic against skin diseases. The leaves are greenish brown, about 2.5-5 cm long, the margin entire and venation pinnate. (2) Henna is reported to contain napthaquinone lawsone. Lawsone is also reported to be an immunostimulant. An ointment prepared from leaves was used to cure the ulcers and wounds. (3) The astringent stem bark of Lawsonia alba is traditionally used in India for treatment of jaundice, enlargement of liver and spleen (4) calculus affliction, and skin diseases (5). One of the major active constituents of leaves of Lawsonia alba are flavonoids(6) The objective of present study was to study hepatoprotective activity and Histopathological studies of leaves of *L.alba*.

MATERIAL AND METHODS

Drugs and chemicals

Carbon tetrachloride was obtained from E.Merck Ltd (India), Mumbai. Silymarin was purchased from Micro labs, India. All other chemicals used in the study were of analytical grades.

The intraperitonial injection of carbon tetrachloride i.e.1.5ml/kg of body weight of carbon tetrachloride is diluted in olive oil (1:1 dilution) was employed for inducing liver toxicity. (7)

Plant material

In present study, the matured leaves of *Lawsonia alba* was collected from local areas of Otur (Pune), Maharashtra. The plan sample was authenticated by Botanical Survey of India, Pune. The voucher specimen (LIDG1) has been preserved in Department of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Otur.

Extract preparation

The collected leaves of *Lawsonia alba were* washed, air dried, powdered and extracted with ethanol as a solvent using a Soxhlet extractor (25 cycles) at 5 batches. After exhaustive extraction, the

collected extract was dried under reduced pressure using rotary flash evaporator and dried at room temperature. The part of extract was used for r fractionation with pet ether and chloroform.

The aqueous extract was being obtained by subjecting the drug to the maceration process carried out for 7 days using the distilled water with occasional stirring and concentrating the supernant liquid to the small volume under the reduced pressure and then evaporated to the dryness.

The aqueous, alcoholic, pet ether and chloroform extracts were subjected for further hepatoprotective studies.

Animals

Male Swiss albino mice weighing between 20-25gm and male albino wistar rats weighing 150-200 gm were used for this study. The animals were obtained from animal house, Raj Biotech, Pune. On arrival, the animals were placed randomly and allocated to treatment groups in polypropelene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{0}C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw diet. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (1197/08c/CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Acute toxicity studies

Acute toxicity studies were performed according to OECD-423 guidelines (8). Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 12 hrs with free access to water only. The various extract and fractions of *Lawsonia alba* were administered orally. The initial dose of 5mg/kg of various extract and fractions of *Lawsonia alba* administered and mortality if any was observed for 24 hrs. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals, then the same dose was

repeated again to confirm the toxic effect. If no mortality was observed, the higher (50,100,250,500,1000,2000,3000mg/kg) doses of various extracts and fraction of *Lawsonia alba* for further studies. One tenth of LD_{50} was used as a maximum dose of extracts tested for acute toxicity. The doses were selected for evaluation of hepatoprotective activity was 300mg/kg.

Experimental Design:

Assessment of the hepatoprotective activity was done Silymarin was taken as standard drug.

Animals were divided into six groups each containing 6 animals.

Group I - received daily-olive oil, i.p. for 7 days (control).

Group II - received CCL4 (1.5ml/kg), i.p. for 7 days.

Group III- received CCL4 (1.5ml/kg) i.p. for 7 consecutive days + Silymarin (100 mg/kg), p.o from day 8 onwards.

Group IV- received CCL4 (1.5ml/kg) i.p. for 7 consecutive days + alcoholic extract of *Lawsonia alba* (300 mg/kg), p.o from day 8 onwards.

Group V - received CCL4 (1.5ml/kg) i.p. for 7 consecutive days + aqueous extract of *Lawsonia alba* (300 mg/kg), p.o from day 8 onwards.

Group VI - simultaneously CCL4 (1.5ml/kg) i.p. and alcoholic extract of *Lawsonia alba* (300 mg/kg), p.o followed by drug alone from day 8.

Group VII - simultaneously CCL4 (1.5ml/kg) i.p. and aqueous extract of *Lawsonia alba* (300 mg/kg), p.o followed by drug alone from day 8.

Group VIII- received CCL4 (1.5ml/kg) i.p. for 7 consecutive days + CHCL3 fraction of *Lawsonia alba* (300 mg/kg), p.o from day 8 onwards.

Group XI- received CCL4 (1.5ml/kg) i.p. for 7 consecutive days + pet ether fraction of *Lawsonia alba* (300 mg/kg), p.o from day 8 onwards.

On the seventh day of the start of respective treatment the rats were anaesthetized by light ether anesthesia and the blood was withdrawn by retro orbital plexus. It was allowed to coagulate for 30 min and serum was

separated by centrifugation at 2500 rpm. The serum was used to estimate serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP).

Biochemical Estimation:

The blood was obtained from all the animals by puncturing retro-orbital plexuses. The blood samples were allowed to clot for 45 min. at room temperature. Serum was separated by centrifugation 10000 rpm for 5min at $30^{o}C$ and utilized for estimation of various biochemical parameters namely SGPT (Aghape, India), SGOT (Teco, US), ALP (Teco, US), total bilirubin (Bayer, India).

Histopathological Examination:

All animals were sacrificed on last day of experimental study, blood was collected and liver was removed and washed with saline. The liver pieces were preserved in 10% formalin for Histopathological studies. The sections were approximately 4-6 micron in thickness. They were stained with hematoxylin and eosin and photographed.

Statistical Analysis:

The data's were expressed as mean ± SEM. For obtaining this data, biochemical and physiological parameters were stastically analyzed using one-way ANOVA followed by Dunnet's test. (Ref Remington) For comparison with control group and CCl4 treated group, $P<0.05$ was considered as significant.

RESULTS :

Table 1: Effect of *Lawsonia alba* Extracts and Fractions

Biochemical Parameters (Mean ±SEM)

Treatment	SGPT	SGOT	ALP	TBIL	Liver Weight
Control (Vehicle Treated)	19.61 ± 1.648	27.61 ± 3.924	50.43 ± 5.708	0.3013 ± 0.01349	6.135 ± 0.1209
CCl4 Treated	84.92 ± 17.46 [a]	61.47 ± 2.435 [a]	110.6 ± 16.40 [b]	0.6017 ± 0.06896 [b]	7.210 ± 0.2250 [c]
Silymarin (100mg/kb b.wt.)	33.88 ± 5.539 [x]	39.14 ± 1.571 [y]	74.57 ± 2.380 [z]	0.6983 ± 0.05986	6.448 ± 0.1242 [x]
Aq. LA (300mg/kb b.wt.)	40.94 ± 2.809 [x]	42.46 ± 2.389 [y]	56.96 ± 6.499 [x]	0.7883 ± 0.08604	6.203 ± 0.08094 [y]
Alc.LA 300mg/kb b.wt.)	31.19 ± 2.217 [x]	40.15 ± 2.232 [y]	53.30 ± 2.956 [y]	0.6483 ± 0.05474	6.345 ± 0.1098 [y]
Sim Aq. LA (300mg/kb b.wt.)	48.32 ± 2.315 [x]	37.91 ± 2.574 [y]	67.82 ± 3.023 [y]	0.6275 ± 0.06411	6.210 ± 0.1978 [y]
Sim Alc. LA (300mg/kb b.wt.)	38.38 ± 4.426 [y]	33.52 ± 3.887 [y]	72.33 ± 1.857 [x]	0.6055 ± 0.08640	6.435 ± 0.1135 [x]
Pet Ether LA 300mg/kb b.wt.)	27.91 ± 1.966 [y]	42.18 ± 1.625 [y]	56.28 ± 3.202 [y]	0.7583 ± 0.03410	6.352 ± 0.07436 [y]
CHCL3 LA 300mg/kb b.wt.)	35.35 ± 4.962 [y]	45.01 ± 2.442 [y]	71.08 ± 3.048 [y]	0.6933 ± 0.04022	6.272 ± 0.07476 [y]

a= P<0.0001 when compared with Control Rats.

b= P<0.001 when compared with Control Rats.

c= P<0.05 when compared with Control Treated Rats.

x= P<0.001 when compared with CCl4 Rats.

y= P<0.0001 when compared with CCl4 Rats.

Z= P<0.05 when compared with CCL4 Treated Rats.

Results:

Carbon tetrachloride intoxication in normal rats elevated the levels of SGOT, SGPT and ALP and total bilirubin indicating acute hepatocellular damage and biliary obstruction.

Similarly simultaneous administration of trial drug and carbon tetrachloride for 7 days showed marked increase in SGOT, SGPT values on the 7^{th} day. The rats treated with aqueous, alcoholic extract, fraction and also silymarin showed a significant decrease in elevated SGOT, SGPT and ALP. There was no significant effect on total bilirubin throughout the study.

The rats treated with pet.ether and chloroform fraction showed marked decrease in SGOT, SGPT and ALP which are nearly equal to the values of standard drug (silymarin). Table no.1

Histopathological examination of liver section of control showed normal cellular architecture with disturbed hepatic cells, sinusoidal spaces and central vein (Figure 1). Disarrangement of normal hepatic cells with intense centrobular necrosis and vacuolization of periportal vein are observed in carbon tetrachloride treated rats. (Figure 2).

The level of fatty degeneration, necrosis, congestation and vacuole formation were obvious after acute carbon tetrachloride treatment. Administration of extracts and fraction of *Lawsonia alba* at the dose of 300mg/kg and 100mg/kg of silymarin for 15 days reduced the hepatic injury score, fatty degeneration and necrosis.

The liver section of the rats treated with aqueous (figure 4), alcoholic extract(figure 5), simultaneous treated by alcoholic extract (figure 6), simultaneous treated by aqueous (figure 7), chloroform fraction (figure 8), pet ether fraction(figure 9) showed 60-100% cell regeneration capacity in the carbon tetrachloride induced rats. Even the standard drug silymarin showed significant results (figure 3).

The result are expressed in the form of hepatic necrosis, inflammation and congestion of cells and fatty changes in table 2

The results of hepatic histopathological examination CCl4-induced hepatotoxicity in adult rats are shown in Table 2.

Groups	Hepatic necrosis	Inflammation	Congestion	Fatty change
CCl4+ Silymarin	--	+	+	+++
CCl4+ Alcoholic	--	--	+	--
CCl4+ Aqueous	--	+	+	+++
CCl4+ pet.ether.	--	+	++	+++
CCl4+ Chloro	+	++	+	++
CCl4+ Aq. (Sim)	--	+	+	+++
CCl4+ Alc. (Sim)	--	+	+	+++
CCl4	+	+	+	+++
Control	--	++	+	--

Gross: H& E stained slides received for evaluation.

Grades:

-- : No change

+ : 30% of the cells show change.

++ : 30-60% of the cells show change.

+++: 60-100% cell show change.

DISCUSSION

Plant extract have been used by traditional medical practitioners for the treatment of liver disorders for centuries (9). In Indian system of medicine certain herbs are claimed to provide relief against liver disorders.

In the present study ethanolic, aqueous extract and fractions of *Lawsonia alba* posses significant ($p<0.0001$) hepatoprotective effect in carbon tetrachloride model of intoxication in rats. Our qualitative phytochemical investigation showed presence of flavonoids in ethanolic extract,

flavonoids, and phenolic glycosides in pet.ether, flavonoids and triterpenes in chloroform fraction. According to this the presence of flavonoids, phenolic glycosides could be considered for the hepatoprotective activity.

The hepatotoxicity of carbon tetrachloride has been reported to be due to formation of highly reactive trichloro free radical which attacks polyunsaturated fatty acid. It produced hepatotoxicity by altering liver microsomal membranes in experimental animals (10)

The effect of hepatotoxicity is observed on 7^{th} day after inducing the carbon tetrachloride. Hence the withdrawing of blood for biochemical parameters were carried out after 13 day in carbon tetrachloride intoxication (11).

From table no 1, it is evident that pet.ether and chloroform fraction extensively reduced the all elevated biochemical parameters due to the hepatotoxin intoxication. The reduction in the level SGOT, SGPT and ALP towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by carbon tetrachloride. The protective effect exhibited by the pet.ether and chloroform fraction is nearly similar to silymarin treatment.

Histopathological examinations of the liver reveal that the normal liver was disturbed by hepatotoxin intoxication. In the section obtained from the rats treated with pet.ether and chloroform fraction and intoxicated with hepatoxin, the normal cellular structure was retained as compared to silymarin and thereby by confirming protective effect of the extracts.

It can be concluded from the investigation that among all aqueous, alcoholic extract, pet.ether and chloroform fractions have shown better hepatoprotective effect, only the little difference are observed in result analysis. Overall the drug is effective.

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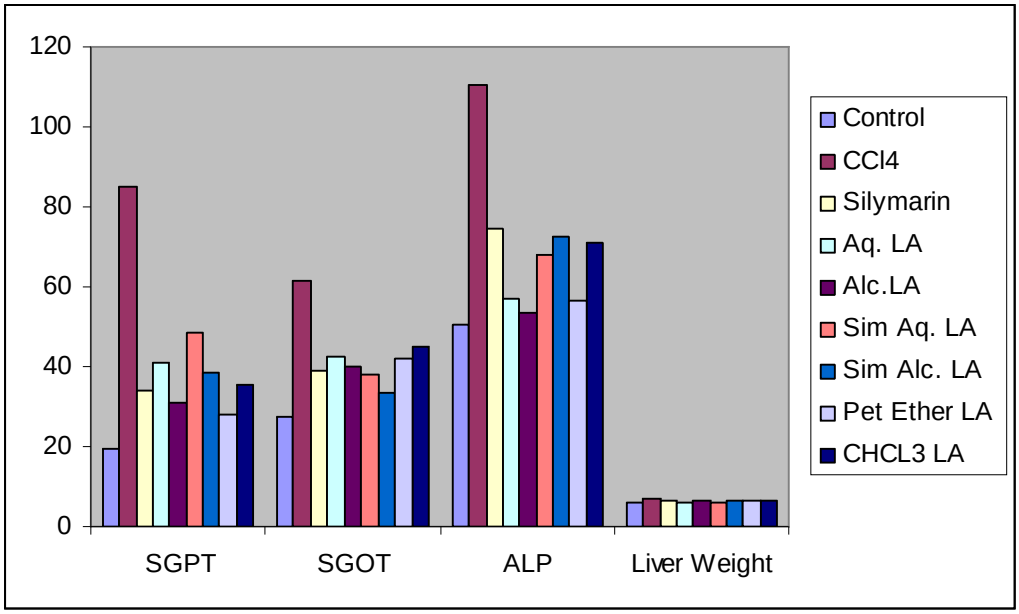
Figure . A Effect of *Lawsonia alba* leaves on serum SGPT, SGOT, ALP and Liver weight in CCL_4 induced hepatotoxicity in rats.



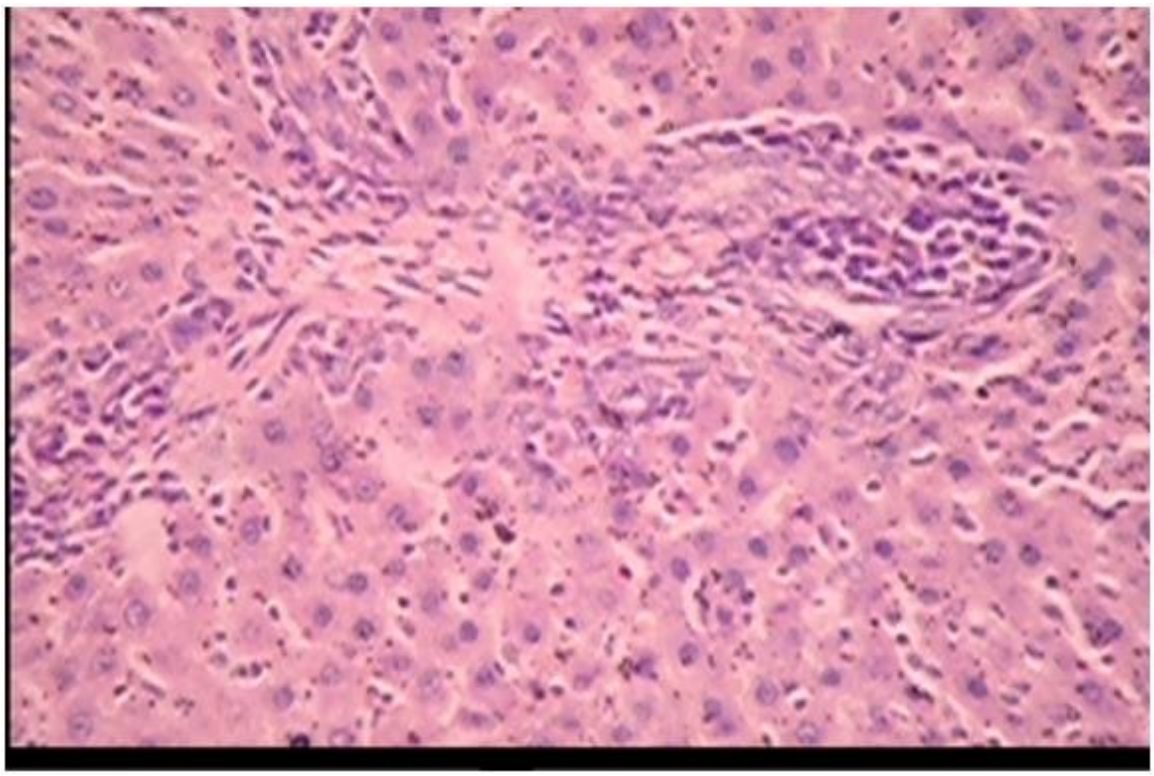


Figure 1. Histopathological examinations of liver section Control treated group

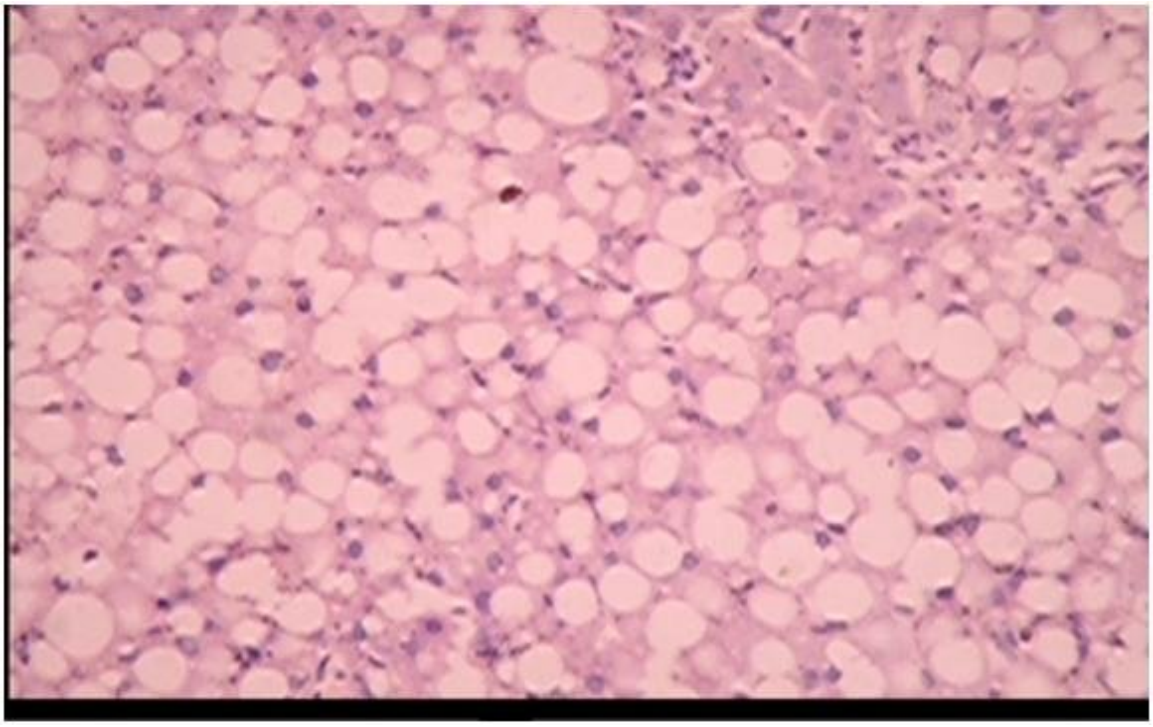


Figure 2. Histopathological examinations of liver section Carbon tetrachloride treated group

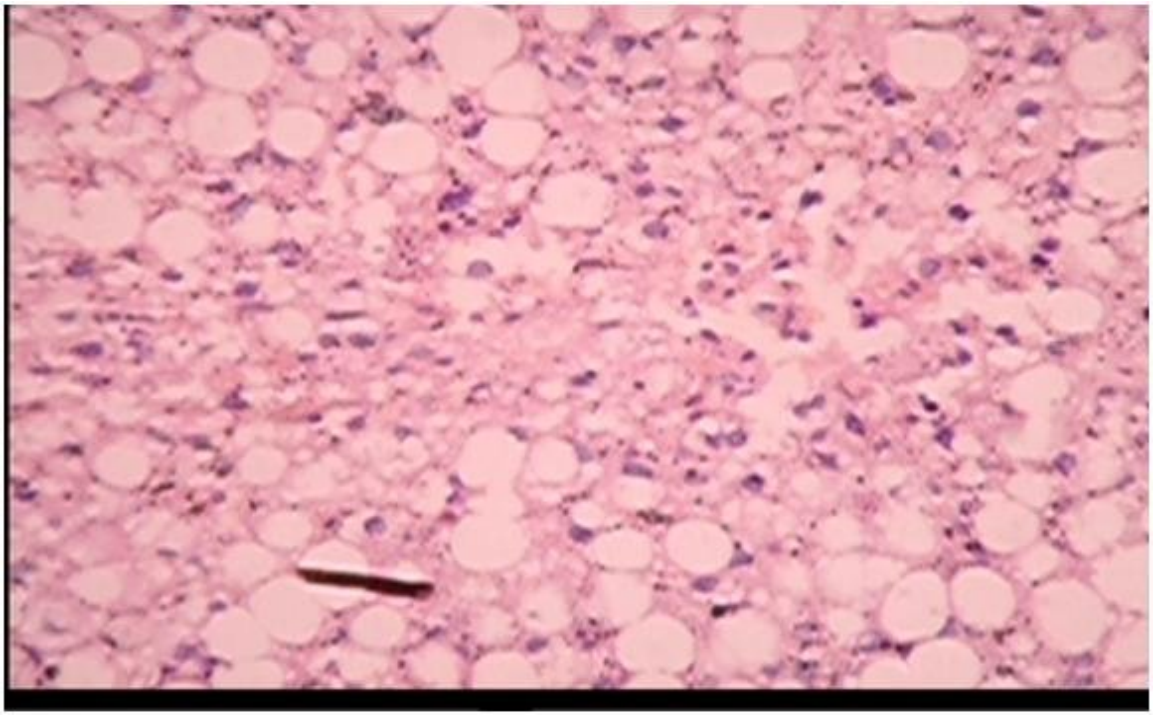


Figure 3. Histopathological examinations of liver section silymarin treated group

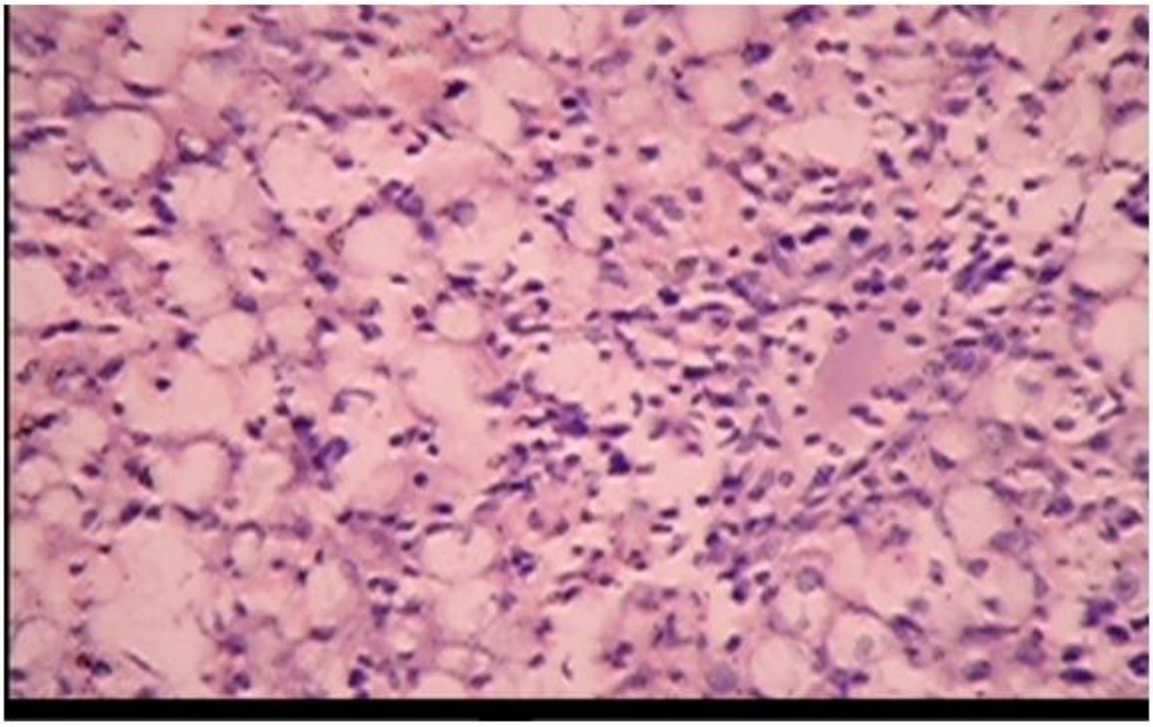


Figure 4: Histopathological examinations of liver section Aqueous extract treated group

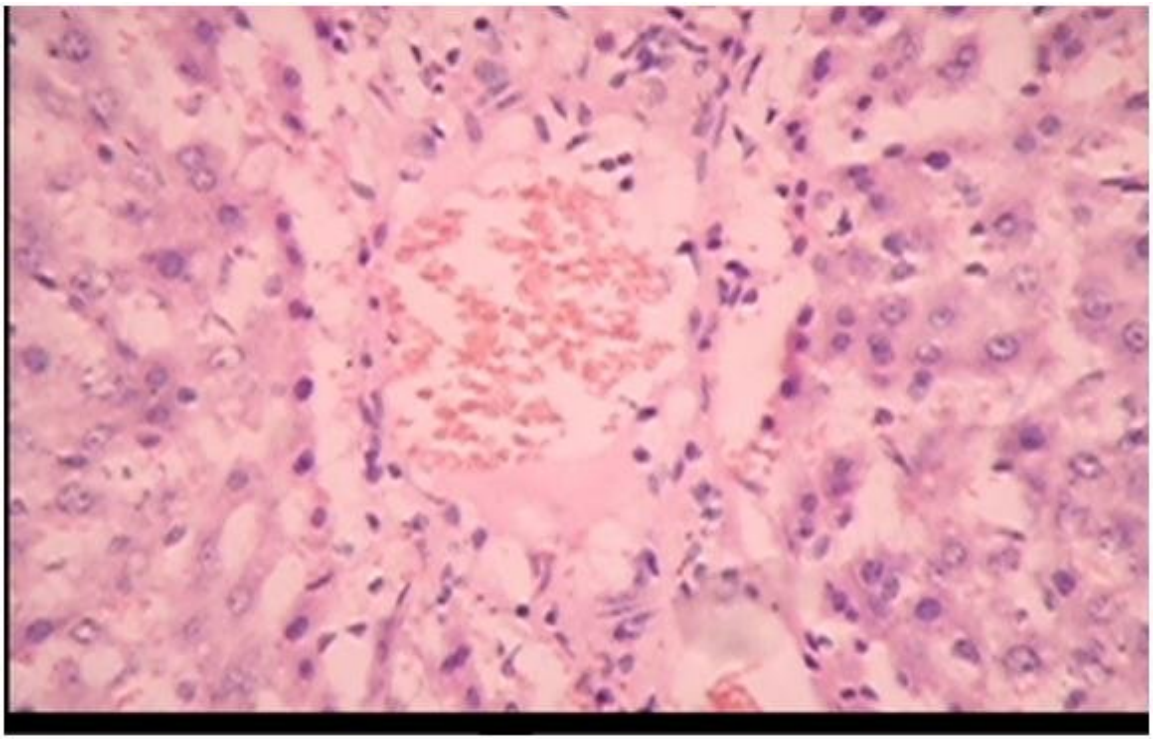


Figure 5: Histopathological examinations of liver section of Alcoholic extract treated group

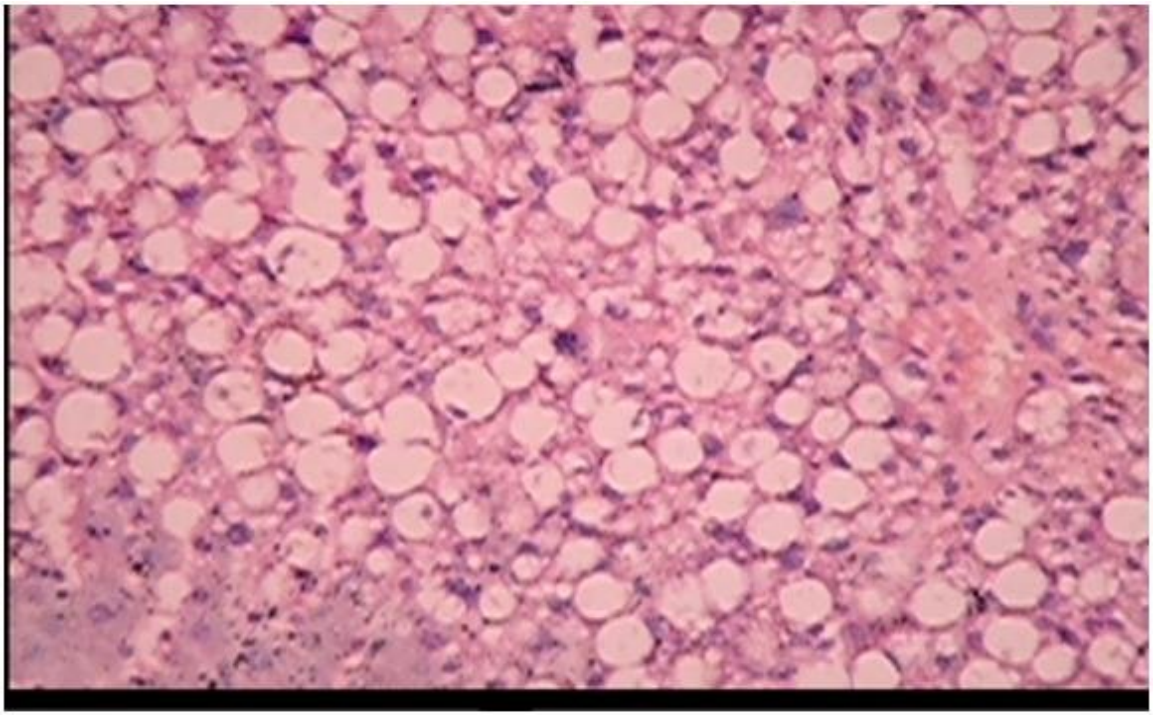


Figure 6 :Histopathological examinations of liver section of Alcoholic extract + simultaneous treated group

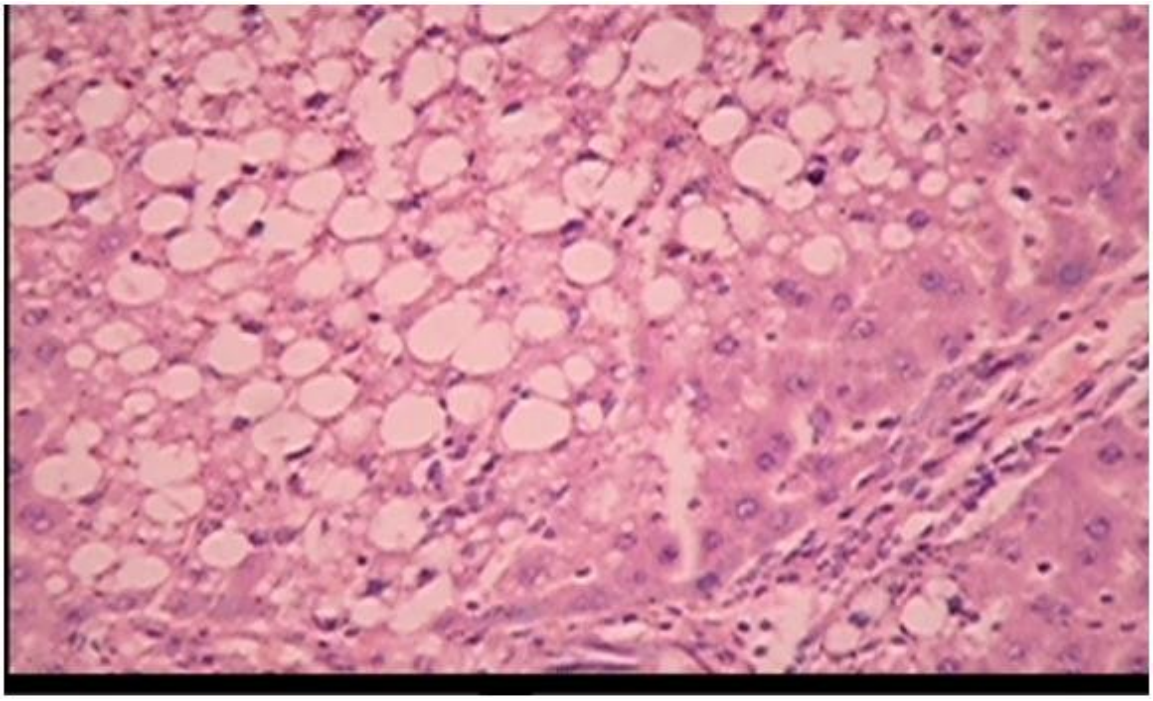


Figure 7: Histopathological examinations of liver section Aqueous extract + simultaneous treated group

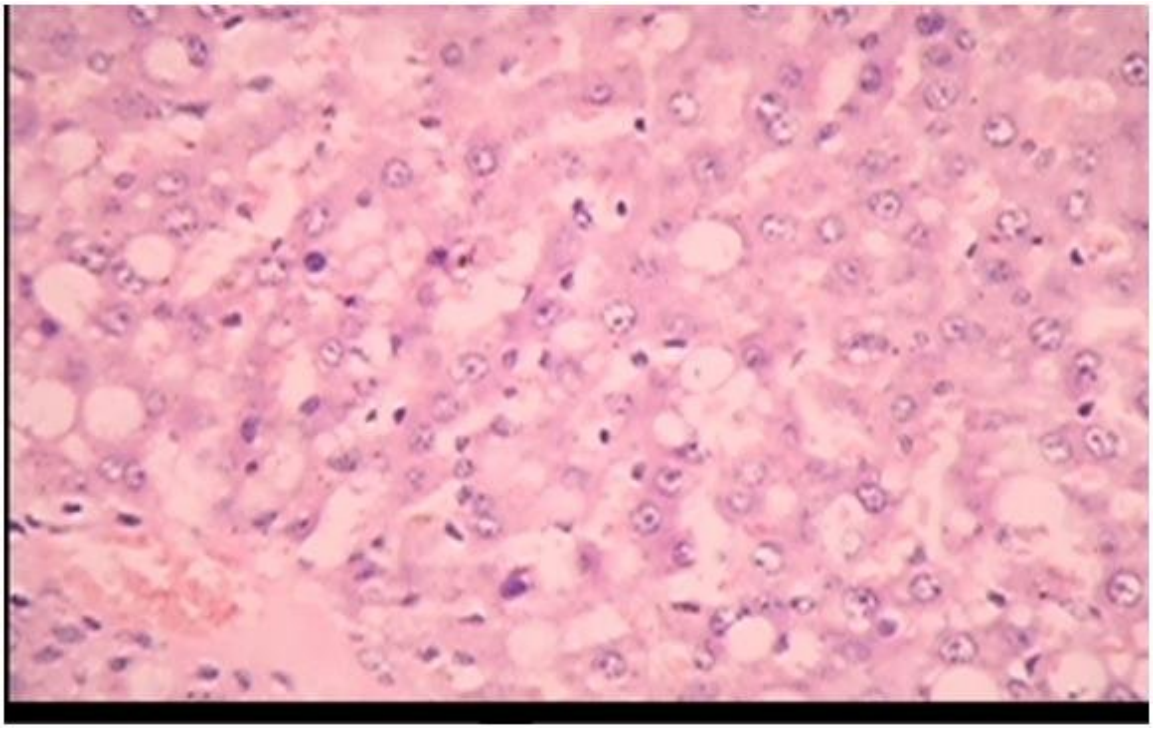


Figure 8: Histopathological examinations of liver section Chloroform fraction treated group

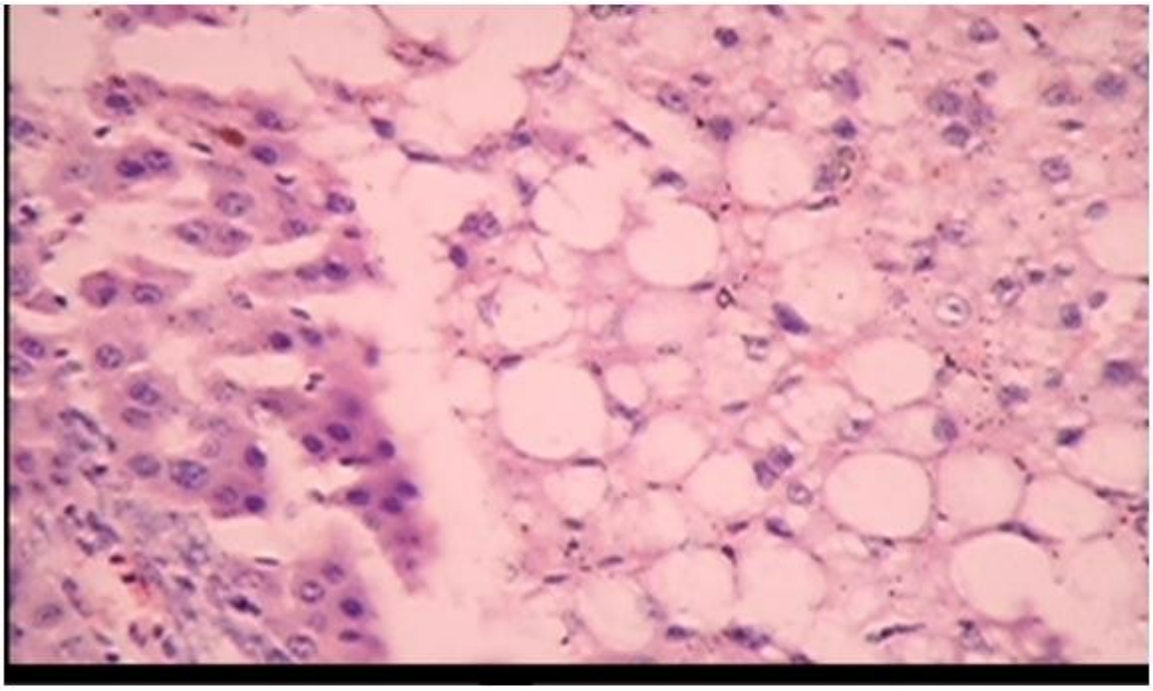


Figure 9: Histopathological examinations of liver section Pet. ether fraction treated group