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Research Article

EVALUATION OF "*LEUCAS LANATA*" AND ASSESSMENT OF ITS HEPATOPROTECTIVE EFFECTS ON ANTITUBERCULAR DRUG-INDUCED HEPATIC DAMAGE

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ABSTRACT

Objective: The objective of this study was to investigate the hepatoprotective activity of ethanolic whole plant extract of *Leucas lanata* (family - Lamiaceae) against rifampicin+isoniazid (RIF+INH) induced hepatic damage in rats.

Methods: Wistar rats of either sex were divided into five groups of six animals each and given orally the following treatment for 28 days. The normal control was given 1% CMC 1 ml/kg body weight (b.w.), RIF+INH antitubercular drug (ATT) at a dose of 50 mg/kg b.w., p.o. was given a toxic dose for inducing hepatotoxicity. Silymarin (100 mg/kg, p.o) was given as reference standard. Two different doses of *Lucas lanata* extract of 200 and 400 mg/kg, p.o were tested for hepatoprotective activity. At the end of the treatment, blood was collected from direct cardiac puncture and analyzed for numerous bodily fluid parameter such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), total protein (TP), and total bilirubin (TB) and oxidative stress parameter lipid peroxidation (LPO), superoxide dismutase (SOD), catalase, glutathione (GSH), malondialdehyde and also evaluated the hydroxyproline content, tumor necrosis factor alpha, and interleukin-1 beta in all groups. Livers were isolated for its essential estimation followed by histopathological studies.

Results: Hepatic serum markers (ALT, AST, ALP, albumins, TP, and TB) were significant ($p<0.01$–$p<0.001$) protective effect was obtained against ATT-induced liver damage, and ATT treatment significantly decreased LPO, SOD, and GSH ($p<0.001$) levels compared with control Group I and given a *Leucas lanata* extract (LLE) ($p<0.01$–$p<0.001$), the oxidative stress markers were markedly reversed. Histopathology of liver tissue showed that LLE attenuated the hepatocellular necrosis, regeneration, and repair of cells toward normal. LLE which was standardized using high-performance thin-layer chromatography revealed the presence of some critical phenolic (gallic acid and quercetin) compound.

Conclusion: The results of this study powerfully indicate the protecting result of LLE against liver injury which can be attributed to its hepatoprotective activity, and thereby scientifically support its traditional use.

Keywords: *Lucas lanata*, Hepatoprotective, Rifampicin+isoniazid, Histological, Biochemical, Antioxidant activity.

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INTRODUCTION

Liver noted as the "metabolic factory" of the body is central to the metabolism of virtually every foreign substance together with antituberculosis medicine [1]. Liver injury induced by varied category of drug, chemicals, and viruses may be a well-recognized cause of toxicologic apprehensions. Within the liver, medicine undergoes protein biotransformation, which boosts metabolite hydrophobicity and clearance from the body [2,3]. Isoniazid (INH) and rifampicin (RIF) area unit the foremost vital 1st line antitubercular drugs (ATT); however, ATT elicited hepatotoxicity (DIH) remains obscure. Thus, although INH+RIF every in itself is probably hepatotoxic, once given in combination, their harmful result is augmented [4]. INH is thought to be directly or indirectly metabolized to the toxicant metabolites (acetyl hydrazine and hydrazine) by N-acetyltransferase and amide hydrolase in the liver [5-7]. The reactive acetylating species generated by monoacetyl hydrazine (MAH) are capable of binding covalently with viscus proteins. Hepatotoxicity induced by hydrazine has additionally been attributed to oxidative stress as a result of the formation of reactive oxygen species (ROS) mediate by cytochrome P450 2E1 (CYP2E1). CYP2E1 a CYP isoform supper family of enzymes concerned in the production of free radicals [8]. RIF is an efficient inducer of a variety of drug metabolizing enzymes of the liver. Hence, concomitant administration of RIF with alternative drugs may lead to drug interactions and adverse aspect effects [9,10]. The combination of these two ATT-induced hepatotoxicities manifested mainly as hepatocellular steatosis and centrilobular necrosis, possibly associated with cholestasis, and it has been suggested that toxic INH metabolites bind covalently to cellular macromolecules in both animal and human case studies [11]. During hepatotoxicity, liver cells are unable to synthesize proteins properly as a result of hepatocellular damage [12].

Leucas lanata Wall. ex Benth. (Family - Lamiaceae) is vernacularly known as Biskapra or Gumma. The juice of the whole plant has been traditionally used by local peoples to treat stomachache [13], headache [14], whooping cough [15], and antidote for reptile poisoning [16]. Leaves and flowers with cold water or milk are also used in cold, cough, and dysentery [17]. Fresh leaves are placed on the affected area for absorbing pus [18] and applied externally for wound healing in the form of paste [19], antibacterial [20], free radical scavenging, antiepileptic, and antiparkinson activities [21]. Allopathic medicines are inadequate to treat the chronic liver diseases [22].

At present, worldwide, much attention has been focused on the use of a natural antioxidant to maintain the health of the individual. As the liver is a major organ attacked by ROS and OS in induced toxicity is considered as the pathological mechanism in initiation and progression of a various liver disease, the present study was designed to evaluate the antioxidant activity of the selected plant extract. This study would be play or important role for further advanced study & development of new drug formulation.

MATERIALS AND METHODS

Chemicals

Silymarin powder (Silbyon), INH and RIF, Standard kits for alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP), 1,1-diphenyl-2-2picrylhydrazyl (DPPH), dimethyl sulfoxide, ethanol, formic acid, and hemotoxylin dyes were obtained from Sigma-Aldrich, USA, and all the other chemicals and solvents were used for analytical grade.

Sample preparation

The disease-free whole plant was collected from the local areas of Pachmarhi, Hoshangabad, and Madhya Pradesh, India, in December 2016. Collected the plants were washed twist, dried at 60°C using the oven and the whole plant material was glinded and obtained powdered (4 kg) was exhaustively extracted by percolation, at 50°C, with 50% ethanol. The resulting extract was evaporated under vacuum (rotavapor), to obtain viscous semisolid masses under the temperature not more than 40°C, until almost free from ethanol. The final concentrate was then subjected to lyophilization to yield a dry greenish-brown powder (90.1 g). The dried *Leucas lanata* extract (LLE) was carefully saved in a dark brown tightly closed container for further biological and phytochemical investigation. The origin of plant material was systemically identified and approved by the National Botanical Research Institute, Lucknow (Specimen No. NBRI/CIF/536/2016).

Preliminary phytochemical screening

The extract was tested for steroids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthraquinone, and amino acids. Phytochemical screening of extract was carried out according to the quality strategies [23].

DPPH radical scavenging activity

The free radical scavenging activities of LLE were conducted using the method of Devi *et al*. [24]. Briefly, 3 mL of extract with different concentration (50 mg/mL, 100 mg/mL, 150 mg/mL, 250 mg/mL, and 500 mg/mL) was mixed with 1 mL of DPPH (0.1 mM) solution in ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature, and the absorbance was then measured with a quartz glass cuvette (Hellma; Mullheim, Germany) at 517 nm against a blank using an ultraviolet (UV)-visible spectrophotometer (Pharma Spec UV-1700; Shimadzu; Kyoto, Japan) [25]. A low absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid was used as positive controls. The capability to scavenge the DPPH radical was calculated using equation:

$$\text{DPPH scavenging effect (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

Where, A_{blank} and A_{sample} are the absorbance of the control reaction (containing all reagents except the test extract) and the absorbance of the test extract, respectively.

Acute toxicity study

Acute oral toxicity of LLE was evaluated in mice using the OECD guideline 423. The extract, at the concentration of 1000, 1500, and 2000 mg/kg, dissolved in distilled water was administered intragastric. After administration, the animals were observed continuously for the first 4 h for behavioral changes, and at the end of 24 h, no any mortality is recorded. The effective dose 50% of LLE was decided $1/10^{th}$ of maximum dose (2000 mg/kg). 50% ethanolic LLE was used at the doses of 100, 200, and 400 mg/kg body weight (b.w.), p.o. for the hepatoprotective activity.

Experimental animals

Wistar rats (aged 3–4 weeks) were used from the animals house of National Botanical Research Institute and the protocol for the experiments on animals was approved by CPCSEA, New Delhi (approval number: 1732/GO/R/S/13/CPCSEA). All animals were maintained in specific-pathogen-free environment with 12 h light/dark cycle. Photoperiod was maintained and offered *ad libitum* access to food and water. After acclimation period, all experimental were randomly divided into 5 groups: Control (Blank group); ATT (50mgkg-1/d); ATT+STD (100mgkg-1/d); LLE1 (200mgkg-1/d) and LLE2 (400mgkg-1/d)[26]. Toxic drug and treatment drug were administered by oral gavage. The entire study lasted for 28 consecutive days. All the doses were determined by preliminary experiments and the control rats treated with equal volume of saline, termination of the experiments, all animals were euthanized and liver, blood, and other tissue sample were harvested for further analysis [27]. Hepatic lobes were used for the preparation of liver homogenates and histopathology sections.

Evaluation of liver injury and oxidative stress

The serum was used for estimating the biochemical parameters, namely ALT, AST, ALP, bilirubin, and total protein (TP) using standard assay kit method. Hepatic tissues of rats were homogenized (10%) in phosphate buffer (pH 7.4) with a Potter-Elvehjem glass homogenizer. Homogenate was centrifuged at 12,000 rpm for 20 min at 4°C to obtain post-mitochondrial supernatant (PMS) and used for the estimation of lipid peroxidation (LPO) [28], TP content was estimated by the method of Lowry *et al*. [29]. Catalase (CAT) (Sinha, 1972) [30], glutathione (GSH), and superoxide dismutase (SOD) of the liver were measured by the methods described by Aebi [31] and Kakkar *et al.* [32].

Determination of hydroxyproline (Hyp) content

Hyp (µg)/protein (mg) was determined by the calorimetrically in duplicates form 0.2 g of liver tissue using a modified method of Jamall *et al*. [33]. Hepatic tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1b) assays cytokines were detected by ELISA kits, and the results were expressed as pg/mL and pg/mg protein, respectively.

Morphologic examination of liver

The tissue was fixed by immersion in 10% natural buffered formalin. The sample was embedded in paraffin. Sections were cut by microtome at 6 mm thickness and stained with hematoxylin and eosin staining followed by a blinded histologic assessment.

Data analysis

The results were analyzed statistically using two-way analysis of variance (ANOVA) followed by Bonferroni post-tests to calculate the level of significance. Values are expressed as mean ± standard error mean (number of animals, n=6); significantly different at *p<0.05, **p<0.01, and ***p<0.001 when compared with the control group.

RESULTS AND DISCUSSION

Preliminary phytochemical and high-performance thin-layer chromatography (HPTLC) analysis

The study revealed that preparatory phytochemical compounds, for instance, alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, and other fragrant compounds are discretionary metabolites that are made in a plant. LLE affirms the occurrence of flavonoids, the major chemical constituents were found as glycoside and the obtained

Table1. Effect of hematological parameter on acute toxicity studies.

Parameter	WBC (10^3/uL)	RBC (10^6/uL)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pq)	MCHC (g/dL)	PLT (10^3/uL)
Ctrl	14.39±1.95	7.54±0.24	13.38±0.64	40.74±1.48	54.06±0.83	17.74±0.39	32.8±0.57	801±76.52*
LLE	14.44±1.73	7.54±0.24	13.14±0.13	40.3±0.62	57.36±1.20	18.72±0.28	32.76±0.29	1005±42.03**

Ctrl: Control, Hb: Hemoglobin, HCT: Hematocrit, RBCs: Red blood cells, WBCs: White blood cells, PLT: Platelets, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, SEM: Standard error mean

quantitative data using HPTLC showed the presence of gallic acid and rutin, quercetin which is helpful in the hepatoprotection against drug-induced liver illness.

DPPH free radical scavenging activity

A number of methods are used to determine the radical scavenging effects of an antioxidant. The DPPH assay is preferred because it is easy and reliable and does not require a special reaction in DPPH radicle scavenging assay; antioxidants react with DPPH and convert into yellow colored α, α-diphenyl-β picrylhydrazine. The degree of decolorization indicates the radicle scavenging potential of antioxidants [34]. It was found of LLE possess the strongest DPPH radicle activity (IC_{50}=122.56 µg/ml, respectively). The IC_{50} value for ascorbic acid was shown to be 42.23 µg/ml. After estimation of LLE was found to contain 0.735±0.017% of total phenolic and 0.21±0.020% of total flavonoid content.

Acute toxicity study

The outcome revealed that acute lethal dose 50% effect of LLE none of the mice died during 14 days at a dose of 2000 mg/kg so did not display any significant signs of clinical toxicity and no signature was found to changing in a gain of b.w. or feed consumption. LLE was estimated as >2000 mg/kg, implying the relative safety when administered acutely.

Hematological parameters

Hematological parameters revealed the degree of injurious impact of outside compound including plant extract on the blood constituents of animals. The outcomes exhibited that LLE was neither lethal to the circulating RBC, WBC, and platelets, nor it interferes with their production. The hematological parameter of mice given an extract up to 2000 mg/kg did not differ significantly ($p<0.01$) form of the respective control group. There were no toxicological effects recognized in any of the hematological parameters present statistically significant ($p<0.05$–$p<0.01$**)** when compared with control.

Effect of LLE on percentage change of body and liver weight in ATT-induced hepatic injury in rats

The rats which were treated by ATT showed a significant reduction in the body and liver weight ($p<0.01$) compared with the control group on the 28th day. However, cotreated ATT and LLE group showed significant enhancement in b.w. and also improvement of liver weight when compared ($p<0.05$–$p<0.01$) to ATT treated group. The mean relative liver weights (LW/BW ratio) of ATT-induced animals exhibited significant decrease compared to the control group ($p<0.05$). However, the group that was pretreated with 400 mg/kg of LLE showed decrease in the value of mean relative liver weights (Table 2).

Evaluated biochemical parameters

The markers of hepatic serum components were AST (228.70±17.66) IU/L, ALT (178.91±9.54) IU/L, and ALP (140.27±7.9) IU/L, TP (2.18±0.56) IU/L, albumins(Ab)(1.080±0.230)IU/L,andtotalbilirubin(TB)(1.78±0.08)mg/dL were significantly increased in ATT treated group ($p<0.001$) (Group-2). Administration of (200 and 400 mg/kg b.w.) both doses of LLE treated rats showed significant reduction level of AST, ALT, ALP, TP, Ab, and TB ($p<0.01$) when compared with ATT treated rats. However, the maximum reduction of AST (142.78±11.28) IU/L, ALT (79.09±4.88) IU/L, ALP (95.60±6.88) IU/L, TP (5.97±0.39) g/dl, Ab (3.31±0.069) IU/L, and TB (1.06 ± 0.18) mg/dL was observed in the high dose group (400 mg/kg b.w.) ($p<0.01$–$p<0.001$) (Fig. 1a and b). The results of the present study demonstrated the ATT could induce a significant liver injury, as evidenced by elevation of serum ALT, AST, ALP, TB levels, nuclear pleomorphism, and liver pathological changes. The extract exhibited significant protection against ATT-induced liver damage by attenuating the elevated ALT, AST, ALP, and TB levels in a dose-dependent manner.

Effect of LLE on malondialdehyde (MDA), LPO, GSH, CAT, and SOD in ATT-induced hepatotoxicity

As demonstrated in Fig. 2a and b, ATT treatment significantly increases the oxidativestresslevelMDA(66.34±1.92)nmol/ml,glutathioneS-transferase (GST) (1.75±0.15) U/mg, and GSH (1.02±0.04) mmol/mg levels compared with control Group I ($p<0.01$) and decline the LPO (4.14±1.21) mmol/mg, CAT (10.47±2.13) U/mg, and SOD (22.25±2.65) U/mg; however, the doses of LLE 400mg/kg significantly ($p<0.01$–$p<0.001$) revised all the oxidative level MDA (45.39±1.79) nmol/ml, GST (1.51±0.15) U/mg, GSH (0.40±0.09) mmol/mg, LPO (0.96±0.97) mmol/mg, SOD (88.16±8.77) U/mg, and CAT (18.28±3.22) U/mg. In our study, the proper balance between oxidant and antioxidant system was found to be disturbed by the ATT drug, as indicated by the increased formation of liver MDA, and depletion of hepatic GSH and antioxidant enzymes responsible for scavenging hydroperoxides including SOD, CAT, and GSH is an intracellular antioxidant that prevents intracellular ROS formation and LPO. The administration of LLE significantly inhibited the increase of hepatic MDA levels, the decrease of tissue GSH levels, and the activities of the antioxidant enzyme, which suggested that the imbalance between the oxidant and antioxidant system was reversed by LLE.

Table 2: Effect of LLE on body and liver weight of ATT-induced hepatotoxicity in Wistar rats represents significant at P<0.05 P<0.01when compared with control Group I

Treatment/dose	Body wt. (g)	Liver wt. (g)	LW/BW
Ctrl	164.90±4.35	6.10±0.09	3.69
ATT	149.57±3.69*	4.20±0.20*	2.80
STD	159.59±3.34*	5.84±0.25*	3.65
LLE_1	160.28±4.40**	5.25±0.16**	3.27
LLE_2	161.43±4.97**	5.69±0.45**	3.52

Ctrl: Control, ATT: Antitubercular drug: 50 mg/kg, LLE1, LLE2 (LLE: 200, 400 mg/kg), STD (Silymarin 100 mg/kg)

Table 3: The Histopathological severity of various features of hepatic injury was evaluated based on those following scoring schemes: – normal, + mild effect, ++ moderate effect, and ++++ severe effect.

Groups	Steatosis	Necrosis	Inflammation	Hemorrhage
CTRL	–	–	–	–
ATT	–	++++	+++	++
STD	–	+	+	–
LLE_1	–	++	+	–
LLE_2	–	+	+	–

LLE attenuated ATT-induced Hyp, TNF-α, and IL-1b intoxicated rats

Collagen deposition on the hepatic injury as markers was determined by the hepatic Hyp content. In case of Hyp, content was significantly rising when given doses of ATT, and the coadministered of ATT + LLE given a promising activity by increasing Hyp level. The protein levels of TNF-α and IL-1b in liver tissue were significantly upregulated by given ATT ($p<0.001$), and LLE significantly attenuated the overproduction of TNF-α and IL-1b, which was consistent with the hepatoprotective effect of LLE on the liver injury induced by ATT [35]. Collagen contended of liver tissue was quantified by the assurance of Hyp content was considerably reduced by LLE in each dose as compared to control group. However, the reduction in Hyp was more outstanding with 400 mg/kg of LLE. The livers of ATT-induced injury model groups were puffy, stiff, and acquired an irregular and granular surface. However, treatment with LLE remarkably promoted the recovery of ATT - damage livers structure as shown in Fig. 5. The degree of some medical specialty parameter TNF-α and IL-1b in each liver was significantly elevated when long-run treatment 28 days with the ATT drug in rats. LLE eased the production of TNF-α and IL-1b-pg/mg protein shows in a dose-dependent manner. In the present study, Silymarin 100 mg/kg was used as the standard to compare the activity of both of the extract, and it was found that Silymarin was significantly ($p<0.001$) more effective than the highest dose 400 mg/kg tested in the study. The available synthetic drugs to treat liver disorders in this condition may also cause further damage to the liver [36].

Histopathologic examination

Here these linens were shifted for histhopathological scoring. (a) Liver segment of normal control rats showing normal architecture; (b) liver

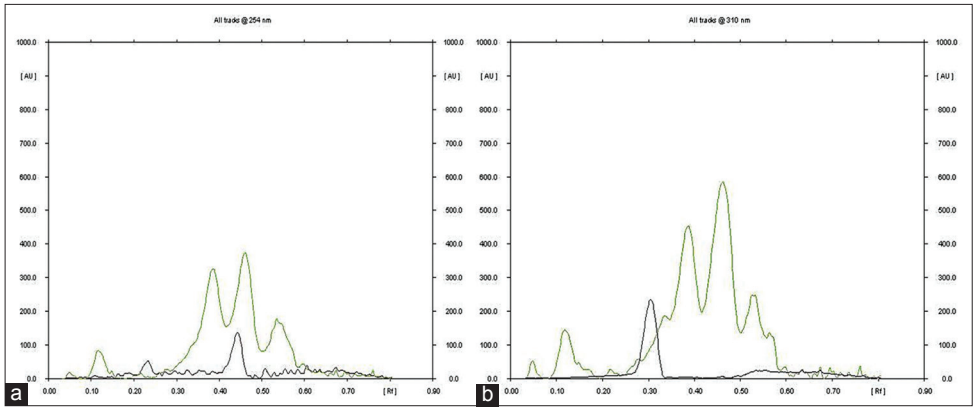

Fig. 1: (a and b) High-performance liquid chromatography analysis of *Leucas lanata* extract with standard quercetin, gallic acid

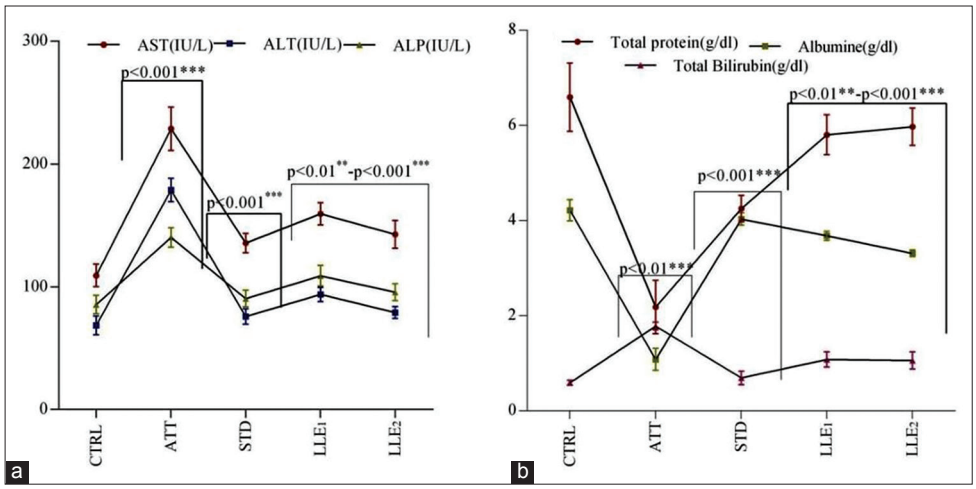

Fig. 2: Effect of LLE on serum in ATT-induced liver injury. (a) Alanine transaminase, aspartate aminotransferase, alkaline phosphate. (b) TP, albumins, total bilirubin (TB) in ATT-induced hepatotoxicity in Wistar rats represents significant at p<0.01 highly significant at p<0.001 when compared with control. Data are mean±strandard error mean (n=6 for CTRL, ATT, STD, LLE_1, LLE_2). CTRL: Control, ATT: Antitubercular drug: 50 mg/kg, LLE_1, LLE_2 (LLE: 200, 400 mg/kg), STD (Silymarin 100 mg/kg)

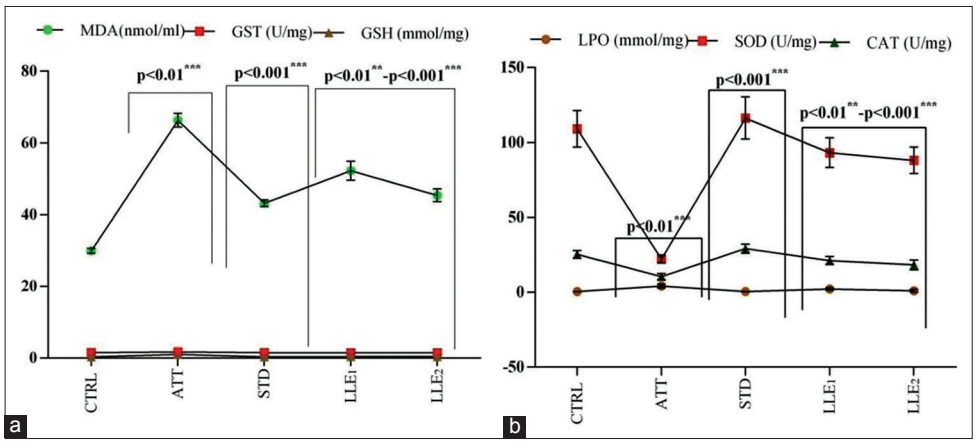

Fig. 3 (A,B): Effect of oxidative stress marker enzyme: Melondialdehyde (MDA), Glutathione-S transferase (GST), Glutathione (GSH), Lipid peroxidation (LPO), Superoxidase Dismutase (SOD), Catalase (CAT) parameter ATT induced hepatotoxicity in wistar rats represents significant at (p<0.01) highly significant at (p<0.001) when compared with control. Data are Mean±SEM (n=6 for CTRL, ATT, STD, LLE1, LLE2). CTRL: Control, ATT (Antitubercular drug: 50mg kg-1), LLE1, LLE2 (*Leucas lanata extract*: 200,400mg kg-1), STD (Silymarin 100mgkg-1).

segment of ATT treated rats showing huge fatty changes, necrosis, ballooning degeneration, and severe infiltration of the lymphocytes and therefore the loss of cellular boundaries; (c) liver section of rats treated ATT and 100 mg/kg of Silymarin showing signs of inflammatory cascade around central vein indicating a light degree of fatty amendment, necrosis, and focal necrosis (dilatation); (d) liver segment of rats treated ATT and 200 mg/kg of LLE_1 showing less inflammatory cells around central vein, absence of necrosis; and (e) liver segment of rats treated ATT and 400 mg/kg of LLE_2 showing: Minimal inflammatory cellular infiltration, large septa of connective tissue flowing together,

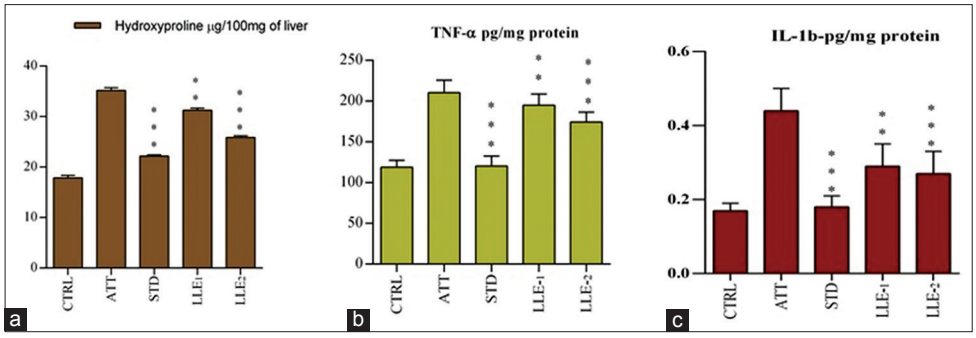

Fig 4 (A,B,C): Effect of LLE on hepatic damage in ATT-induced liver homogenate in Wistar rats on Hydroxyproline (Hyp) content, Tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-Ib) represent significant at (p<0.01) highly significant at (p<0.001) when compared with control. Data are Mean±SEM (n=6 for CTRL, ATT, STD, LLE1, LLE2). CTRL: Control, ATT (Antitubercular drug: 50mg kg-1), LLE1, LLE2 (*Leucas lanata extract*: 200,400mg kg-1), STD (Silymarin 100mgkg-1).

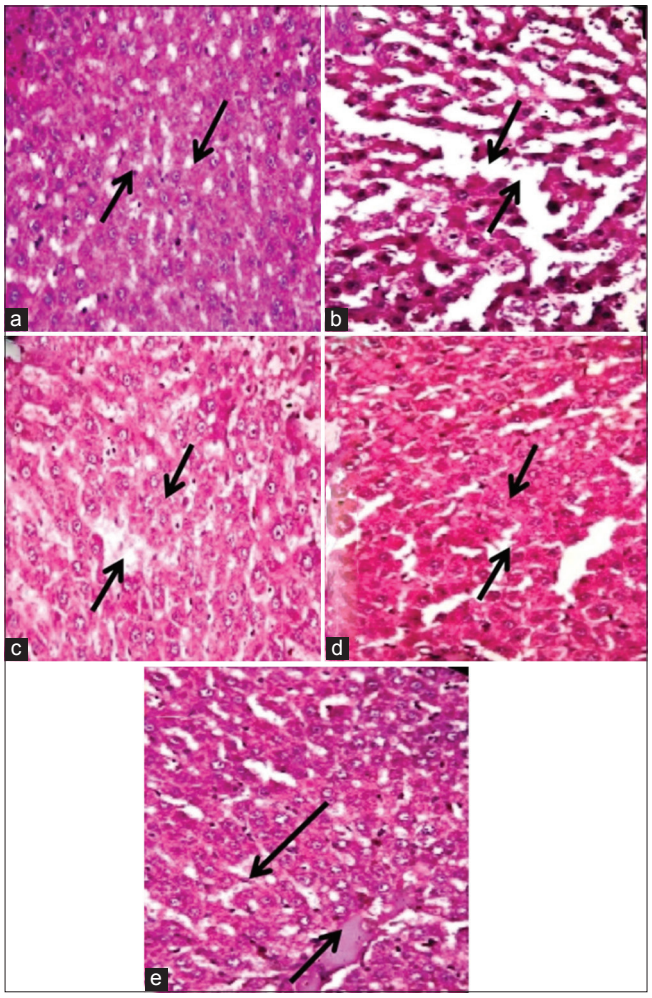

Fig. 5: (a-e) Histopathological studies of representative photomicrographs of liver sections

and penetrating into the parenchyma. There is a regeneration of hepatocytes evident.

Histopathological scoring of the tissue of ATT-induced hepatic injury rats after pre-treatment with LLE.

While histopathological changes including nuclear pleomorphism, increased cellular size, degeneration in hepatocytes and hepatic cords, cytoplasm dissolution, and inflammatory cell infiltration were also remarkably improved by LLE.

CONCLUSION

The study incontestible that the progression of ATT evoked liver toxicity can be prevented or reduced victimization the ethanolic LLE. The extract exhibited its hepatoprotective result by preventing the harmful cascade of event induced by ATT toxicity. The protecting capability of LLE secured the livers and effectively attenuated the intense oxidative stress elicited throughout ATT. Moreover, the power to cut back the elevated level of elevation ALT, AST, ALP, TP, and TB suggesting that these biochemical restorations could be owing to the extracting ability. LLE bonded further study of an enticing pharmacological target for the advancement of latest drug and potential in treating the liver injury.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS OF AUTHORS

Pritt Verma: Acute toxicity and histopathological study. Literature search, study design, data collection, data analysis, and data interpretation writing. Dr. Ch. V Rao: Data interpretation, manuscript review, and data analysis. Manuscript review and data analysis. Acute toxicity and histopathological study.

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