Hepatoprotective activity of alcoholic and aqueous extracts of fruits of *Luffa cylindrica* Linn in rats

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ABSTRACT

In the present study, we have chosen a plant named Luffa cylindrica Linn (cucurbitaceae) to evaluate for its hepatoprotective activity in experimental animals. Different pharmacological activities are reported with different parts of this plant. Some of its medicinal properties reported as an anthelmintic, stomachic and antipyretic, Extract of the fruits showed antioxidant activity, iron deficiency, anaemia, expulsion of worms and abnormal stoppage of menses. From the literature we found that L. cylindrica Linn has also been traditionally indicated for treatment of hepatic disorders. This prompet us to select fruit extracts of this plant for the study of hepatoprotective activity in paracetamol induced hepatotoxicity in experimental animals. Phytochemical Chemical screening of L. cylindrica revealed the presence of Saponins, carbohydrate, Flavonoids. The present study was made to evaluate the hepatoprotective effect of alcoholic and aqueous extracts of fruits of Luffa cylindrica in paracetamol induced hepatic injury in experimental animal rats. Hepatoprotective activity of L. cylindrica was studied by estimating the serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), direct and total bilirubin, cholesterol, triglycerides, total proteins and albumin. The treatment with AEFLC and AQEFLC showed significant reduction of paracetamol induced elevated serum enzyme levels and increase in total protein and albumin. Histopathology of liver shows hepatoprotective effect of these extracts.

Key words: Paracetamol, Hepatoprotective activity, Fruit extracts, *Luffa cylindrica*.
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INTRODUCTION

Liver plays an important role in regulation of physiological processes, involved in several vital functions such as storage, secretion and metabolism. It also detoxifies a variety of drugs and xenobiotics and secretes bile that has an important role in digestion. Liver plays a central role in

transforming, clearing the chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when administered within therapeutic ranges may injure this organ.

Liver diseases are among the most serious ailments classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (fibrosis of liver). Liver diseases are mainly caused by toxic chemical drugs eg. paracetamol, anti-tubercular, anticancer agents or alcohol, some natural toxins such as peptides of *Amanita phalloides*, pyrrolidizines and the toxin of cycad nut[1]. Most of the hepatotoxic chemicals damage liver cells by inducing lipid peroxidation and others by oxidative cell damage. It has been estimated that about 90% of acute hepatitis is due to viruses and major viral agents involved are hepatitis A, B, C, D, E and G. Among these, hepatitis B infection often results in chronic liver diseases and cirrhosis of liver.

Paracetmol is probably the most versatile and widely used analgesic and antipyretic drug worldwide. Its potential hepatotoxicity was not suspected until the first clinical reports of severe and fatal liver damage following over dosage was reported by David and Eastham (1966). PCM taken in over doses results in hepatotoxicity in men and in experimental animal[2].

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures[3].

In contrast to the narrow spectrum of activity of synthetic drugs with their possible risk of side effects and also chemophobia, herbal drugs as traditionally preferred however, herbal drugs are often mild in action and need to be taken for a long period to be effective especially in chronic conditions.

Jaundice and hepatitis are two major liver disorders, with a high toll of death rate. At present only one (Silybon-Microlabs, Bangalore) hepatoprotective allopathic drug is available for the treatment of liver disorders. Hence people are adapting different preparations from the plants, crude powder extract for the treatment of liver disorders. In the present study, we have chosen a plant named *L. cylindrica* Linn (*cucurbitaceae*) to evaluate for its hepatoprotective activity in experimental animals. From the literature we found that *L. cylindrica* Linn has also be traditionally indicated for treatment of hepatic disorders. This prompet us to select stem extracts of this plant for the study of hepatoprotective activity in different models of experimental animals.

Luffa cylindrica Linn, Family, Roem (Cucurbitaceae), is used in traditional medicine; fruits are used in the traditional Chinese medicine as an anthelmintic, stomachic, antioxidant and antipyretic phytomedicinal drug. Saponins from the leaves and fruits possess effect on lanoxia and fatigue and immunological activity. Extract of the fruits showed antioxidant activity.

Chemical constituents isolated are from the classes of Lucyosides A–R, triterpenoid saponins, and Apigenin from the leaves, seeds and fruits. *L. cylindrica* Linn also reported to have the constituents like Bryonolic acid from cell suspension cultures. 4-O-methyl-D-glucurono-D-xylan from fruit fibres and Constituent like Diosmetin 7-O-β-D-glucuronide methyl ester (0.04% from freeze-dried fruits)[4].

MATERIALS AND METHODS

Drugs and chemicals: Silymarin was obtained from Micro Labs, Bangalore. The chemical kits for all biochemical parameters were obtained from Transasia Biomedicals Ltd., Solan H.P, India. The solvents and other chemicals were used were of analytical grade.

Plant material and extracts: Fruits of *Luffa cylindrica* is obtained from fields of Kanpur, Uttar Pradesh, India; the fruits were cut into small pieces and shade dried at room temperature. The plant was authenticated by a Botanist, Gandhi College Nonari, Kanpur. Dried fruits were powdered and then subjected for alcoholic extraction process with 95% v/v ethanol for 18 hour, using Soxhlet apparatus. The aqueous extract was prepared by taking 100 g of powder in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50°C. Their percentage yield of AEFLC and AQEFLC were 11.14% and 18.12%, respectively.

Phytochemical Screening: A preliminary phytochemical screening of AEFLC and AQEFLC was carried out as described by Khandelwal[5].

Animals: Wistar albino rats (150-200g) of either sex were procured from Venkateshwara Enterprises, Bangalore and were acclimatized for 10 days under standard housing conditions maintained at a room temperature of 24±1°c; relative humidity 45-55% with 12:12 hour light and dark cycle. The animals were maintained on standard pellet diet and tap water *ad libitum*. The animals were habituated to laboratory conditions for 48 hour prior to the experimental protocol to minimize any nonspecific stress. The institutional animal ethics committee of V.L. College of Pharmacy, Raichur, India, approved the experimental protocol in accordance with the guidelines provided by committee for the purpose of control and supervision of experiments on Animals (CPCSEA) with registration number 557/02/C/CPCSEA.

LD_{50} Determination: The acute toxicity of AEFLC and AQEFLC were determined by using non-pregnant female albino mice (18-22g) those maintained under standard husbandry conditions. The animals were fasted 3 h prior to the experiment and "up and down" (OECD guideline No.425) method of CPCSEA was adopted for toxicity studies. Animals were administered with single doses of extracts dissolved in 2% w/v Tween 80 and observed for their mortality during 48 h study period (short term toxicity). Based on the short-term profile of drug the doses for the next animals were determined as per OECD guideline No. 425. All the animals were observed for long term toxicity (14days) and then $1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of the maximum (2000mg/kg) dose was selected for the present study[6].

Hepatoprotective activity: Determination of hepatoprotective activity in (curative aspect) Paracetamol induced hepatotoxicity[7].

Wistar albino rats weighing between 150-200 g and each group containing 6 animals will be divided into 9 groups.

Group 1-   Normal control (vehicle treated, p.o for 10 days)
Group 2-   Toxicant (Paracetamol 2g/kg daily p.o for 03 days, and from 4th-10th day vehicle only)
Group 3-   Paracetamol 2g/kg daily p.o for 03 days, Standard Silymarin 100 mg/ kg p.o from 4th-10th day.
Group 4-   Paracetamol 2g/kg daily p.o for 03 days, AEFLC (100 mg/kg) Low dose p.o from 4th-10th day.
 Group5-   Paracetamol 2g/kg daily p.o for 03 days, AEFLC (200 mg/kg) Medium dose p.o from 4th-10th day.
Group 6-   Paracetamol 2g/kg daily p.o for 03 days, AEFLC (400 mg/kg) Higher dose p.o from 4th-10th day.
Group 7-   Paracetamol 2g/kg daily, p.o for.03 days, AQEFLC (100 mg/kg) Low dose p.o from 4th-10th day.
Group 8-   Paracetamol 2g/kg daily, p.o for 03 days, AQEFLC (200 mg/kg) Medium dose p.o from 4th-10th day.
Group 9-   Paracetamol 2g/kg daily, p.o for 03 days, AQEFLC (400 mg/kg) Higher dose p.o from 4th-10th day.

Experimental procedure: Hepatoprotective study was carried out as described by Kim Y W et al[6]. Wistar albino rats of either sex (150-200 g) were selected and divided into nine groups of six animals each. The animals were treated with paracetamol (2000 mg/kg) for three days except normal control group which was treated with vehicle (1% gum acacia). From day 4th -10th animals in group1, 2 treated with vehicle, Group 3 treated with Silymarin (100 mg/kg/day p.o.) which was served as standard. Animals in Group 4, 5, and 6 were treated with three different doses (low 100 mg/kg, medium 200 mg/kg, high 400 mg/kg) of AEFLC and group 7, 8 and 9 were treated with three different doses (low 100 mg/kg, medium 200 mg/kg, high 400 mg/kg) of AQEFLC once daily p.o. On day 11th, thiopentone sodium (40 mg /kg, i.p) was injected and the sleeping time recorded in al l the animals.

The same animals were then anesthetized using anesthetic ether, 1 hour after complete recovery from thiopentone sodium effect, blood collected by retro orbital puncture. The levels of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), direct and total bilirubin, cholesterol, triglycerides, total proteins and albumin were estimsted as per the standard procedures prescribed by the manufacturer (Transasia Biomedicals Ltd., Solan H.P, India). Immediately after the collection of blood, the animals were euthanized with an over dose of ether; their livers removed, washed in saline and the wet weight and volume determined. Histopathology of liver was carried out by a modified method of Luna[8]. In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 hour. Then the livers were paraffin embedded and 5 μ thickness microtome sections made. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

Statistical analysis: The data are expressed as mean ±SEM. Statistical differences between means were determined by one- way ANOVA followed by Dunnett's 't' test test. Values of $P<0.05$ were considered as significant.

RESULTS

Preliminary Phytochemical studies revealed the presence of alkaloids, carbohydrate, steroids, Saponins and triterpines in AEFLC while alkaloids, carbohydrates and Saponins were noticed in AQEFLC. The AEFLC and AQEFLC were found to be nontoxic up to a dose of 2000 mg/kg.

135

Treatment of rats with paracetamol produced an increase in the weight and volume of wet liver. Rats treated with silymarin, AEFLC and AQEFLC showed significant decrease in wet-liver weight and volume compared to toxicant control group (Figure 1).

Paracetamol treatment resulted in significant elevation of AST, ALT, SALP, Triglycerides, cholesterol, direct bilirubin and total bilirubin levels, while total protein and albumin were found to be decreased compared to normal control group. Groups treated with silymarin, AEFLC, AQEFLC significantly prevented the biochemical changes induced by paracetamol. The hepatoprotective effect offered by AEFLC was found to be significantly greater than AQEFLC treatment (Table 1).

A significant reduction in thiopentone-induced sleep time was observed with both the etracts as compared to the paracetamol-treated toxicant control group (Table 2). The AEFLC treated group greatly reduced the thiopentone sleep time as compared to AQEFLC (Figure 2).

Hepatocyte of the normal control group showed a normal lobular architecture of the liver. In the paracetamol-treated group showed microvascular fatty changes surrounded by large number and hydropic degeneration with loss of nuclear architecture, increased number of kupffer cells and lymphocytic degeneration. Groups treated with Silymarin, AEFLC and AQEFLC showed minimal centrilobular necrosis hydropic degeneration with retention of normal lobular architecture, which confirms hepatoprotective activity of these extracts. However, AEFLC showed more microvascular changes than AQEFLC. The hepatoprotective activity of the extracts were in the order of silymarin> AQEFLC> AEFLC.

DISCUSSION

Most of the hepatotoxic chemicals damage liver cells either by lipid peroxidation or by other oxidative stress mechanisms induced cellular damage. Many chemicals damage mitochondria and intracellular organelle that produce energy and their dysfunction releases excessive amount of oxidants, which in turn injure hepatic cells. Activation of some enzymes in the Cytochrome P-450 system such as CYP-2E1 also leads to oxidative stress and injury to hepatocytes, bile duct cells causing accumulation of bile acid inside liver and which in turn promotes further liver damage.

Acetaminophen (APAP) is a safe, effective and widely used antipyretic–analgesic drug however; an overdose can induce severe hepatotoxicity in experimental animals and humans. Excessive administration of APAP can cause over production of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450. This mechanism has been suggested to participate in the development of oxidative stress and injury in APAP-induced hepatotoxicity.

Chronic administration of drug (paracetamol) to rats increased the levels of marker enzymes like ALT, AST and ALP as these are stored in the liver cells and increase in the levels of these marker enzymes in serum indicate damage to the liver cells. Treatment with AEFLC and AQEFLC decreased the levels of ALT, AST, ALP, BILD, BITD, CHO, TG levels and increased

PRO and ALB levels, an indication for the hepatoprotective activity of these extracts against drug induced hepatotoxicity.

Intoxication with drugs cause increase in cholesterol and triglyceride levels. AEFLC and AQEFLC prevented elevated cholesterol and triglyceride levels due to hepatic lipid peroxidation occurred after drug intoxication.
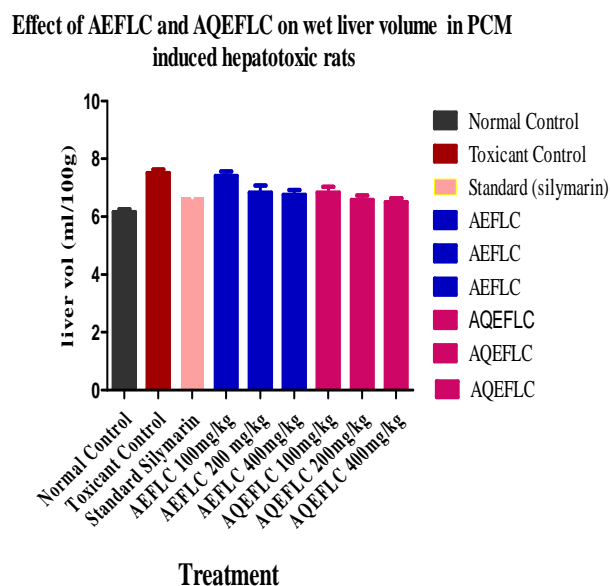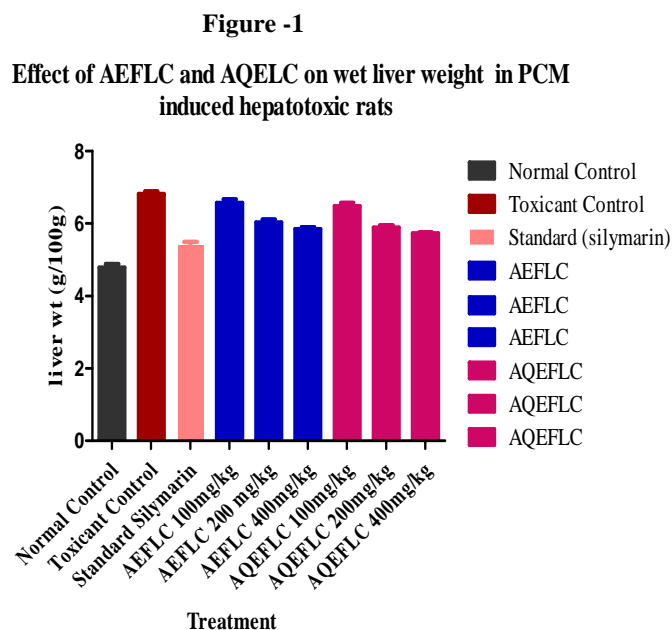
Figure -1

Effect of AEFLC and AQELC on wet liver weight in PCM induced hepatotoxic rats

Effect of AEFLC and AQEFLC on wet liver volume in PCM induced hepatotoxic rats

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Table No.1 Effect of AEFLC and AQEFLC on biochemical parameters in paracetamol induced hepatotoxic rats

Serum biochemical parsmeters	Normal	Toxicant	Standard	AEFLC 100 mg/kg	AEFLC 200 mg/kg	AEFLC 400 mg/kg	AQEFLC 100 mg/kg	AQEFLC 200 mg/kg	AQEFLC 400 mg/kg
ALT	45.06±1.69	153.80±11.04	61.32±2.06**	141.92±1.92^{ns}	84.81±1.32**	70.66±0.85**	136.89±1.47*	80.31±1.42**	63.11±0.36**
AST	111.08±2.42	132.49±20.52	122.85±1.47**	125.46±11.56^{ns}	189.74±10.10*	143.07±6.38**	196.13±1.72*	187.88±5.29**	139.39±1.35**
ALP	113.11±2.67	243.51±4.50	130.14±0.95**	135.57±1.22^{ns}	160.25±2.05**	141.53±1.27**	132.91±1.25*	154.91±1.86**	138.18±2.06**
BILD	021±0.01	0.70±0.10	0.37±0.01**	0.63±0.07^{ns}	0.56±0.02^{ns}	0.41±0.02**	0.60±0.51^{ns}	0.43±0.02**	0.40±0.01**
BILT ±S	0.25±0.02	1.65±0.20	0.54±0.03**	1.41±0.11^{ns}	1.11±0.07**	0.70±0.04**	1.18±0.02**	1.06±0.02**	0.62±0.02**
ALB	4.57±0.46	2.46±0.1916	4.07±0.19**	2.28±0.11^{ns}	3.37±0.15*	3.80±0.21**	3.35±0.14*	3.81±0.08**	3.90±0.07**
PRO	14.24±1.01	8.35±0.26	11.84±0.20**	9.07±1.03^{ns}	10.86±0.55*	11.34±0.30**	10.92±0.22*	11.33±0.48**	11.42±0.25**
CHO	134.54±2.91	232.73±2.92	170.88±3.03**	229.63±1.04^{ns}	198.58±1.95**	183.48±3.70**	219.32±2.26**	192.76±1.68**	176.93±1.33**
TG	32.80±1.00	199.74±5.69	87.52±2.58**	191.20±4.64^{ns}	169.95±8.93**	102.44±2.80**	185.54±2.16^{ns}	113.87±1.66**	101.42±3.41**

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant
AEFLC-Alcoholic extract of fruits of L. cylindrica Linn, AQESFLC- Aqueous extract of fruits of L.cylindrica.

Table No. 2 Effect of AEFLC and AQEFLC on thiopentone induced sleeping time (TST), wet liver weight and volume in paracetamol induced hepatotoxic rats
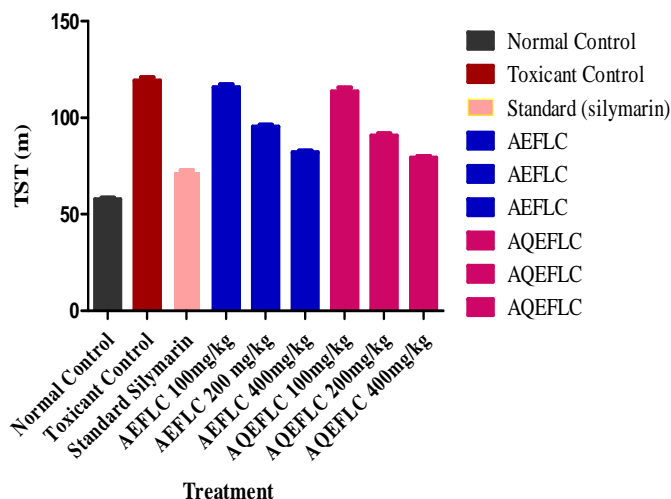
Physical parameters	Normal	Toxicant	Standard	AEFLC 100 mg/kg	AEFLC 200 mg/kg	AEFLC 400 mg/kg	AQEFLC 100 mg/kg	AQEFLC 200 mg/kg	AQEFLC 400 mg/kg
Duration of sleep (min)	57.83±0.70	119.33±1.67	71.83±0.98**	115.83±1.35^{ns}	95.33±0.99**	82.00±0.89**	113.50±2.13*	90.66±1.26**	79.33±0.80**
Liver weight (g/100 g)	4.78±0.10	6.82±0.07	5.40±0.02**	6.58±0.09^{ns}	6.03±0.08**	5.85±0.05**	6.48±0.09*	5.89±0.06**	5.74±0.02**
Liver volume (ml/100 g)	6.16±0.11	7.50±0.13	6.66±0.11**	7.41±0.15^{ns}	6.83±0.25	6.75±0.17*	6.83±0.21*	6.58±0.15**	6.50±0.13**

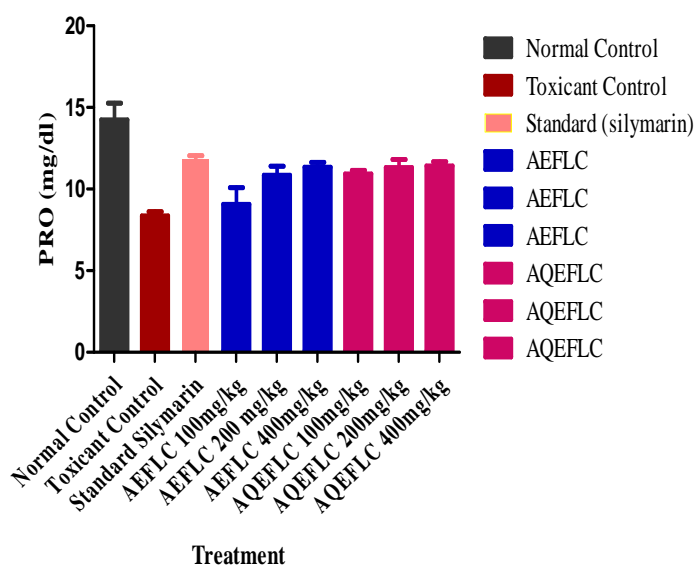*AEFLC-Alcoholic extract of stem fruits of L.cylindrica,   AQEFLC- Aqueous extract of fruits of L.cylindrica*
n = 6, Significant at P < 0.05*, 0.01** and 0.001***,   ns = not significant

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138

Figure -2

Effect of AEFLC and AQEFLC on thiopentone induced sleeping time (TST) in PCM induced hepatotoxic rats
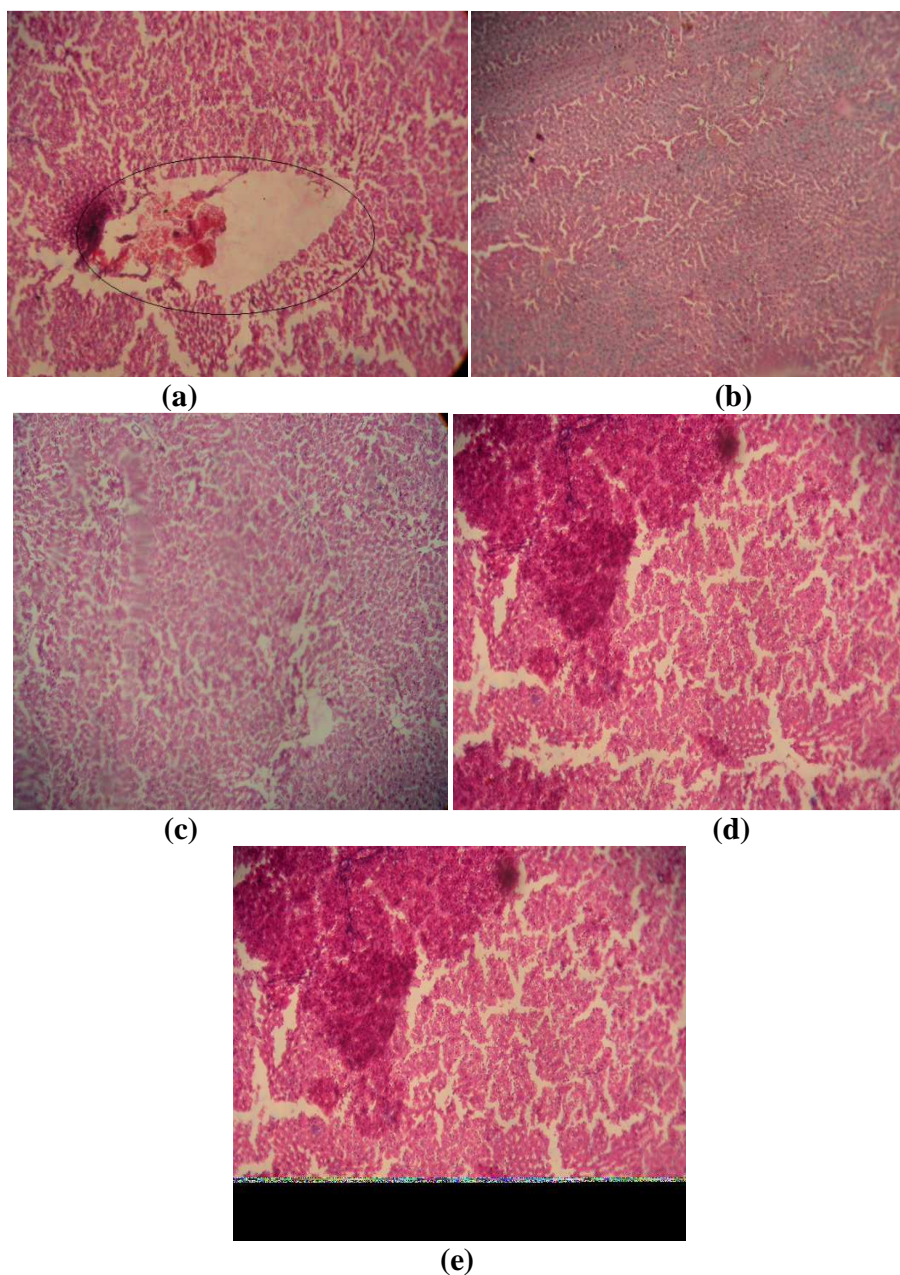

Effect of AEFLC and AQEFLC on serum PRO levels in PCM induced hepatotoxic rats

In chronic drug induced hepatotoxicity models, administration of thiopentone sodium results with an increased duration of sleeping time, as liver is the primary site for the metabolism of xenobiotics like barbiturates and functional damage to liver requires longer time to inactivate thiopentone resulting with an increased duration of action of this drug. Groups treated with AEFLC and AQEFLC have decreased the thiopentone induced sleeping time as compared to toxic control indicating their protection of liver function against drug induced toxicity in rats. Liver weight and volume gets increased in toxicant control group. Where in standard and AEFLC and AQEFLC treated groups these were decreased which confirms the hepatoprotective activity of both the extracts.

139

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The protective effect shown by the extracts in functional parameters (Thiopentone induced sleeping time), physical parameters (wet liver weight and wet liver volume), biochemical parameters (ALT, AST & ALP) followed by histological parameters clearly depicts that Fruit extracts of *L. cylindrica* possess hepatoprotective activity.

Histopathology of Liver shows normal hepatic tissue **(a)**, PCM induced damage in hepatic tissue **(b)** Effect of Silymarin on PCM induced hepatic damage **(c)**, Effect of AESCR (high) dose on PCM induced hepatic damage **(d)** Effect of AQESCR (High) dose on PCM induced hepatic damage **(e)**

(a) **(b)**

(c) **(d)**

(e)

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CONCLUSION

These observations suggest that aqueous and alcoholic extracts of *L. cylindrica* exhibited significant hepatoprotective activity. So the hepatoprotective effect of extracts may be due to its saponins content. Further, this study might highlights phytochemical profile of *L. cylindrica* and finds the effective leads from natural resources for the desired therapeutic benefit.

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