RESEARCH ARTICLE

Effectiveness of *Anaphalis triplinervis* on Carbon Tetrachloride-induced Hepatic Injury in Rats by Interfering with the Inflammatory, Cell Death and Oxidative-stress Pathways

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ABSTRACT

The continuous practice of traditional medicines provides scientific validation of the use of herbal medicines, which eventually leads to widespread recognition among the socioeconomic healthcare systems. *Anaphalis triplinervis*, (Asteraceae) is native species being used to prevent, treat, and cure the hepatic ailment, in Chinese and Indian traditional systems of medicine. we investigated the hepatoprotective effect of *A. triplinervis* extracted with hydroethanolic solvent (HEEAT) a potent natural anti-oxidant against carbon tetrachloride-induced hepatic injury in rats and elucidate the inflammatory cascade leading to apoptosis in adult rat liver. Adult Wistar rats were randomized into 6 groups and each group contains six animals, (n=6). Group I: normal control; animals treated with vehicle. Group II: toxin control; rats administered with CCl_4+Olive oil 1:1 *i.p*., Group III: standard; silymarin (125 mg/kg), Group IV, V, and VI administered with HEEAT 100, 200, 300 mg/kg up to 14 days. The body weight and hematological assessment were performed further the antioxidant activity was determined by assay for catalase, glutathione reductase, superoxide dismutase, and malondialdehyde in the hepatic tissue. Increased oxidative stress, cytokines (TNF-alpha and IL-1beta), NF-kappa β, and caspase-3 levels in CCl_4-treated rats. Co-administration with HEEAT significantly prevented all the biochemical and molecular alterations in the hepatic tissue of CCl_4-treated rats in a dose-dependent manner. The hepatoprotective effect was further confirmed by examining the liver organ weight and histopathological analysis using H&E staining. Collectively the findings from the current study demonstrate the possible involvement of oxidative- stress-mediated activation of inflammatory cascade and apoptosis in chronic CCl_4-induced liver injury and suggest the effectiveness of HEEAT in mitigating the liver toxicity associated with chronic CCl_4 administration. Further, our study using morphological, hematological, biochemical, histopathological data analysis suggest that HEEAT may serve as an effective antioxidant and hepatoprotective candidate.

Keywords: *Anaphalis triplinervis*, Antioxidant enzymes, Hepatoprotective, Liver toxicity.

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INTRODUCTION

The liver is a major organ for metabolizing nutrients, drugs, medications including alcohol along with detoxifying different systemic endogenous and exogenous toxins.[1] The hepatic system may involve in the biotransformation of xenobiotics by synchronizing the synthesis of various physiochemical and physiological functions of the body.[2] The type I and type II biotransformation reaction are well balanced by the liver.[3] Not only, etiologically different microorganisms such as *Toxoplasma gondii, Staphylococcus,* etc, are involved in the development of liver infections[4] but also Ecological pollutants are responsible for diverse sorts about the hepatic injury, including liver destruction, alcoholic liver disease, fatty liver encephalitis, hepatocellular carcinoma, etc.[5]

Oxidative stress along with reactive oxygen species (ROS) and reactive oxygen nitrite (RON) system is one of the main factors that contribute to various liver injuries such as hemochromatosis and hemosiderosis.[6] The cellular lipid peroxidation due to the generation of free radicals precipitates hepatic fibrogenesis, notably via aldehyde peroxidation on kupffer cells and lipocytes.[7] The literature and scientific shreds of evidence disclose that many chemicals are hepatotoxic in nature like carbon tetrachloride (CCl_4), NSAIDS, etc.[8]

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Here we have used the CCl_4 experimental paradigm for the induction of liver injury while elucidating the cellular and molecular mechanisms behind it.[9] For assessment of the antioxidant effect and hepatoprotective role of drugs this model is most popular and routinely used in the experimental setup.[10] At the cellular level, CCl_4 primarily forms the ROS and RNS, which are metabolized in the liver. CCl_4 enhances the production of fast generating trichloromethyl free radicals (CCl_3) in the hepatic tissue, further the cytochrome P450 converts the free radicals into trichloromethyl peroxy radical (CCl_3OO) which via oxidative phosphorylation ultimately leads to the hepatic injury.[11] The generated CCl_3O free radicals disrupt the structural and functional integrity of hepatic tissue and thus produce toxicity.[13] Neutralization of free radicals by antioxidants is an effective approach to restore liver physiology.

From age's nature has been a source of a large number of potent therapeutic herbs. Among various natural antioxidants, polyphenolic-containing herbs are also the most important signs. For example, *A. triplinervis* DC belonging to the family *asteraceae*, authenticated by Botanical Survey of India, Dehradun, Uttarakhand having specimen no-115900. The distribution of these medicinal herbs is widely in both tropical as well as the temperate region of Asia especially found in Uttarakhand, India. It is acknowledged underneath the local name of pilijari in the garhwal region, naga guining meadow rue, and mamira in the Kumaun region.[14] Divergent bioactive constituents are isolated from this plant. Major two bioactive constituents benzylisoquinoline alkaloids with chloro group, falisasumines A (1) and B (2), also including eight major isoquinoline alkaloids (3–10) were isolated which has significant pharmacological activity.[15] With the help of basic analysis including 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) have been used to elucidate the structure of these compounds based on the establishment of these finding, we draw the inference these constituents is accountable for protective profile for the liver disorder.[16] This bioactive constituent-rich medicinal plant is being traditionally used for treating liver disorders such as jaundice and hepatitis.[16,17] Other uses of this plant are antipyretic, antimalarial,[18] antivenom, dyspepsia, febrifuge,[19] and antimicrobial properties are scientifically validated.[17] The present study was designed to explore the mechanisms behind the CCl_4-induced liver damage and to investigate the therapeutic effect of HEEAT.

METHODOLOGY

Animals

Wistar rats (150220 g) of either sex were obtained from the Animal House of Anand College of Pharmacy, Agra, Uttar Pradesh India. Animals were purchased from Indian Veterinary Research Institute Bareilly, India (IVRI). All experimental rats were housed under specific conditions; room temperature 24 ± 2°C and humidity 45–55% and 12:12 hours light/dark cycle. The animals had free access to food (pallets purchased from VRS Foods Limited, New Delhi, India) and water *ad-libitum*. All experimental protocol was designed to give minimum pain to animals and the same were approved as per the norms of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (registration no 125/PO/Re/s/10/CPCSEA). All experimental animals were randomized to different groups.

Drugs and Chemicals

Silymarin as a standard drug was procured from Yarrow chem. products, Mumbai, India. The biochemical kits were acquired from Transasia BioMedical, Himachal Pradesh, India. All the solvents and chemicals were of analytical grade. *A. triplinervis* was collected from the valley of Kedarnath, Uttarakhand, India during the winter season. The collected plant was recognized by Professor S. K. Srivastava, Department of Botanical Survey of India, Dehradun, Uttarakhand, India. The account number assigned is 115900. Furthermore, the whole plant of *HEEAT* was dried (25°C) and extracted in the ratio of 40:60 distilled water with ethyl alcohol (95% v/v) for 24 hours with the help of hot soxhlation method and the extract was allowed to dry on a water bath (50°C) and finally, the % yield was calculated.

LD_{50} Determination

Determination and conclusive statements for the acute toxicity test study were done as per the OECD guideline No. 425 fixed-dose procedure.[20] Ten experimental rats were assigned into two groups; normal control and test group (5 females and 5 males). Animals received a HEEAT 2000 mg/kg per oral route post 16 hours of fasting. The normal control group received vehicle (sterile saline n = 5), test group received HEEAT 2000 mg/kg followed by clinical observation with different periods (0.25, 0.5, 1, and 4 hours). Every sign of toxicity was observed as well as recorded and registered. All animals were kept under keen clinical observation for fourteen consecutive days and body weighed was measured at different time intervals (day 0, 7, and 14 post-treatment). Moreover, we have distinguished parameters for assessing the toxicity including the cumulative weight change (%) which was estimated based on the initial weight. Also, we have calculated the mean food and water consumption for rats. At the end of the observation period, the blood samples were collected by retro-orbital plexus. Blood samples were further processed for performing hematological assessment and biochemical assays. The plasma was separated by centrifugation at 8000 rpm. The mortality rate was also observed in comparison to the vehicle-treated rats. Finally, the animals were euthanized and an autopsy was performed.[20]

Experimental Design

The animals were randomly divided into six groups each group containing 6 animals, Group I: normal control (NC). Group II: Toxin control 1:1CCl_4+Olive oil i.p. Inducing group. Group III: Positive Control (silymarin 125 mg/kg) p.o. +CCl_4+ Olive oil i.p. Group IV: HEEAT (100 mg/kg) p.o. +CCl_4+Olive oil i.p. Group V: HEEAT (200 mg/kg) p.o. +CCl_4+Olive oil i.p. Group VI: HEEAT (300mg/kg) p.o.+CCl_4+Olive oil i.p. for 14 days. At the end of the experiments (day 14th), test and standard drugs were administered; CCl_4 was given within 30

minutes' post-drug administration. All experimental rats were anesthetized using thiopentone sodium (40 mg/kg i.p) and sleeping time was recorded to its natural arousal, i.e., loss of righting reflex to its recovery. After recovery the same animals were anesthetized using ketamine (20 mg/kg) + xylazine (80 mg/kg) for euthanasia.

Hematological Analysis

Parameters such as hemoglobin, complete blood counts (CBC) were determined using an auto analyzer.

Biochemical Estimation

All the isolated liver samples were immediately washed with normal saline, and each sample was homogenized in the PBS (7.4) on ice to prevent tissue damage (w/v; 1 g tissue with 3 mL PBS) and centrifuged at 10000×g for 15 minutes, at 4°C.

Quantification of Liver Lipid Peroxidation (LPO)

The assessment of the lipid peroxidation was carried out by estimating the amount of malondialdehyde (MDA) formed, using the method described earlier.[21] MDA is the end product of lipid peroxidation which is the marker for free radical-mediated damage and oxidative stress. The principle behind the above-mentioned method includes the reaction between MDA and thiobarbituric acid (TBA) under an acidic environment at a temperature of 90–100°C to yield a pink color MDA-(TBA) 2 conjugate, which was quantified with a spectrophotometer (530 nm). In the procedure, 0.5 mL of 20% TCA was added to 0.5 mL of the tissue homogenate, and then there was an addition of 1-mL of 0.67% TBA. The mixture was incubated at 100ºC for 15 minutes in a water bath, cooled, and then added with 4 mL of n-butanol and centrifuged at 3000 rpm for 15 minutes. The absorbance of the clear pink supernatant was then read against a blank at 532 nm spectrophotometrically. The concentration of MDA is expressed in nmol/g of the tissue.[22]

Determination of Liver Reduced Glutathione (GSH):

Reduced glutathione was determined by the method described previously.[23] The assay is based on the oxidation of GSH by 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), DTNB, and glutathione (GSH) react to generate 2-nitro-5- thiobenzoic acid (TNB) which has a yellow color. Therefore, GSH concentration can be determined by measuring absorbance at 412 nm. For this assay, 50 µL of the tissue homogenate was taken and further diluted with 10 mL of phosphate buffer (0.1 M, pH 8). To 3 mL of the above-diluted mixture, 20 µL of DTNB (0.01 M) was also added and absorbance was taken at 412 nm, the blank was prepared under the same conditions.

Quantification of Liver Superoxide Dismutase (SOD)

The assessment of the superoxide dismutase activity was done via adopting the experimental protocol as described, the xanthine-xanthine oxidase system was employed for generating a superoxide flux, and nitro blue tetrazolium (NBT) was used as a marker indicating production of superoxide. The activity of SOD is a measure of the degree of inhibition of the reaction unit of enzyme providing 50% inhibition of NBT reduction. Results are further expressed in U/mg.[24]

Quantification of Liver Catalase Activity

The catalase activity was quantified in the serum by employing the protocol.[6] Briefly, we have taken a serum (10 μL) in a test tube that contains 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). Furthermore, the reaction began after the addition of 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was determined by using a spectrophotometer with a wavelength of 240 nm for 5 min. A molar extinction coefficient of 0.041 $mM^{-1} cm^{-1}$ was used for the estimation of catalase activity.

Quantification of Liver of IL-1β and TNF-alpha

The assay was performed on liver tissues homogenate as per manufacturer instructions using R&D Systems Quantikine Rat IL-1β and TNF-α ELISA kits.

Quantification of Liver of NF kappa β p65 unit

That assay was performed on liver tissues homogenate as per manufacturer instruction using NF-κβ/p65 Active ELISA (Imgenex, San Diego, USA) ELISA kit.

Quantification of Liver Caspase-3 using a Colorimetric Assay

A colorimetric assay was performed for the estimation of caspase-3 levels. The protease activity was measured in tissue homogenates samples using a caspase-specific peptide conjugated with a chromophore (p-nitroaniline). The peptide cleavage done by caspase was analyzed by using the spectrophotometric technique at 405 nm. The percentage of control was taken as a measure to express the results.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism 8.3.3 software (GraphPad, San Diego, CA). Results were presented as mean ± SD and the $p < 0.05$ value was considered as statistically significant. Hematological and biochemical data were analyzed using two-way ANOVA, or repeated measures one-way ANOVA where appropriate, followed by Turkey post hoc test analysis.

RESULTS

Preliminary Phytochemical Screening

Hydroethanolic extract of *Anaphalis* contains different chemical constituents such as saponins, carbohydrates, alkaloids; glycoside and iso-flavonoid, etc. percentage yield of the extract was found to be 11.37%.

Toxicity Studies for the HEEAT

We found no sign of toxicity in clinical observation of the test group as compared to the normal control group. Our finding reveals from the data of hematology, biochemical and organ toxicity suggests that the dose up to 2000 mg/kg of HEEAT can be regarded as a maximum LD_{50} in comparison to the rats treated with vehicle (normal control). This proofs the wide margin of safety of *HEEAT.* The extract obtained from the natural source was found to be in safer limits up to a dose of 2 gm/kg body weight. Further, the test drug doses were decided which are 100, 200, and finally 300 mg/kg for conducting the remaining studies.

Body Weight

The CCl4 negatively affects the bodyweight of rats and a significant decline in body weight was observed (Figure 1) this decline in body weight was reversed significantly with HEEAT at 100, 200, 300 mg/kg p.o. dose-dependently and with silymarin (125 mg/kg p.o.).

Liver Weight

The weight of the liver in the CCl_4 control group was found to be increased significantly (*p <0.01), as compared to the normal control group. Moreover, the HEEAT and the silymarin treated group showed significantly enhanced liver weight with the comparison to CCl_4 treated rats as shown in Figure 2.

Hematological Parameters:

The complete blood analysis showed that hemoglobin concentration, RBC, WBC, platelet count, MCV, MCHC%, and RDW% were decreased in CCl4 group rats as compared to the control rats. HEEAT 300 mg/kg and silymarin 125 mg/kg respectively as shown in Table 1.

Biochemical Observations and Interpretation

The hepatic biochemical markers such as SGOT, SGPT, cholesterol, ALP, triglycerides, total bilirubin, and direct bilirubin in blood serum were found to be increased in CCl_4 rats as compared to the control rats. These data indicate the CCl_4 induced toxicity in the liver. Findings were shown in Table 2. Serum glutamic-oxaloacetic transaminases (SGOT), serum glutamic pyruvic transaminase (*SGPT*), alkaline *phosphatase* (*ALP*), direct bilirubin (DB), total bilirubin (TB), total protein (TP), albumin, triglycerides, and cholesterol were determined using an auto analyzer.

Chronic HEEAT Treatment Enhances Liver Antioxidant Enzymatic Activity in Rats with CCl4 Administration

Chronic CCl_4 administration in rats significantly (p <0.05) reduces the liver enzymatic activity of catalase as compared

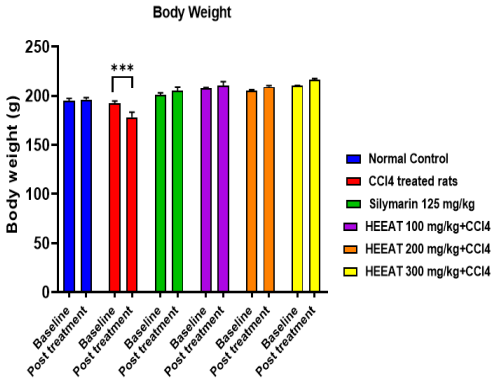

Figure 1: The effect of hydroethanolic extract of *AT* on CCl_4 induced alteration in the bodyweight of rats: Values were expressed as mean ± S.E.M n= 6 animals in each group. One-way ANOVA followed by Tukey's post-hoc analysis was used. **p < 0.001 represents CCl_4 treated rats with compared to normal control group.

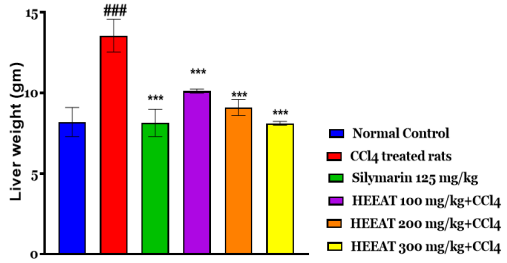

Figure 2: Effect of hydroethanolic extract of *AT* on the liver weight of CCl_4 administered rats. Values are presented as mean ± S.E.M., n = 6 animals in each group. Symbols represent statistical significance. One-way ANOVA followed by Tukey's post-hoc analysis was used. **p < 0.01 and *p < 0.001* groups compared to normal control group.

Table 1: Protective effects of HEEAT (100, 200, 300 mg/kg; oral gavage) on various hematological parameters in rats with CCl_4-induced hepatotoxicity on day 14. Values were expressed as mean ± S.E.M. (n=6-per group). Two-way ANOVA followed by multiple compassion analysis was used.

	Group I	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*
Groups	*Normal control*	*CCl_4 treated rats*	*Silymarin treated rats*	*HEEAT 100 mg/kg +CCl4*	*HEEAT 200 mg/kg +CCl4*	*HEEAT 300 mg/kg +CCl4*
Hemoglobin (gm/dL)	13.35 ± 0.35	9.26 ± 0.31^{++}	14.73 ± 0.09	12.90 ± 0.22	13.87 ± 0.16	14.81 ± 0.17**
WBC 10^3/uL	11.15 ± 0.29	18.56 ± 0.29^{++}	13.77 ± 0.33	8.92 ± 0.19	12.77 ± 0.31	13.70 ± 0.45**
RBC 10^6/uL	7.30 ± 0.12	5.93 ± 0.12^{++}	11.06 ± 0.19**	7.13 ± 0.14	7.20 ± 0.04	10.03 ± 0.18**
Packed cell volume (HCT)%	40.82 ± 0.23	33.93 ± 0.67^{++}	40.48 ± 0.58**	37.32 ± 0.76	38.28 ± 0.24	40.05 ± 0.35**
Mean corpuscular volume	54.45 ± 0.42	46.65 ± 1.58^{++}	52.32 ± 0.15**	47.95 ± 0.36	50.95 ± 0.19	52.19 ± 0.38**
Mean corpuscular Hemoglobin	18.35 ± 0.14	13.83 ± 0.48^{++}	17.30 ± 0.19**	15.82 ± 0.38	17.58 ± 0.44	17.00 ± 0.19**
Mean corpuscular Hemoglobin concentration %	32.90 ± 0.36	28.66 ± 0.68^{++}	33.77 ± 0.52**	31.65 ± 0.40	32.07 ± 0.98*	33.30 ± 0.58**
RDW %	13.41 ± 0.67	11.41 ± 0.38^{++}	14.18 ± 0.19**	11.58 ± 0.32	12.05 ± 0.13*	12.05 ± 0.13**
Platelet count 10^5/UL	11.30 ± 0.62	6.78 ± 0.19^{++}	11.43 ± 0.46**	10.07 ± 0.13	12.58 ± 0.24**	12.58 ± 0.24**

$^{++}p < 0.001$ represents as compared to the control group.**p < 0.01, **p < 0.01, ***p < 0.001* represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

to the rats in the control group. A significant effect across the groups was observed on catalase activity in the liver after one-way ANOVA followed by Brown-Forsythe test [(F (5,24) = 53.47; DF= 5, p < 0.0001) (Table 3). A similar trend was observed in levels of other major anti-oxidant enzymes GSH was shown in the liver was depicted in Table 3. A significant effect across the groups was observed on GSH activity in liver tissue after one-way ANOVA followed by Bartlett's test [(F (5,24) = 21; DF= 5, p < 0.0001). However, in the case of SOD in the liver tissue after one-way ANOVA followed by Bartlett's test [(F (5,24) = 51; DF= 5, p < 0.0001), was shown in Table 3. Moreover, chronic treatment with HEEAT (100, 200, and 300 mg/kg) significantly reverses these enzymatic activities in the liver of rats with CCl_4 administration (p < 0.0001).

Chronic HEEAT Treatment Suppressed Liver LPO levels in Rats with CCl_4 Administration

Rats administered with chronic CCl_4 were found to have a significant (p < 0.001 increase in lipid peroxidation in the liver (3.57 fold) as compared to the control group rats. A significant effect across the groups was observed on LPO activity in the liver after one-way ANOVA followed by Bartlett's test [(F (5,24) = 75.44; DF= 5, p < 0.0001). The HEEAT treated (100, 200, and 300 mg/kg) rats were observed to have a significant (p < 0.001) reduction in lipid peroxidation in the liver tissue of CCl_4 administered rats. (Table 3).

Chronic HEEAT treatment mitigates liver tumor necrosis factor-alpha TNF-α in rats with CCl_4 administration

Chronic CCl_4 administration in rats significantly (p < 0.05) increased the liver (3.47 fold) TNF-α levels as compared to the control group rats. A significant effect across the groups was observed on *TNF-α* expression in the liver after one-way ANOVA followed by Bartlett's test [(F (5,24) = 320.8; DF= 5, p < 0.0001. The HEEAT (100, 200, and 300 mg/kg) treatment decreases the TNF-α levels significantly (p < 0.05) in the liver of HEEAT (100, 200 and 300 mg/kg) CCl_4 administered rats dose-dependently. (Figure 3).

Chronic HEEAT treatment suppressed liver interleukin-1β (IL-1β) in rats CCl_4 administration

The IL-1β levels were significantly (p < 0.05) increased in the liver (4.33 fold) of rats with chronic CCl_4 administration when compared to the rats in control. A significant effect across the groups was observed on *IL-1β* expression in the liver after one-way ANOVA followed by Bartlett's test (F (8,24) = 326.8; DF= 5; p < 0.0001) respectively. Moreover, rats treated with HEEAT (100, 200 and 300 mg/kg) showed a significant

Table 2: Protective effects of HEEAT (100, 200, 300 mg/kg; oral gavage) on various biochemical parameters in rats with CCl_4-induced hepatotoxicity on day 14. Values were expressed as mean ± S.E.M. (n=6-per group). Two-way ANOVA followed by multiple compassion analysis was used.

	Group I	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*
Groups	*Normal control*	*CCl_4 treated rats*	*Silymarin 125 mg/kg treated rats*	*HEEAT 100 mg/kg +CCl4*	*HEEAT 200 mg/kg +CCl4*	*HEEAT 300 mg/kg +CCl4*
Urea (mg/dL)	37.6 ± 0.84	58.82 ± 2.23^{+++}	40.97 ± 3.17***	57.8 ± 4.56**	50.32 ± 2.53**	39.97 ± 3.57***
Creatinine (mg/dL)	0.5 ± 0.03	0.70 ± 0.12^{+++}	0.47 ± 1.14***	0.7 ± 0.06*	0.60 ± 0.09**	0.52 ± 0.04**
SGOT(U/L)	148.3 ± 4.94	353.6 ± 6.57^{+++}	153.15 ± 10.95**	184.5 ± 5.47**	182.97 ± 6.59**	149.32 ± 7.21**
SGPT(U/L)	96.8 ± 1.46	269.15 ± 7.51^{+++}	124.9 ± 3.07***	144.8 ± 5.74**	141.20 ± 4.05**	122.65 ± 3.77**
ALP (U/L)	76.0 ± 1.77	180.93 ± 1.78^{+++}	87.85 ± 2.12***	101.0 ± 2.38**	95.30 ± 2.48*	88.33 ± 2.53***
Cholesterol (mg/dL)	74.8 ± 3.80	142.97 ± 3.87^{+++}	87.93 ± 4.81***	99.5 ± 1.07**	92.07 ± 3.27**	88.98 ± 3.30***
Triglyceride (mg/dL)	123.8 ± 8.52	180.85 ± 2.43^{+++}	125.58 ± 4.04**	162.3 ± 5.68**	139.73 ± 7.45*	125.30 ± 4.38**
Direct bilirubin (mg/dL)	1.1 ± 0.06	2.47 ± 0.17^{+++}	1.16 ± 0.04***	1.6 ± 0.09**	1.35 ± 0.09*	1.17 ± 0.06***
Total bilirubin (mg/dL)	2.2 ± 0.16	4.37 ± 0.18^{+++}	2.09 ± 0.10***	3.0 ± 0.04*	2.62 ± 0.13**	2.12 ± 0.16***
Albumin (g/dL)	4.1 ± 0.07	2.69 ± 0.17^{+++}	6.10 ± 0.13**	4.4 ± 0.24*	5.44 ± 0.17*	6.37 ± 0.12***
Total protein (g/dL)	8.5 ± 0.32	5.25 ± 0.46^{+++}	9.74 ± 0.17***	7.8 ± 0.58**	9.54 ± 0.21**	9.74 ± 0.16***

$^{+++}p < 0.001$ represents as compared to the control group. *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$ represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

Table 3: Protective effects of HEEAT (100, 200, 300 mg/kg; oral gavage) on various antioxidant parameters in rats with CCl_4-induced hepatotoxicity on day 14. Values were expressed as mean ± S.E.M. (n = 6-per group). Two-way ANOVA followed by multiple compassion analysis was used. $^{++}p < 0.001$ represents as compared to the control group.

	Group I	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*
Groups	*Normal control*	*CCl_4 treated rats*	*Silymarin treated rats*	*HEEAT 100 mg/kg +CCl4*	*HEEAT 200 mg/kg +CCl4*	*HEEAT 300 mg/kg +CCl4*
SOD (U/mg protein)	75.12 ± 1.34	46.60 ± 2.12^{+++}	67.82 ± 1.90**	71.91 ± 2.13**	68.88 ± 2.34***	65.70 ± 2.60***
CAT (U/mg protein)	46.83 ± 1.10	25.66 ± 0.65^{+++}	39.31 ± 1.91**	43.55 ± 1.08**	41.03 ± 0.63***	37.46 ± 2.27***
GSH (mg/g protein)	2.51 ± 0.12	1.38 ± 0.13^{+++}	1.76 ± 0.12**	1.64 ± 0.16**	1.83 ± 0.06***	1.60 ± 0.06***
MDA (nmol/mg protein)	1.28 ± 0.07	2.44 ± 0.20^{+++}	1.46 ± 0.08**	1.81 ± 0.17**	1.55 ± 0.09***	1.19 ± 0.08***

*$p < 0.01$, **$p < 0.01$, ***$p < 0.001$ represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

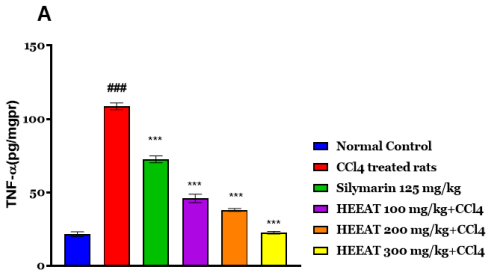

Figure 3: Effect of HEEAT (25, 50, 100 mg/kg; oral gavage) on TNF-α level in rats chronically administered CCl_4. Values were expressed as mean ± S.E.M. (n = 6-per group). One-way ANOVA followed by Tukey's post-hoc analysis was used. $^{###}p < 0.001$ represents as compared to the control group. $^{***}p < 0.001$ represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

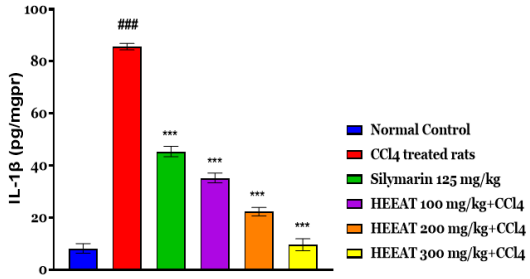

Figure 4: Effect of HEEAT (25, 50, 100 mg/kg; oral gavage) on *IL-1*β level in rats chronically administered CCl_4. Values were expressed as mean ± S.E.M. (n = 6-per group). One-way ANOVA followed by Tukey's post-hoc analysis was used. *### p < 0.001* represents as compared to the control group. $^{***}p < 0.001$ represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

($p < 0.05$) reduction in liver IL-1β levels. This effect was dose-dependent (Figure. 4).

Chronic HEEAT treatment ameliorated liver nuclear factor kappa beta (NF-κβ) p56 subunit levels in rats with CCl_4 administration

Rats administered with chronic CCl_4 were observed to have significantly ($p < 0.05$) elevated NF-κβ p65 subunit levels in the liver (7.62 fold) when compared to the control group. The significant effect across the groups was observed on *NF-κβ* expression in the liver after one-way ANOVA followed by Bartlett's test ($F(8,24) = 98$; DF= 5; $p < 0.0001$). Treatment with HEEAT (100, 200, and 300 mg/kg) significantly ($p < 0.001$) reduced the liver NF-κβ p56 subunit expression of chronic CCl_4 administered rats and this effect was dose-dependent. (Figure. 5).

Chronic HEEAT treatment suppressed liver caspase-3 levels of rats with chronic CCl_4 administration

The rats administered with chronic CCl_4 were found to have a significant ($p < 0.05$) increase in caspase-3 level in the liver (3.62 fold) as compared to the rats in the control group. The

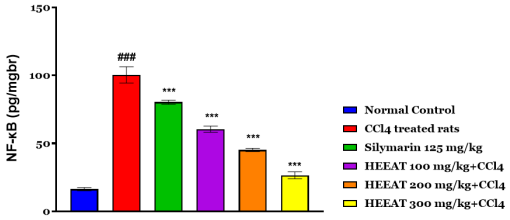

Figure 5: Effect of HEEAT (25, 50, 100 mg/kg; oral gavage) on NF-kβ level in rats chronically administered CCl_4. Values were expressed as mean ± S.E.M. (n = 6-per group). One-way ANOVA followed by Tukey's post-hoc analysis was used. $^{###}p < 0.001$ represents as compared to the control group. $^{***}p < 0.001$ represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

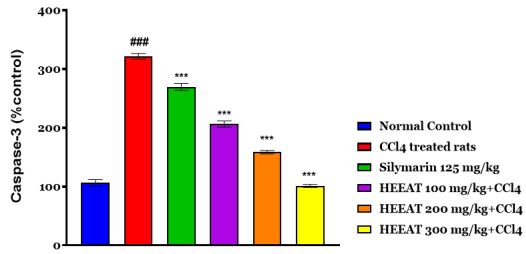

Figure 6: Effect of HEEAT (25, 50, 100 mg/kg; oral gavage) on caspase-3 level in rats chronically administered CCl_4. Values were expressed as mean ± S.E.M. (n = 6-per group). One-way ANOVA followed by Tukey's post-hoc analysis was used. *### P < 0.001* represents as compared to the control group. $^{***}p < 0.001$ represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

significant effect across the groups was observed on caspase-3 expression in liver after one-way ANOVA followed by Bartlett's test ($F(5,24) = 3342$; DF= 5; $p < 0.0001$). A significant ($p < 0.05$) reduction was observed with HEEAT (100, 200, and 300 mg/kg) in caspase-3 levels of the liver in a dose-dependent mode. (Figure. 6).

Histopathological or Inferences of hepatocellular necrosis/protection

Slide (A) Hepatic tissues of vehicle-treated rats, assign as normal control shows normal histology architecture such as portal triad, normal portal vein, hepatic artery, and biliary canaliculi system (H×E 10 xs).

Slide (B) Fraction of the hepatic tissues of rats treated with silymarin showing normal histology and portal triad showing normal portal vein, hepatic artery, and bile duct. (H×E 10x).

Slide (C) Fraction of the hepatic tissues of CCl_4 treated rats was showing significant necrosis in the central vein as well as liver parenchyma cells also shows higher necrosis among fatty acid vacuoles in sinusoids (H×E 10x).

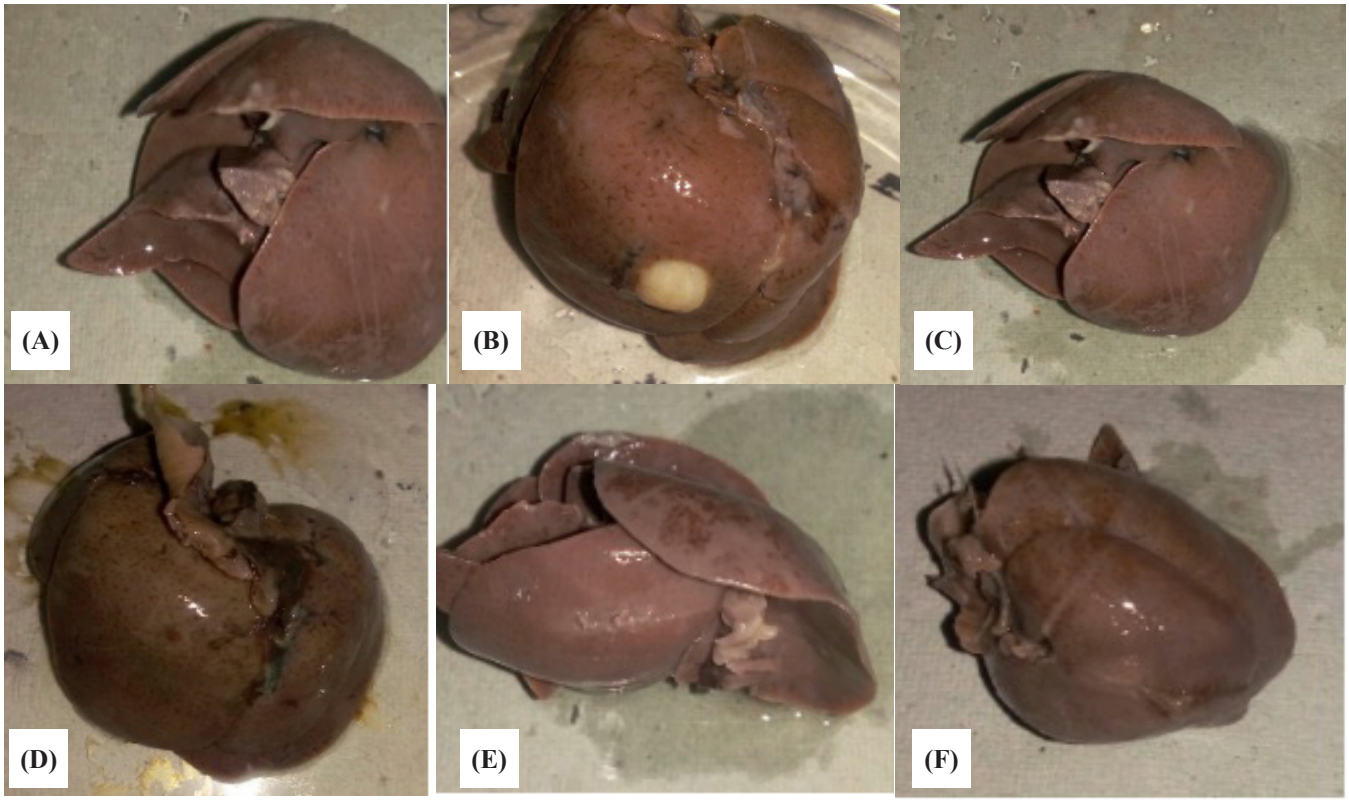

Figure 7: Visual appearance of necrosis in rat's liver. (A) vehicle-treated rats (B) rats exposed to CCl_4 (C) silymarin treated rats (D) HEEAT 100mg/kg treated rats (E) HEEAT 200mg/kg treated rats (F) HEEAT 300mg/kg treated rats.

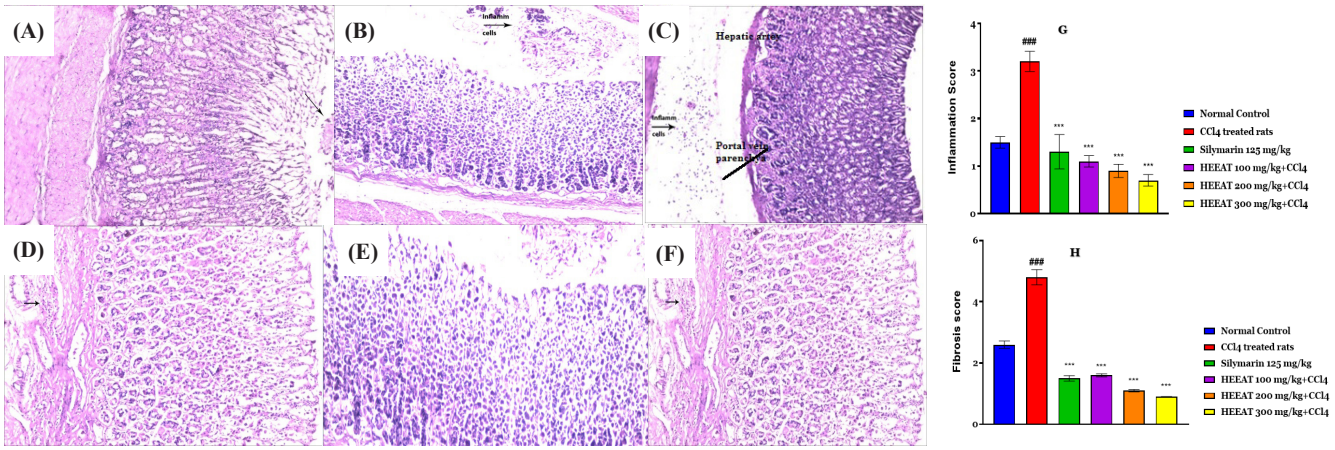

Figure 8: Histopathological changes of hepatic tissue: **(A-F)** Interference diagram of the hepatocytes. **G)** Inflammation score **H)** Fibrosis score. Values were expressed as mean ± S.E.M. (n=6-per group). One-way ANOVA followed by Tukey's post-hoc analysis was used. $^{\#\#}p < 0.001$ represents as compared to the control group. $^{**}p < 0.01$; represents HEEAT (100, 200 and 300 mg/kg) as compared to the CCl_4 treated rats.

Slide (D) Fraction of the liver tissue of rats treated with HEEAT at a dose of 100 mg/kg, shows no abnormalities in the arrangement of hepatocytes parenchymal cells around the portal vein and hepatic artery but little necrosis found in space of disse (H×E 10x).

Slide (E) Fraction of the hepatic tissue of animals treated with HEEAT at dose 200 mg/kg shows the normal arrangement of the hepatocytes, and the absence of necrosis in portal veins and bile duct but some fatty vacuoles necrosis found (H×E 10x).

Slide (F) Segment of the liver tissue of animals treated with HEEAT at dose 300 mg/kg displays the normal arrangement of the hepatocytes parenchymal cells and the absence of necrosis in portal veins, biliary canaliculi as well as the absence of fatty vacuoles necrosis (H×E 10x). All slides are depicted in Figure 8.

DISCUSSION

This study explores the mechanisms behind the CCl_4- induced liver damage and to investigates the hepatoprotective effect and elucidated the mechanism of HEEAT to prevent liver injury and also deamination the therapeutic effect of HEEAT. *HEEAT*DC, a hepatoprotective agent reported in Ayurveda. Based on our

finding of the biochemical estimation suggested that there was an elevation of the serum enzyme, such as ALT, AST, ALP, TB, DB, triglycerides, and cholesterol serves as a marker of cell leakage and loss of functional integrity of the liver membrane. Co-administration of HEEAT exerted its protective function by decreasing the serum levels of ALT and AST towards their respective normal value that is an indication of stabilization of the plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. On the other hand, ALP, TB, DB, Triglycerides, and cholesterol act as a marker of pathological alteration in the biliary flow. Elevation of serum ALP is in line with high levels of serum bilirubin by the induction of CCl4.

Effective control of ALP and bilirubin levels in the HEEAT treatment group points towards an early improvement in the secretary mechanism of hepatocytes. Moreover, histopathological tests have further confirmed these biochemical results. CCl_4 produces experimental damage that histologically resembles viral hepatitis.[25] Toxicity begins with the transitional change in the endoplasmic reticulum, along with the loss of metabolic enzymes located in the intracellular structures.[26]

CCl_4 has contributed to a substantial increase in liver weight as the secretion of hepatic triglycerides is blocked to plasma.[27] The decreased uric acid level is probably due to the increased use of uric acid concerning rising free radicals' production. The toxic metabolite CCl_3 radical is also formed as well as further converted into a trichloromethyl peroxy radical with help of cytochrome P450 2E1 enzyme and it causes peroxidative cell membrane degradation leading to necrosis of the hepatocytes due to the binding nature of radical with macromolecules.[28] A rise in MDA levels, as demonstrated in our research, indicates that enhanced lipid peroxidation contributes to tissue damage and failure of antioxidant defense mechanisms to prevent the development of excessive free radicals.[29] HEEAT treatment has dramatically reversed these changes. Moreover, the administration of HEEAT produced a significant increase in activities of enzymatic antioxidants, SOD and CAT as well as a non-enzymatic biological antioxidant, GSH which is present in the liver. This shows that HEEAT can scavenge reactive free radicals, which can minimize oxidative damage to the hepatic tissue and enhance the activity of hepatic antioxidant enzymes. Desire and lead compounds derived from medicinal plants display the vast potential as pharmaceuticals due to a wide range in their major structural attributes and activity,[30] specifically antioxidant, hepatoprotective, and anticancer drugs.[31] The previous investigation along with a literature survey of the Thalictrum genus indicated, it has many secondary metabolites present such as isoquinoline alkaloids and their dimers, particularly showing their activity against yellow fever virus in hepatitis, anti-influenza virus, another potential effect of this genus such as preventing platelet aggregation.[16] Pro-inflammatory cytokine levels are increased with liver injury or pathological conditions. Activation of inflammatory signaling in rats with CCl_4 administration contributes to tissue damage. Recently a report suggested that the CCl_4 treated rats showed persistent alterations of cytokine levels and altered microglia morphology providing evidence of CCl_4-induced inflammation.[32] Other reports had demonstrated that the CCl_4-associated liver damage is linked with increased Il-1β and TNF-α levels in the liver of rats.[33] The current study is in line with previous findings, as we had observed the significantly increased IL-1β and TNF-α levels in the liver of chronic CCl_4 administered rats. Moreover, this elevation in levels of IL-1β and TNF-α in the liver of CCl_4-exposed rats was significantly reduced with the chronic HEEAT treatment dose-dependently. Activation of NF-kB unit stimulates the enzymes that are responsible for ROS production such as NADPH oxidase, NOS, and COX2. These ROS-producing enzymes are also up-regulated in chronic CCl_4 exposure thus there is a possible involvement of NF-kB activation leading to these oxidative events.[34] Moreover, our results from the present study showed that the treatment with HEEAT significantly suppressed the levels of NFκβ and caspase-3 in the rat's liver after CCl_4 was administrated. This plant is highly implicated for treat jaundice and hepatitis as per traditional medicine of the system of India. Hence, we are taking this plant extract for hepatoprotective.[35] Due to limited research available on bioactive constituents of *AT*, only a few active constituents were isolated and reported such as alkaloids including isoquinolines, bisbenzylisoquinoline; protoberberine-derived, aporphine-benzylisoquinoline, aporphine, and quaternary alkaloids. Finally, this study strongly promotes the use of HEEAT as the source of major antioxidants from nature and the same may be brought into use as part of food supplement for the benefit of the health of individuals, as these constituents from nature possess excellent hepatoprotective activity.

CONCLUSION

In a nutshell, our study demonstrated the HEEAT treatment prevents the development of chronic CCl_4-associated liver injury by suppressing the oxidative stress-dependent facilitation of cell death signaling in the liver. Similar studies are required further so more constituents from *A. triplinervis* can be further explored for their therapeutic potential. Further, the data obtained from the current study and the activities reported would be more useful and pioneer for attracting the major attention and focus of researchers for future studies.

ABBREVIATIONS

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCl_4, carbon tetrachloride. °C, degree Celsius, b.w, body weight CAT, Catalase, CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals, GSH: Glutathione, LPO: Lipid peroxidation, MDA, malondialdehyd, ROS, Reactive oxygen species, SGOT, serum glutamate oxaloacetate transaminase, SGPT, Serum glutamate pyruvate transaminase, SOD, Superoxide dismutase, TBARS, thiobarbituric acid reacting substances, HEEAT, hydroethanolic extract of *A. triplinervis.*

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REFERENCES

1. Asadi-Samani M, Kafash-Farkhad N, Azimi N, Fasihi A, Alinia-Ahandani E, Rafieian-Kopaei M. Medicinal plants with hepatoprotective activity in Iranian folk medicine. Asian Pacific Journal of Tropical Biomedicine. 2015 Feb 1;5(2):146-157. https://doi.org/10.1016/S2221-1691(15)30159-3.
2. Delgado-Montemayor C, Cordero-Pérez P, Salazar-Aranda R, Waksman-Minsky N. Models of hepatoprotective activity assessment. Medicina universitaria. 2015 Oct 1;17(69):222-228. https://doi.org/10.1016/j.rmu.2015.10.002.
3. Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. Digestive and liver disease. 2007 Apr 1;39(4):293-304. https://doi.org/10.1016/j.dld.2006.11.004.
4. Tatiya AU, Surana SJ, Sutar MP, Gamit NH. Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats. Pharmacognosy Research. 2012 Jan;4(1):50-56. https://doi.org/10.4103/0974-8490.91040.
5. Valenti G, Vitale SG, Tropea A, Biondi A, Laganà AS. Tumor markers of uterine cervical cancer: a new scenario to guide surgical practice?. Updates in surgery. 2017 Dec;69(4):441-449. https://doi.org/10.1007/s13304-017-0491-3.
6. Parashar S, Uplanchiwar V, Gautam RK, Goyal S. *In vitro* antioxidant and in vivo hepatoprotective activity of ethanolic extract of Ziziphus rugosa Lam. leaves, Indian Drugs. 2019;56: 69-75. http://www.indiandrugsonline.org/issuesarticle-details?id=OTU0 (accessed February 7, 2020).
7. Seki S, Kitada T, Sakaguchi H, Nakatani K, Wakasa K. Pathological significance of oxidative cellular damage in human alcoholic liver disease. Histopathology. 2003 Apr;42(4):365-371. https://doi.org/10.1046/j.1365-2559.2003.01599.x.
8. Ranawat L, Bhatt J, Patel J. Hepatoprotective activity of ethanolic extracts of bark of Zanthoxylum armatum DC in CCl4 induced hepatic damage in rats. Journal of ethnopharmacology. 2010 Feb 17;127(3):777-780. https://doi.org/10.1016/j.jep.2009.10.019.
9. Wahid A, Hamed AN, Eltahir HM, Abouzied MM. Hepatoprotective activity of ethanolic extract of Salix subserrata against CCl4-induced chronic hepatotoxicity in rats. BMC complementary and alternative medicine. 2016 Dec;16(1):263. https://doi.org/10.1186/s12906-016-1238-2.
10. Ali S, Sai C, Krishna GV, AshaJyothi U, Sowmya S. Hepatoprotective Activity of Hydroalcholic Extract of Leaves of Urena Lobata Plant on Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats, Int. J. Pharma Res. Heal. Sci. 2018;6:2792–95. https://doi.org/10.21276/ijprhs.2018.05.08.
11. Singh A, Bisht M. Evaluation of in-vitro antioxidant potential and in-vivo hepatoprotective activity of root extract of Quercus oblongata D. DON. Journal of Drug Delivery and Therapeutics. 2018 Oct 15;8(5-s):152-611. https://doi.org/10.22270/jddt.v8i5-s.1925.
12. Subedi CK, Gurung J, Ghimire SK, Chettri N, Pasakhala B, Bhandari P, Chaudhary RP. Variation in structure and composition of two pine forests in Kailash Sacred Landscape, Nepal. Banko Janakari. 2018 May 26;28(1):26-36. https://doi.org/10.3126/banko.v28i1.21453.
13. Wahid A, Hamed AN, Eltahir HM, Abouzied MM. Hepatoprotective activity of ethanolic extract of Salix subserrata against CCl4-induced chronic hepatotoxicity in rats. BMC complementary and alternative medicine. 2016 Dec;16(1). https://doi.org/10.1186/s12906-016-1238-2.
14. Singh H, Srivastava A, Kumar A, Sundaresan V, Husain T. Thalictrum nainitalense (Ranunculaceae), a new species from the Uttarakhand Himalaya, India. Folia Geobotanica. 2018 Dec;53(4):449-455. https://doi.org/10.1007/s12224-018-9326-3.
15. Naman CB, Gupta G, Varikuti S, Chai H, Doskotch RW, Satoskar AR, Kinghorn AD. Northalrugosidine is a bisbenzyltetrahydroisoquinoline alkaloid from Thalictrum alpinum with in vivo antileishmanial activity. Journal of natural products. 2015 Mar 27;78(3):552-556. https://doi.org/10.1021/np501028u.
16. D. hong Li, J. Guo, W. Bin, N. Zhao, K. bo Wang, J. yong Li, Z. lin Li, H. ming Hua, Two new benzylisoquinoline alkaloids from *Anaphalis triplinervis* and their antioxidant and in vitro antiproliferative properties, Arch. Pharm. Res. 2016;39:871–877. https://doi.org/10.1007/s12272-016-0724-x.
17. Kumari P, Joshi GC, Tewari LM. Assessment of availability of traditionally used flora in curing Jaundice in central Himalayan region. Journal of Phytology. 2011 Sep 18;3(9):26–32. http://updatepublishing.com/journal/index.php/jp/article/view/2288 (accessed June 27, 2019).
18. Walter NS. Antimalarial Efficacy of *Anaphalis triplinervis* (Meadow rue) Against Chloroquine-Resistant P. falciparum, J. Trop. Dis. 2015;03. https://doi.org/10.4172/2329-891x.1000163.
19. D. hong Li, J. Guo, W. Bin, N. Zhao, K. bo Wang, J. yong Li, Z. lin Li, H. ming Hua, Two new benzylisoquinoline alkaloids from *Anaphalis triplinervis* and their antioxidant and in vitro antiproliferative properties, Arch. Pharm. Res. 2016;39:871–877. https://doi.org/10.1007/s12272-016-0724-x.
20. Botham PA. Acute systemic toxicity - Prospects for tiered testing strategies, Toxicol. Vitr. 2004;18:227–230. https://doi.org/10.1016/S0887-2333(03)00143-7.
21. Youssef FS, Ashour ML, Sobeh M, El-Beshbishy HA, Singab AN, Wink M. Eremophila maculata—Isolation of a rare naturally-occurring lignan glycoside and the hepatoprotective activity of the leaf extract. Phytomedicine. 2016 Nov 15;23(12):1484-1493. https://doi.org/10.1016/j.phymed.2016.08.006.
22. Sadashiva CT, Hussain HF, Nanjundaiah S. Evaluation of hepatoprotective, antioxidant and cytotoxic properties of aqueous extract of turmeric rhizome (Turmesac). Journal of Medicinal Plants Research. 2019 Oct 31;13(17):423-430. https://doi.org/10.5897/JMPR2019.6824.
23. Miftahudin M, Hasibuan R, Chikmawati T. Antioxidant activity of ethanolic extract of three Selaginella species from Java Island, Indonesia. Biodiversitas Journal of Biological Diversity. 2019 Nov 26;20(12):3715–3722. https://doi.org/10.13057/biodiv/d201234.
24. Sobeh M, Youssef FS, Esmat A, Petruk G, El-Khatib AH, Monti DM, Ashour ML, Wink M. High resolution UPLC-MS/MS profiling of polyphenolics in the methanol extract of Syzygium samarangense leaves and its hepatoprotective activity in rats with

CCl4-induced hepatic damage. Food and Chemical Toxicology. 2018 Mar 1;113:145-153. https://doi.org/10.1016/j.fct.2018.01.031.
25. Taamalli A, Feriani A, Lozano-Sanchez J, Ghazouani L, El Mufti A, Allagui MS, Segura-Carretero A, Mhamdi R, Arráez-Roman D. Potential hepatoprotective activity of super critical carbon dioxide olive leaf extracts against CCl4-induced liver damage. Foods. 2020 Jun;9(6):804. https://doi.org/10.3390/foods9060804.
26. Teng H, Chen M, Zou A, Jiang H, Han J, Sun L, Feng C, Liu J. Hepatoprotective effects of licochalcone B on carbon tetrachloride-induced liver toxicity in mice. Iranian journal of basic medical sciences. 2016 Aug;19(8):910-915. https://doi.org/10.22038/ijbms.2016.7474.
27. Toppo R, Roy BK, Gora RH, Baxla SL, Kumar P. Hepatoprotective activity of Moringa oleifera against cadmium toxicity in rats. Veterinary world. 2015 Apr;8(4):537-540. https://doi.org/10.14202/vetworld.2015.537-540.
28. Lorenzer C, Dirin M, Winkler AM, Baumann V, Winkler J. Going beyond the liver: progress and challenges of targeted delivery of siRNA therapeutics. Journal of Controlled Release. 2015 Apr 10;203:1-5. https://doi.org/10.1016/j.jconrel.2015.02.003.
29. Mahaldar K, Hossain A, Islam F, Islam S, Islam MA, Shahriar M, Rahman MM. Antioxidant and hepatoprotective activity of Piper retrofractum against Paracetamol-induced hepatotoxicity in Sprague-Dawley rat. Natural Product Research. 2020 Nov 16;34(22):3219-3225. https://doi.org/10.1080/14786419.2018.1550768.
30. Li RW, Lin GD, Myers SP, Leach DN. Anti-inflammatory activity of Chinese medicinal vine plants. Journal of ethnopharmacology. 2003 Mar 1;85(1):61-67. https://doi.org/10.1016/S0378-8741(02)00339-2.
31. Adebajo AC, Iwalewa EO, Obuotor EM, Ibikunle GF, Omisore NO, Adewunmi CO, Obaparusi OO, Klaes M, Adetogun GE, Schmidt TJ, Verspohl EJ. Pharmacological properties of the extract and some isolated compounds of Clausena lansium stem bark: anti-trichomonal, antidiabetic, anti-inflammatory, hepatoprotective and antioxidant effects. Journal of ethnopharmacology. 2009 Feb 25;122(1):10-19. https://doi.org/10.1016/j.jep.2008.11.015.
32. Gnanadesigan M, Ravikumar S, Anand M. Hepatoprotective activity of Ceriops decandra (Griff.) Ding Hou mangrove plant against CCl4 induced liver damage. Journal of Taibah University for Science. 2017 May 1;11(3):450-457. https://doi.org/10.1016/j.jtusci.2016.07.004.
33. Sobeh M, Youssef FS, Esmat A, Petruk G, El-Khatib AH, Monti DM, Ashour ML, Wink M. High resolution UPLC-MS/MS profiling of polyphenolics in the methanol extract of Syzygium samarangense leaves and its hepatoprotective activity in rats with CCl4-induced hepatic damage. Food and Chemical Toxicology. 2018 Mar 1;113:145-153. https://doi.org/10.1016/j.fct.2018.01.031.
34. M. Sobeh, F.S. Youssef, A. Esmat, G. Petruk, A.H. El-Khatib, D.M. Monti, M.L. Ashour, M. Wink, High resolution UPLC-MS/MS profiling of polyphenolics in the methanol extract of Syzygium samarangense leaves and its hepatoprotective activity in rats with CCl4-induced hepatic damage, Food Chem. Toxicol. 113 (2018) 145–153. https://doi.org/10.1016/j.fct.2018.01.031.
35. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. Journal of ethnobiology and ethnomedicine. 2006 Dec;2(1):1-8. https://doi.org/10.1186/1746-4269-2-14.