Asian Journal of Pharmaceutical Education and Research
Vol -11, Issue-1, January- March 2022
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

ANTIOXIDANT ACTIVITY AND HEPATOPROTECTIVE EFFECTS OF *LUFFA ECHINATA* ROXB. SEEDS EXTRACTS AGAINST ALCOHOL INDUCED HEPATIC DAMAGE

Vikash Rajak, Harshita Jain, Sunil K. Jain, Arpit Shrivastava*

Department of Pharmacology, Adina Institute of Pharmaceutical Sciences, Sagar (M.P.)-470001

*Corresponding Author's E mail: arpitshrivastava54@gmail.com

Received 11 Oct. 2021; Revised 17 Nov. 2021; Accepted 22 Nov. 2021, Available online 10 Jan. 2022

Cite this article as: Rajak V, Jain H, Jain SK, Shrivastava A. Antioxidant activity and hepatoprotective effects of luffa echinata roxb. Seeds extracts against alcohol induced hepatic damage. Asian Journal of Pharmaceutical Education and Research. 2022; 11(1): 65-78.

https://dx.doi.org/10.38164/AJPER/10.1.2022.65-78

ABSTRACT

The present study was done to determine the antioxidant activity and hepatoprotective effects of *Luffa echinata* (LE) seeds extracts/fractions against alcohol-induced hepatotoxicity in *in vivo* models. Total flavonoids content (TFC) and total phenolics contents (TPC) were determined using quercetin and tannic acid equivalents, as standard while antioxidant activities of different extracts/fractions against DPPH, hydroxyl ($OH^{\bullet}$) and inhibition of lipid peroxidation (LPO) were evaluated by using standard *in vitro* assays methods. *In vitro* active extract/fractions were subjected for the determination of hepatoprotective effects against alcohol induced liver damage. Among all extract/fractions, *n*-butanolic fraction (LEBF) showed marked possess potent antioxidant activity *viz.* DPPH (104.1µg/ml), hydroxyl ($OH^{\bullet}$) (114.5 µg/ml) and inhibition of lipid peroxidation (LPO) (148.6 µg/ml), followed by methanolic extract (LEME) 158 µg/ml**,** 184.7 µg/ml and 207.4 µg/ml, respectively. Pre-treatment with LEBF significantly ($p<0.001$) restores the alcohol-induced altered serum liver enzymes activities as well as tissue antioxidant enzyme level, which is comparable to silymarin. Hepatoprotective potential was further supported by hepatic tissue histopathological results. Study results suggest that antioxidant activity and hepatoprotective effect of LEBF might be due to presence of polyphenols *viz.* TFC (49.68±0.72 mg QE/gm of extract) and TPC (112.17±0.72 mg GAE/gm of extract). In this study advocated that marked antioxidant and hepatoprotective activity of *Luffa echinata* seeds (*i.e.* LEBF) was exhibited due to the presence of polyphenols along with other compound.

Keywords: Alcohol; antioxidant; *Luffa echinata*; free radicals; polyphenols.

INTRODUCTION

Liver injury and disease caused by alcohol is a common complication to human health worldwide. Alcoholic liver disease (ALD) alludes to changes inside the liver caused by excessive alcohol. ALD is caused by over-production of reactive oxygen species (ROS), lipid peroxidation injury, cytokine damage, and inflammation [1]. Lately, the occurrence of ALD has expended, representing a genuine danger to

human wellbeing. Subsequently, research on drugs that are effective in interfering with the course of ALD is crucial. ALD has a complex pathogenesis, and the molecular mechanisms underlying its progression remain unclear. A number of studies showed that oxidative stress and inflammation assumed as key part in the development of ALD and that compounds with anti-oxidative or antiinflammatory activities ameliorated the progression of ALD in animal models [2, 3].

Luffa echinata Roxb. (Cucurbitaceae) is a spreading climbing herb of tremendous medicinal importance. Traditionally various parts of the plant are being used for the treatment of different ailments such as jaundice, intestinal colic, enlargement of liver and spleen [5], leprosy [6], diabetes [7], bronchitis, nephritis, rheumatism, cirrhosis, dropsy, anthelmintic, stomach ache, snake bite, dog bite, fever, diarrohea and hemorrhoid disorder [8]. Antioxidant [4], analgesic and anti-inflammatory [9], antidepressant, anxiolytic, antiepileptic [10], hepatoprotective [11], antiulcer [12] and anticancer [13] activity have been reported. Seeds powder of the *L. echinata* has been used traditionally in the treatment of alcoholic liver disease [14]. Analgesic and antiinflammatory activity of methanolic extract of LE seeds have been reported [15]. There are no previous studies on the antioxidant and hepatoprotective effects of LE seeds extract on animal models; therefore, this study aims to investigate liver protective effects of LE seeds against alcoholic liver injury in rat models.

MATERIALS AND METHODS

Chemicals and Reagents

Quercetin, gallic acid, vitamin C, trichloroacetic acid, thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich, Mumbai. The alcohol and Folin–Ciocalteu reagents (FCR) were purchased from SD Fines Chemicals Pvt. Ltd., Mumbai. The Silymarin was received from Micro Lab, Baddi as gift sample. All other chemicals and solvents of analytical grade used in this study.

Plant Material Collection and Authentication

Fruits of LE were purchased from Satbhaiya drug house, Sagar (Madhya Pradesh), India. The plant specimen was authenticated by Prof. Pradeep Tiwari, Department of Botany, Dr. H. S. Gour Central University, Sagar (Madhya Pradesh), India. A voucher specimen was prepared and deposited in the herbarium of the department.

Plant Material Extraction:

Seeds of LE were separated from the fruits manually. Dried powdered of LE seeds was extracted with methanol using Soxhlet's apparatus. Filtrates were dried under vacuum using rotary evaporator (Superfit Pvt Ltd., Mumbai, India) at 40±2°C. Further methanolic extract (LEME) (8.26% *w/w*) was suspended in

water and then fractionated with petroleum ether (40-60ºC.) and *n*-butanol, to yield petroleum ether fraction (LEPEF) (0.72% *w/w*), *n*-butanol fraction (LEBF) (3.83% *w/w*) and aqueous fractions (LEAF) (5.18% *w/w*).

Determination of Phytoconstituents

Preliminary phytochemical screening

The LEME, LEPEF, LEBF, and LEAQF extracts/ fractions of L. echinata were subjected to determine the presence of various phytoconstituents using previously reported qualitative chemical tests [16].

Total flavonoid content (TFC)

Determination of TFC was based on measurement of the intensity of red color complex formed due to reaction between flavonoids and aluminum trichloride (AlCl3) [17]. Briefly, the $AlCl_3$-methanolic solution (1 ml, 2% *w/v*) was mixed to various diluted extracts or standard (1 ml) and allowed to stand for 1 h at 25 ± 2°C before the absorbance was measured at λmax 420 nm against blank using ultravioletvisible (UV-Vis) spectrophotometer (Shimadzu 1800, Kyoto, Japan). Extract samples were evaluated at a final concentration of 1 mg/ ml. The TFC was compared to quercetin equivalent (QE) (mg/g of extract) using the regression equation Y=0.063X-0.079, r^2=0.992, where x is the absorbance and y is the QE.

Total phenolic content (TPC) and total tannin content (TTC)

The TPC was determined by the reported Folin-Ciocalteu method [18] Briefly, various extracts (1 ml, 1% *w/v*) were mixed with FCR (10 ml, previously diluted with distilled water in 1:10 ratio), vortexed and set aside for 5 min, then sodium carbonate solution (10 ml, 7% *w/v*) was added and diluted up to 25 ml with distilled water. The mixture was allowed to stand for 1.5 h at 25±2°C and absorbance was measured at λmax 765 nm against blank using UV-Vis spectrophotometer (Shimadzu 1800, Kyoto, Japan). The results were compared to gallic acid equivalent (GAE) (mg/g of extract) using the regression equation y=0.032x+0.042, r^2=0.998, where x is the absorbance and y is the GAE.

Determination of Antioxidant Activity

DPPH radical scavenging assay

The DPPH• radical scavenging ability of extracts was determined by measuring the intensity of yellow colored complex formed due to reaction between proton donar and DPPH• radical [19]. In short, the methanolic solution of DPPH (3 ml, 0.01 mM) was mixed with extracts or vitamin C (3 ml, 10-200 µg/ ml) and incubated for 30 min in the dark. The absorbance of DPPH solution was measured at λmax 517 nm using UV-Vis spectrophotometer (Shimadzu 1800, Kyoto, Japan). The DPPH • radical scavenging ability was calculated using the following equation:

$$\text{Scavenging effect (\%)} = (A_0 - A_t / A_0) \times 100 \quad \text{(Equation 1)}$$

Where, A_0 is the absorbance of the control and A_t is the absorbance of the sample.

Hydroxyl radical scavenging assay

Hydroxyl radicals ($OH^{\bullet}$) scavenging ability of the extracts was determined by measuring the intensity of hydroxylated salicylate complex formed by the reaction of $OH^{\bullet}$ radical and sodium salicylate [17]. The reaction mixture containing ferrous sulfate (1 ml, 1.5 mM), hydrogen peroxide (H_2O_2) (0.7 ml, 6 mM), sodium salicylate (0.3 ml, 20 mM), and varying concentrations of extracts or vitamin C (1 ml, 10-200 µg/ ml) were incubated for 1 h at 37 ± 2°C. After incubation absorbance of the reaction mixture was measured at λmax 562 nm using UV-Vis spectrophotometer. The OH• radical scavenging ability was calculated using the Equation 1.

Inhibition of lipid peroxidation in rat liver homogenate

Inhibition of lipid peroxidation activity of extracts was determined by measuring the intensity of pink colored complex formed by the reaction between malondialdehyde and TBA [20]. Swiss albino rats (180-240 g) of either sex were sacrificed, and their livers were dissected out, washed properly with potassium chloride solution (0.15 M), homogenized and centrifuged at 3000 rpm for 10 min at 40±2°C and discarded the precipitate. In liver homogenate (1 ml, 1% w/v), ferrous chloride ($FeCl_2$) (0.5 ml, 0.5 mM), H_2O_2 (0.5 ml, 0.5 mM), and various concentrations of extracts or vitamin C (1 ml, 20-150 µg/ ml) were added and incubated at 37±2°C for 60 min. After incubation 1 ml of each trichloroacetic acid (15%) and TBA (0.67%) were added in reaction mixture, heated on boiling water bath for 25 min. Intensity of pink color formed was measured at λmax 535 nm. Percent inhibition of LPO was calculated using Equation 1.

Determination of *in vivo* Hepatoprotective Effect

Animals

Swiss albino rats of either sex, weighing between 180 and 240 g were used. The animals were procured from College of Veterinary Sciences and Animal Husbandry, Mhow, Madhya Pradesh, India. Animals were allowed to acclimatize for 2 weeks before commencing the study and maintained under standard laboratory conditions (25±2°C temperature, 45-65% relative humidity and 12 h light and 12 h dark cycle). The animals were fed with standard laboratory animal feed and water *ad libitum* throughout the study. The animal experimental protocols were duly approved by the Institution Animal Ethical Committee of Adina Institute of Pharmaceutical Sciences, Sagar (M.P.).

Acute oral toxicity

The acute toxicity study was carried out in adult female albino rats by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420 [21]. The animals (non-

pregnant female Wistar albino rats) were divided into different groups consisting three animals each. The all animals were fasted overnight with free access to water, weighed and a single dose (2000 mg/kg) of test extracts/fractions of LE (suspended in 5% *v/v* Tween-80 solution) was administered. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days.

***In vivo* Hepatoprotective Effect of LEBF and LEME**

Swiss albino rats of (180-250 gm) were used to study the protective effects against alcohol with slight modification [22]. Each group contains six animals (n=6). Group I-normal control; Group II-ethanol (5 g/kg b.w.) (physiological saline was used as the vehicle); Group III-Silymarin (100 mg/kg b.w.) + ethanol; Group IV-LEBF (100 mg/kg b.w.) + ethanol; Group V-LEBF (200 mg/kg b.w.) + ethanol; Group VI-LEBF (400 mg/kg b.w.); Group VII-LEME (100 mg/kg b.w.) + ethanol; Group VIII-LEME (200 mg/kg b.w.) + ethanol; Group IX-LEME (400 mg/kg b.w.). Treatment with extracts (LEBF and LEME) and silymarin was given daily for 7 days while alcohol was given once only on 7^{th} day.

Biochemical Analysis

The animals receiving different treatments were euthanized on the 8^{th} day and blood samples were collected by retroorbital puncturing method. Blood samples collected from the animals were centrifuged at 3000 rpm for 15 minutes, and plasma from each sample was collected for analysis for various biochemical parameters including ALP, SGOT, SGPT and TB using Erba Chem 5 Plus V2 biochemistry analyzer (Transasia, Germany).

Anti-oxidant Enzyme estimation in tissue

Biochemical analysis of tissue enzymes including inhibition of lipid peroxidation (LPO) [23], catalase (CAT) [24], reduced glutathione (GSH) [25] and superoxide dismutase (SOD) [26] enzyme activity were performed in order to assess the *in vivo* antioxidant activity as per standard methods reported.

Histopathological examination

Livers of rats receiving different treatments were isolated, fixed in formaldehyde (10% *v/v*) and histopathologically examined using hematoxylin and eosin dyes. The cellular images were captured at suitable magnification.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test using Graph Pad Instat Software (Version 5.0, Graph Pad Software, San Diego, California, USA). A probability $p \leq 0.05$ was considered as significant. The results were expressed as mean ± standard error of mean (n = 6) or specified in the study.

RESULTS

Determination of Phytoconstituents

Preliminary phytochemical screening

Results of preliminary phytochemical screening were showed in Table 1

Table 1: Phyotchemical screening of *L. echinata* extract/fractions

	LEME	LEPEF	LEBF	LEAF
Alkaloids	+	-	-	+
Carbohydrate	+	-	-	+
Glycosides	+	-	+	+
Flavonoids	+	-	+	+
Phytosterols & triterpenoids	+	+	+	-
Protein and amino acids	-	-	-	-
Tannins	+	-	+	+
Fixed oils and fat	+	+	-	-

*(-), Absent; (+), Present

Estimation of TPC and TFC

Results demonstrated that amount of TPC and TFC differed significantly among various extracts as given in Table 2. The TPC and TFC were found more in LEBF 112.17±0.72mg GAE/g, 49.68± 0.72 mg QE/g of extract, respectively, followed by LEME, LEAF and LEPEF. However, this method has some limitation as FCR reacts with other non-phenolic reducing compounds like organic acids, sugar leading to over evaluation of phenolic compound [27].

Table 2: Amount of TPC and TFC present in LE extracts/fractions

Extract	TPC (mg GAE/g of extract)	TFC (mg QE/g of extract)
LEME	87.12±0.86	26.48± 0.32
LEPEF	10.16±0.24	0.97± 0.48
LEBF	112.17±0.72	49.68± 0.72
LEAF	34.38±0.84	11.16± 0.56

Values are presented as mean±SEM; (n=3)

Estimation of Antioxidant Activity

DPPH radical scavenging assay

DPPH radical scavenging assay measured hydrogen donating nature of extracts [28]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of LEME, LEPEF, LEBF and LEAF

extract was found to be 158±1.64, 567.8±2.17, 104.1±0.69 and 303.1±1.14 µg/mL, respectively, as compared to that of vitamin C (11.9 ± 0.42 µg/mL) [Table 3 and Figure 1a].

Hydroxyl radical scavenging assay

OH• radical is one of the most reactive radicals, which has the capacity to conjugate with the nucleotides of DNA resulting in the strand breakage and leads to carcinogenesis, mutagenesis, and cytotoxicity [17]. However, the presence of antioxidant prevents the cellular damage either by quenching off or chelating transition metals [29]. OH• radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of LEME, LEPEF, LEBF and LEAF extract was found to be 184.7±0.64, 589.7±1.48, 114.5±0.96 and 345.7±0.69 µg/mL, respectively, as compared to that of vitamin C (19.4±0.37µg/mL) [Table 3 and Figure 1b].

Inhibition of lipid peroxidation assay

The LEME, LEPEF, LEBF, LEAF and vitamin C showed inhibition of lipid peroxidation with IC50 value of 207.4±0.47, 675.9±2.14, 148.6±1.19, 364.5±1.68 and 81±0.26 µg/mL, respectively [Table 3 and Figure 1C]. LEBF demonstrated significant inhibition of LPO as compared to other extracts/fractions.

Table 3: Antioxidant activity of *L. echinata* seeds extracts/fraction in terms of IC_{50} value

Assay	IC_{50} value of extracts/fractions (µg/mL)				
	Vit.C	LEME	LEPEF	LEBF	LEAF
DPPH	11.9±0.42	158±1.64	567.8±2.17	104.1±0.69	303.1±1.14
OH	19.4±0.37	184.7±0.64	589.7±1.48	114.5±0.96	345.7±0.69
Inhibition of LPO	81±0.26	207.4±0.47	675.9±2.14	148.6±1.19	364.5±1.68

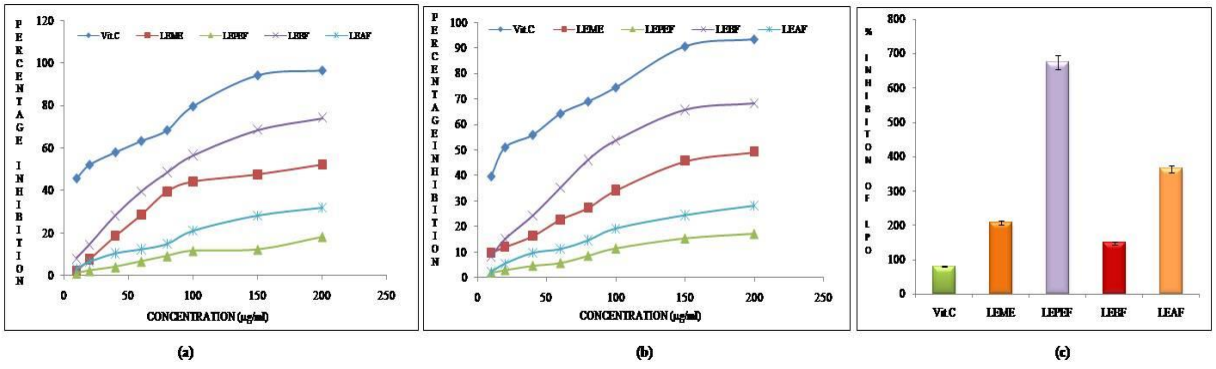

Figure1. Estimation of antioxidant activity of *L. echinata* seeds extracts/fraction by different assays; (a) DPPH radical scavenging assay, (b) OH° radical scavenging assay, and (c) inhibition of lipid peroxidation. Values are represented as mean ± SEM; (n=3)

Hepatoprotective Effects

Acute toxicity studies

No adverse changes and mortality were observed in animals, which orally received extracts up to 2000 mg/kg of body weight. So 1/20th, 1/10th and 1/5th of the maximum safe dose were selected for studying *in vivo* hepatoprotective effect.

Biochemical Analysis

Treatment with LEBF extract more significantly ($p< 0.001$) decreased the elevated enzyme levels of SGOT, SGPT, ALP and TB, as compared to that of LEME and were found comparable to the standard control, silymarin ($p< 0.001$) [Tables 4].

Table 4: Protective effects of LEBF and LEME extract/fractions on SGOT, SGPT, ALP and TB against alcohol-induced hepatotoxicity in rats

Groups	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB (mg/dl)
Group I (Normal control)	42.5±3.76***	33.6±2.77***	46.3±3.27***	0.44±0.05***
Group II (Toxicant control) (5 gm/kg)	96.3±3.95	86.4±3.58	106.2±3.52	1.80±0.11**
Group III (Standard control) (50 mg/kg)	49.3±2.25*** (87.36)	50.6±2.42*** (67.80)	60.1±2.38*** (76.75)	1.18±0.09 (45.58)
Group IV (LEBF treated) (100 mg/kg)	82.1±4.06* (26.39)	73.4±2.41** (24.62)	89.4±2.93** (28.05)	1.62±0.21 (13.24)
Group V (LEBF treated) (200 mg/kg)	74.0±2.84** (41.44)	63.3±1.69*** (43.75)	78.2±3.72*** (46.74)	1.50±0.14** (22.05)
Group VI (LEBF treated) (400 mg/kg)	56.6±2.63*** (73.79)	53.3±4.12*** (62.68)	66.0±2.14*** (67.11)	1.12±0.17*** (50.00)
Group X (LEME treated) (100 mg/kg)	86.2±4.72* (18.77)	75.2±2.61** (21.21)	94.4±5.41* (19.69)	1.69±0.31 (8.08)
Group XI (LEME treated) (200 mg/kg)	83.0±2.68* (24.72)	72.1±2.23* (27.08)	90.7±6.43** (25.87)	1.61±0.16* (13.97)
Group XII (LEME treated) (400 mg/kg)	75.5±4.12** (38.66)	65.6±3.84*** (39.39)	84.5±4.97*** (36.22)	1.44±0.08** (26.47)

Each value present the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with Toxicant control group (alcohol) (one-way ANOVA followed by Dunnett's test).Values in parentheses indicate percent hepatoprotective activity (H), calculated as 100 x (value of alcohol control - value of treatment)/ (value of alcohol control - value of normal control).

Anti-oxidant Enzyme in tissue

Treatment with LEBF extract more significantly ($p< 0.001$) restored the altered enzyme levels of DA, GSH, SOD and CAT, as compared to that of LEME and were found comparable to the standard control, silymarin ($p< 0.001$) [Tables 5].

Table 5: Protective effects of LEBF and LEME extract/fraction on MDA, GSH, SOD and CAT against alcohol-induced hepatotoxicity in rats

Group	MDA (nM/mg protein)	GSH (µM/mg protein)	SOD (U/mg)	CAT (U/mg)
Group I (Normal control)	0.49±0.08***	9.71±0.07***	36.36±1.93***	50.78±1.92***
Group II (Toxicant control) (5 gm/kg)	1.09±0.03	4.25±0.08	15.56±0.87	27.64±0.99
Group III (Standard control) (50 mg/kg)	0.59±0.05*** (83.33)	6.83±0.13*** (47.25)	29.77±1.13*** (68.31)	42.28±1.48*** (63.26)
Group IV (ZXEAE treated) (100 mg/kg)	0.96±0.06 (21.66)	5.11±0.13** (15.75)	20.53±1.00* (23.89)	31.04±1.3 (14.69)
Group V (ZXEAE treated) (200 mg/kg)	0.80±0.06* (48.33)	5.70±0.18** (26.56)	24.2±0.94*** (41.53)	37.4±1.47*** (42.17)
Group VI (ZXEAE treated) (400 mg/kg)	0.63±0.08** (76.66)	6.56±0.13*** (42.30)	26.38±0.86*** (52.02)	41.39±0.26*** (59.42)
Group X (SIEAE treated) (100 mg/kg)	1.01±0.21 (13.33)	4.66±0.06 (7.42)	17.95±0.60 (11.49)	29.24±0.05 (6.92)
Group XI (SIEAE treated) (200 mg/kg)	0.97±0.08* (20.00)	5.16±0.17** (16.73)	18.85±0.54 (15.81)	31.04±0.24 (14.69)
Group XII (SIEAE treated) (400 mg/kg)	0.88±0.07** (35.00)	5.50±0.09** (22.89)	21.07±0.86* (26.49)	34.16±0.31** (28.17)

Each values presents the mean±SEM; (n=6), *p<0.05, **p<0.01, ***p< 0.001 respectively when compared with Toxicant control group (alcohol) (one-way ANOVA followed by Dunnett's test).Values in parentheses indicate percent hepatoprotective activity (H), calculated as 100 x (value of alcohol control – value of treatment) / (value of alcohol control – value of normal control).

DISCUSSION

The liver plays a pivotal role in the biological system that is responsible for the metabolism and clearance of drugs and xenobiotics, including ROS [30]. Liver has become the central organ for detoxification as the liver cells (hepatocytes), the main components that make up the organ, contain majority of enzymes that are responsible for drug metabolism of the entire body. However, when the amount of drugs or

xenobiotics that is encountered has exceed the maximum metabolic capability of the liver; damaging effect of the toxins may lead to various liver ailments [31]. Overconsumption of alcohol had been associated to a spectrum of liver injuries with varying degree of severity, with some common pathologies including steatosis, foamy degeneration, steatonecrosis, venous lesion, and cirrhosis [32].

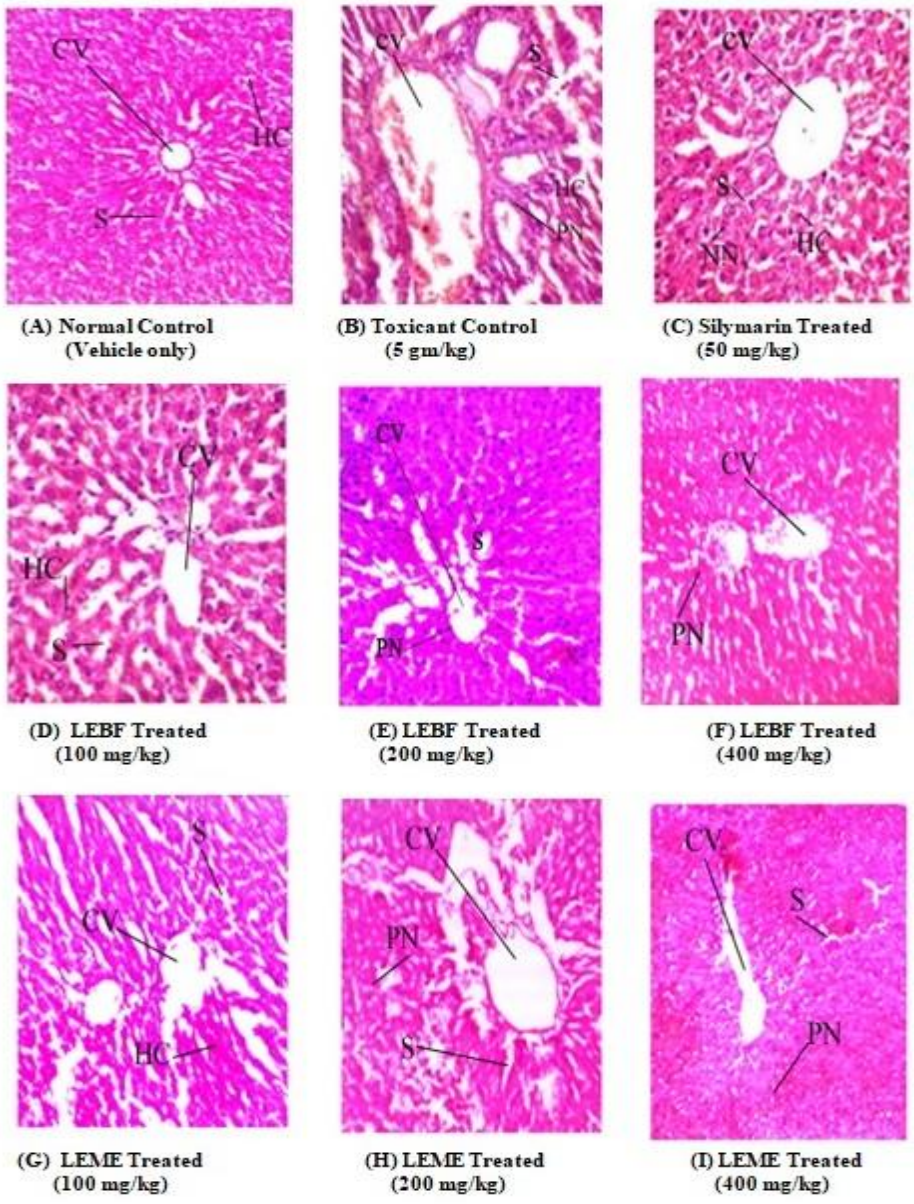

Figure 2. Histopathology of rats liver shows protective effects of LEBF and LEME (X100); CV: Centrilobular vein; HC: Hepatic cord; S: Sinusoids; PN: Pyknotic nucleus

In this study, antioxidant activity of different LE seeds extracts have been determined by different *in vitro* methods like DPPH•, OH• radical scavenging assay and well as inhibition of lipid peroxidation. *In vitro* antioxidant activity results demonstrated that among all extracts/fractions, LEBF demonstrated significantly less IC_{50} value or more antioxidant activity, which was comparable to vitamin C. Order of

antioxidant activity was found to be Vitamin C> LEBF > LEME> LEAF> LEPEF [Figure No. 1; Table 3]. Result of antioxidant activity were supported by phytochemical analysis [Table No.1] as well as quantitative estimation of phytoconstitutents [Table No 2]; which clearly demonstrated that antioxidant effect of extracts are attributed due to presence of antioxidant principal compounds such as phenolics and flavonoids along with other phytoconstitutents. Based on *in vitro* antioxidant studies results; only *in vitro* active extracts of LE i.e. LEME and LEBF were selected for *in vivo* study to evaluate hepatoprotective effects against alcohol induced liver damage.

Rats treated with ethanol showed increased level of enzymes SGOT (2.0 fold), SGPT (2.6 fold), ALP (2.3 fold) and TB (4.1 fold) in serum as well as increase in MDA and decrease in GSH, SOD, CAT enzymes levels in liver cells; as compared to normal control. Treatment with LEBF (100, 200 and 400 mg/kg) extract showed significant ($p<0.001$) dose dependent protective effects [Table No. 4 and 5]. At a dose of 400 mg/kg, LEBF showed percentage protection of 73.79%, 62.68%, 67.11%, 50% and 76.66%, 42.30%, 52.02%, 59.42% against increased level of SGOT, SGPT, ALP and TB as well as altered level of MDA, SOD, GSH, CAT; respectively as comparable to the silymarin. However treatment with LEME (100, 200 and 400 mg/kg) extract showed mild protective effects; as compared to ethanol control group. At a dose of 400 mg/kg, LEME showed percentage protection of 38.66%, 39.39%, 36.22%, 26.47% and 35%, 22.89%, 26.49%, 28.17% against increased level of SGOT, SGPT, ALP and TB as well as altered level of MDA, SOD, GSH and CAT, respectively. Serum enzyme and *in vivo* antioxidant enzyme level study proved that LEBF produces significant ($p<0.001$) dose dependent protective effects; while LEME extract produces mild/moderate protective effects. Histopathological studies [Figure 2] supports the serum enzyme level as well as *in vivo* antioxidant enzyme results, which proves that treatment with LEBF extract almost reveres the toxic effects of alcohol on liver cells, which is comparable to silymarin.

CONCLUSION

Based on the phytochemical screening, *in vitro* antioxidant and *in vivo* hepatoprotective screening studies, we can say that *n*-butanol fraction (LEBF) of *Luffa echinata* have noticeable hepatoprotective activities in the tested models. Results also suggested that the activities may be due to antioxidant property of the extracts; which is attributed due to presence of phenolics, flavonoids, triterpenoids along with other phytoconstitutents.

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