Hematological, antioxidant and protective performance of *Usnea longissima* on chemical induced hepatotoxicity in experimental animals

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**ABSTRACT**

**Objective:** To investigated the hematological, antioxidant and protective performance of *Usnea longissima* (U. longissima) on CCl4 induced hepatotoxicity in experimental animals.

**Methods:** Hepatotoxicity was induced by CCl4 (1 mL/kg body weight 1:1 CCl4 i.p.), ethanolic *U. longissima* extracts at doses (200 and 400 mg/kg body weight) were administered to and compared with Silymarin (25 mg/kg body weight) and hematological, antioxidant and enzymatic, non-enzymatic parameters were assayed through the liver functions test. All the observation was also supplemented with histopathological examination of liver sections.

**Results:** Phytochemical investigation showed that ethanolic extract contains poly phenolic compounds tannins, flavonoids, alkaloids and saponins and acute toxicity study shows that ethanolic extract was safe up to 2000 mg/kg body weight. The toxicant induced a rise in the plasma enzyme levels of ALT, AST, ALP and total bilirubin level. This increased level was significantly decreased by the extract at 400 mg/kg body weight than 200 mg/kg body weight. The animals were prevented (partly or fully) which was showed in the histopathological changes using ethanolic *U. longissima* extract.

**Conclusions:** The outcome of this study reveals that, there is a powerful antioxidant and hepatoprotective activity of *U. longissima*. It is believed that the present constituents are responsible for curting the hepatic disease and alternative components have the power to act as free radical scavenging properties.

1. Introduction

Lichen epiphytes are numerous and functionally necessary in forest ecosystems also as being a key cluster for looking environmental modification[1,2]. Lichens are sophisticated plants, living in dependent relationships with fungi and algae, and the pertinent partners are made public as mycobiont and phycobiont, severally. The plant forms a plant structure or lichenized stroma which will contain characteristic secondary metabolites in all lichens[3]. They present on barks, stems, leaves and within the soil, however typically grow in habitats less favorable for higher plants[4]. *Usnea longissima* (U. longissima) is a lichen species, widely utilized in the treatment of the legs and joints injuries, bone fractures, and skin eruptions in the folk’s medication of assorted countries and its metabolites have manifold biological activities including antiviral, antibiotic, antitumor, allergenic, plant growth inhibitors, antioxidant activities, anti-microbial, anti-herbivore, ecological roles and protein inhibitors[5,6]. The *Usnea* (Usneaceae) is massive, hanging hair lichen (fungus, algae) that grows throughout the northern temperate zones, particularly the sub-Arctic and Europe, Asia and North America[7].

Lichens contain giant amounts of usnic acid, lichesterinic acid, and alternative substances that possess potent antiseptic and medicinal drug activities. Lichen polysaccharides have been receiving abundant attention for their biological activities, especially antitumor, immunostimulatory properties[8,9].

Susceptibility of the liver to injury by such agents is much higher than the other organs as a result of its central role in metabolism in addition as its ability to concentrate and biotransform xenobiotics[10]. The liver demonstrates a serious role in metabolism of xenobiotics by regulating the synthesis, secretion and metabolism...
of xenobiotics. Various physiochemical functions of the body including oxidation, reduction, hydroxylation, hydrolysis, conjugation, sulfation, acetylation, etc. are well balanced by the liver alone[11]. Liver disease is considered to be a serious health problem as liver is an important organ for the detoxification and deposition of endogenous substances. Administration of vaccines, corticosteroids and antiviral drugs has been found to be the best remedial choice for the treatment of liver diseases in conventional medicine. These treatments are not free from serious and adverse effects, particularly when given for a prolonged period[12].

Liver diseases representing a major drawback of worldwide proportions resolvent (CCl₄) may be a well-known poisonous substance that is widely accustomed induce acute-toxic liver injury in a huge vary of laboratory animals[13].

2. Materials and methods

2.1. Preparation of the lichen extract

The lichen samples were dried in the shade in an airy place and then stored in paperbags and kept at natural room temperature. Then the lichen material was milled by an electrical mill. The dried ground thallic of the investigated lichens U. longissima (50 g) was extracted using 60% ethanol in a Soxhlet extractor. Then extract was filtered and concentrated under reduced pressure in a rotary evaporator (Buchi R-200 USA) at 45 °C and then freeze-dried in lyophilizer (Labconco, USA) to obtain solid residue (ASE, yield 20.0% w/w). The extract was dissolved in 5% dimethyl sulphoxide (DMSO) for the experiments[14].

2.2. Acute oral toxicity

Swiss albino mice weighing 20–25 g were used for the acute toxicity study. They were randomly distributed into one control group and five treated groups, containing 5 animals per group and maintained on standard animal diet and provided water spontaneously. They were allowed to acclimate for 7 days to the laboratory conditions before the experiment. After fasting the animal’s over-night, the control group received 0.1 mL of distilled water orally. Solution of the extract was ready and every treated group received the extract dose (2000 mg/kg p.o.). The animals were ascertainment continuously for the first 4 h and for each hour for the next 24 h, and then 6 h for 48 h when administering the extract to watch any changes normally behavioral (restlessness, dullness, and agitation) or different physiological activities and any harmful symptoms.

2.3. DPPH free radical scavenging activity

Antioxidant activity of the plant extract and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical[15]. The diluted working solutions of the test extract were prepared in ethanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 10 μg/mL. About 0.005% DPPH solution in ethanol was prepared. Then 2 mL of this solution was mixed with 2 mL of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a Shimadzu spectrophotometer against ethanol. The control was used is 2 mL of ethanol with 2 mL of DPPH solution (0.005%). The optical density was recorded and percent of inhibition was calculated using the formula given below[16]:

\[
\% \text{Inhibition of DPPH activity} = \left( \frac{A - B}{A} \right) \times 100
\]

where, A is optical density of the control and B is optical density of the sample.

2.4. Experimental animals

Sprague-Dawley rats (160–200 g) of either sex were procured and were kept beneath controlled conditions of temperature (27 ± 20) °C and ratio 44%-56%, light/dark cycles of 12 h severally for one week before and through the experiments. Animals were provided with a standard rodent pellet diet (Amrut, India) and the food was withdrawn 18–24 h before the experiment though water was allowed ad libitum. The protocol for this study has been approved by the Institutional Animal Ethics Committee as per the guidance of committee for the purpose of control and supervision of Experiments on Animals CPCSEA, New Delhi, with number (IAEC CPCSEA/07/2014).

2.5. Preliminary phytochemical analysis

The ethanolic extract of U. longissima was screened for the presence of various phytochemical constituents such as alkaloids (wager’s reagent), flavonoids (Shinoda test), glycoside (anthraquinone test), saponins (hemolysis test), tannins (ferric chloride test) and steroids (acetoc anhydride test) as described by Anyasor et al.[17] and Sani et al.[18] according to a previously described method for extraction.

2.6. Carbon tetrachloride-induced liver toxicity

Animals were divided into five groups (n = 6). Group I served as a vehicle control, which received liquid paraffin. Groups II-V were treated with CCl₄ in liquid paraffin (1:2) at the dose of 1 mL/kg body weight intraperitoneally once in every 72 h for 16 days[19]. Groups III and IV were treated with U. longissima extract at the doses of 200 and 400 mg/kg body weight, respectively, and Group V was administered with silymarin at a dose of 25 mg/kg body weight orally. U. longissima extract treatment was started 10 days prior to CCl₄ administration and continued until the end of the experiment.

Group I: CCl₄ control received liquid paraffin;
Group II: CCl₄ as natural recovery group (1 mL/kg, i.p.);
Group III: CCl₄ + treated with a daily dose of Silymarin (25 mg/kg body weight);
Group IV: CCl₄+ treated with a daily dose of U. longissima extract (200 mg/kg, p.o.);
Group V: CCl₄ + treated with a daily dose of U. longissima extract (400mg/kg, p.o.).

2.7. Sample preparation

The end of the experiment blood samples were collected from the animal, by puncturing the vein. Plasma is ready by spinning a tube of contemporary blood containing associate degree anti-coagulant during a centrifuge till the blood cells fall to bottom of the tube. The separated serum was holding on at 80 °C for more determination of the biochemical parameter for GSH, GPX, SOD and CAT.
2.8. Evaluation of liver damage

Livers of all rats were excised at once assortment of the blood and liver tissues were divided into two portions. The primary portion was used for histopathological examination and therefore second was homogenized in normal physiological isosmotic solution (0.9% NaCl) (1:9 w/v). The homogenate was centrifuged at 4 °C for 5 min at 3000 r/min and the supernatant used for estimation of viscus oxidative stress markers. The opposite liver tissue specimens were used for histopathological examination.

2.9. Hematological analysis

Red blood cell (RBC) count, hemoglobin (Hb), white blood cell (WBC) count, platelet (PLT) and pack cell volume (PCV) were determined according to Nawwar et al.[20]. Whereas, alkaline phosphatase (ALP) was determined by adopting the procedure of Rosalki et al.[21].

2.10. Quantitative analysis of enzyme activities

Aspartate and alanine aminotransferase (AST and ALT) activities were measured according to Nawwar et al.[20]. Whereas, alkaline phosphatase (ALP) was determined by adopting the procedure of Rosalki et al.[21].

2.11. Vitamin E in liver

The level of vitamin E was determined by the method of reduction in Fe$^{3+}$ to Fe$^{2+}$ by the tocopherols with the formation of a red complex of the Fe$^{2+}$ with α, n-dipirydyl. The transmissions were measured by calorimeter and its expressed in terms of μg/mg protein.

2.12. Lipid peroxidation (LPO)

A total of 900 mg of liver tissue was collected from each experimental rat, washed with normal saline and soaked in filter paper. Tissues were then homogenized in 3.0 mL 0.15 mol/L Tris HCl buffer (pH 7.4) and centrifuged at 3000 r/min at 4 °C for 1 h[22]. The supernatant was collected and estimated for lipid peroxidation. The level of lipid peroxides was expressed.

2.13. Glutathione content (GSH)

A 30% w/v liver homogenate was prepared in 0.15 mol/L Tris-HCl buffer (pH 7.4) and trichloroacetic acid was added to precipitate proteins. Samples were centrifuged at 15000 rpm at 4 °C for 1 h. The supernatant was analyzed for content of reduced glutathione and expressed as μg/g of liver tissue[23].

2.14. Catalase activity (CAT)

A total of 900 mg liver tissue was homogenized in 3.0 mL M/150 phosphate buffer in ice and centrifuged at 30000 r/min for 1 h at 4 °C. The supernatant was taken to determine catalase activity[23].

2.15. Determination of malondialdehyde (MDA)

The liver tissues were removed and each tissue was homogenized in 0.15 mol/L KCl (at 4 °C; Potter-Elvehjem type C homogenizer) to give a 10% w/v homogenate. Aliquots of homogenate (1 mL) were incubated at 37 °C for 3 h in a metabolic shaker. Then 1 mL of 10% aqueous trichloroacetic acid was added and mixed. The mixture was then centrifuged at 800 g for 10 min. One milliliters of the supernatant was removed and mixed with 1 mL of 0.67% thioibarbituric acid and placed in a boiling water bath for 10 min. The mixture was cooled and diluted with 1 mL distilled water. The absorbance of the solution was then read at 535 nm. The content of malondialdehyde (nmol/g wet tissue) was then calculated, by reference to a standard curve of malondialdehyde solution[24].

2.16. Determination of superoxide dismutase activity (SOD)

The reaction mixture (3 mL) contained 2.95 mL 0.05 mol/L sodium carbonate buffer pH 10.2, 0.02 mL of serum and 0.03 mL of epinephrine in 0.005 mol/L hydrochloric acid. Serum superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described enzyme activity was calculated by measuring the change in absorbance[25].

2.17. Histopathological studies

Livers were isolated, processed, and embedded in paraflin wax. The sections stained in hematoxylin and eosin and permanently were mounted for viewing and reporting [26].

2.18. Statistical analysis

Results were expressed as mean ± SEM. The data were subjected to One-way analysis of variance (ANOVA) test and the differences between samples were determined by Dunnett’s multiple comparison tests, using the graph pad prism statistical software (Graph Pad Software Inc., CA, and USA). Results were considered significant at P < 0.05.

3. Results

3.1. Effect of U. longissima extract on weekly feed intake, water intake and body weight in Swiss albino mice

In this study, the feed and water intake in case of acute toxicity study of U. longissima extract at a dose of 2000 mg/kg on observation for 14 days. After completion of study there was no changes found on feed intake, water intake and body weight (Figure 1).

3.2. Acute (oral) toxicity study of ethanolic U. longissima extract

The U. longissima extract did not produce any mortality when administered orally at various doses of 250 to 2000 mg/kg body weight (it’s a maximum does) but 2 h post treatment reduced locomotion and dullness were observed in some animals treated with higher doses of 2000 mg/kg (Table 1).

3.3. Determination of IC50 value for U. longissima extract and ascorbic acid (standard)

The effect of antioxidants on DPPH radical scavenging was thought
to be due to their hydrogen donating ability. The study conclusively depicted that IC\textsubscript{50} value of DPPH assay for ascorbic acid and \textit{U. longissima} extract was respectively 340.33 μg/mL and (2.63 ± 0.06) μg/mL, which was positive stand for antioxidant activity of plant extract (Figure 2).

Table 1

| Group | Dose (mg/kg) | D/T | Period of signs observation (h) | Signs of toxicity observed |
|-------|--------------|-----|---------------------------------|----------------------------|
| A     | 0            | -   | 24                              | No toxicity observed      |
| B     | 250          | 0/5 | 24                              | No toxicity observed      |
| C     | 500          | 0/5 | 24                              | No toxicity observed      |
| D     | 1000         | 0/5 | 24                              | No toxicity observed      |
| E     | 2000         | 0/5 | 24                              | Slight dullness was observed in the first 2 h |

Total number of mice (n = 5).

3.4. Effect of \textit{U. longissima} extract on hematological parameters

No significant difference was observed in hemoglobin, red blood cell, white blood cell, erythrocyte sedimentation rate and PVC in the \textit{U. longissima} extract treated groups compared with control (Table 2).

3.5. Effect of ethanolic \textit{U. longissima} extract on liver weight in CCl\textsubscript{4} induced hepatotoxicity

The study showed that the liver weights were significantly decreased in CCl\textsubscript{4} group. However ULE showed a dose dependent protection in liver weight. The result of the high dose (400 mg/kg) was compared with standard drug Silymarin (25 mg/kg) (Figure 3).

3.6. Effects of \textit{U. longissima} extract on SGPT, SGOT, ALP, and TBL in CCl\textsubscript{4} induced hepatotoxicity

CCl\textsubscript{4} caused a significant elevation of liver serum markers. In the CCl\textsubscript{4} treated group, the levels of SGPT, SGOT, ALP, and total bilirubin (TBL) were significantly raised. In contrast, the groups treated with ULE in doses of (200 and 400 mg/kg body weight) once daily for 16 days prohibited the hepatotoxicity in a dose dependent manner (Figure 4).

3.7. Effect of \textit{U. longissima} extract on GGTP, TP, TG, and TC in CCl\textsubscript{4} induced hepatotoxicity

The effects of \textit{U. longissima} extract at two doses 200 mg/kg, 400 mg/kg on liver biochemical assessment in CCl\textsubscript{4} induced hepatic injury are represented in Figure 5. CCl\textsubscript{4} induced toxicity caused fall in the activities of GGTP, TP (total protein), TC (total cholesterol),
Figure 4. Effects of ethanolic U. longissima extract on biochemical markers in CCl₄ induced hepatotoxicity. 1: Normal control; 2: CCl₄ (1 mL/kg); 3: Slymarin (25 mg/kg) + CCl₄; 4: ULE (200 mg/kg) + CCl₄; 5: ULE (400 mg/kg) + CCl₄. * Significant at P < 0.05, **: Highly significant at P < 0.01 when compared with control.

Figure 5. Effects of ethanolic U. longissima extract on biochemical markers in CCl₄ induced hepatotoxicity. 1: Normal control; 2: CCl₄ (1 mL/kg); 3: Slymarin (25 mg/kg) + CCl₄; 4: ULE (200 mg/kg) + CCl₄; 5: ULE (400 mg/kg) + CCl₄. * Significant at P < 0.05, **: Highly significant at P < 0.01 when compared with control.
TG (Tri-glyceride) respectively. Nevertheless, *U. longissima* extract administration has substantially improved their activities.

3.8. Effect of *U. longissima* extract on LPO, MDA, GSH, CAT, and SOD in CCl₄ induced hepatotoxicity

The administration of CCl₄ led to elevation in the levels of LPO and drop in enzymatic scavenger viz. CAT, SOD levels in the liver homogenate. Treatment of rats with *U. longissima* extract in doses of (200 and 400 mg/kg) noticeably prohibited the CCl₄ induced alterations of various parameters LPO, CAT, SOD, MDA and GSH. The degree of protection by *U. longissima* extract (200,400 mg/kg) was ascertained statically similar to the quality drug (Figure 6).

![Graph A: LPO (mmol/mg) vs CCl₄ doses](image1)

![Graph B: MDA (mmol/ml) vs CCl₄ doses](image2)

![Graph C: GSH (mmol/mg) vs CCl₄ doses](image3)

![Graph D: CAT (U/mg) vs CCl₄ doses](image4)

![Graph E: SOD (U/mg) vs CCl₄ doses](image5)

**Figure 6.** Effect of ethanolic *U. longissima* extract on liver homogenates parameters in CCl₄ induced hepatotoxicity.

1: Normal control; 2: CCl₄(1 mL/kg); 3: Sylmarin (25 mg/kg) + CCl₄; 4: ULE (200 mg/kg) + CCl₄; 5: ULE (400 mg/kg) + CCl₄. *: Significant at P < 0.05; **: Highly significant at P < 0.01 when compared with control.

### Table 2

| Treatment | Dose (mg/kg) | Hb (g/dL) | RBC 10⁶/mil/mm³ | WBC 10³/mil/mm³ | ESR mm/h | Platelets in lakhs | Clotting time (Sec) | PCV (%) |
|-----------|--------------|-----------|-----------------|-----------------|----------|-------------------|---------------------|---------|
| Control   | -            | 14.18 ± 1.46 | 5.43 ± 1.23     | 4.63 ± 0.34     | 4.15 ± 1.16 | 350.00 ± 15.13     | 150.40 ± 2.49       | 60.00 ± 2.33 |
| ULE       | 100 mg/kg    | 11.12 ± 1.25 | 5.10 ± 1.70     | 5.10 ± 0.97     | 4.22 ± 1.28 | 400.00 ± 16.14     | 113.30 ± 2.12       | 50.00 ± 3.26 |
|           | 400 mg/kg    | 12.52 ± 1.20 | 4.96 ± 1.38     | 8.46 ± 0.26     | 4.00 ± 1.30 | 425.00 ± 17.18     | 110.30 ± 0.38       | 57.00 ± 2.16 |
|           | 800 mg/kg    | 13.30 ± 0.14 | 5.69 ± 1.65     | 7.93 ± 0.14     | 4.52 ± 2.18 | 459.00 ± 10.15     | 120.10 ± 1.20       | 55.00 ± 1.27 |

All values are expressed as mean ± SEM (n = 6). One-way ANOVA was used. *: Significant at P < 0.05; **: Highly significant at P < 0.01 when compared with control.
3.9. Histopathological studies

The histopathological evaluations are direct means of assessing the defensive effect of the drug. The groups received CCl₄ alone; the spoils of cells around the central vein were well evident. While, the amount of damage was found minor in the studies included treatment of *U. longissima* extract. The results of the histopathological study supported and well associated with data obtained from an assessment of the enzymatic parameters (Figure 7).

![Histopathological studies of representative photomicrographs of liver sections. A: Liver segment of normal control rats showing normal architecture; B: Liver segment of CCl₄ 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 CCl₄ and 25 mg/kg of silymarin showing signs of inflammatory cascade around central vein indicating a light degree of fatty amendment, and necrosis and focal necrosis (dilatation); D: Liver segment of rats treated CCl₄ and 200 mg/kg of ULE showing less inflammatory cells around central vein, absence of necrosis; E: Liver segment of rats treated CCl₄ and 400 mg/kg of ULE showing: minimal inflammatory cellular infiltration, large septa of connective tissue flowing together and penetrating into the parenchyma. There is regeneration of hepatocytes evident.](image)

**Figure 7.** Histopathological studies of representative photomicrographs of liver sections.

4. Discussion

World Health Organization survey indicates that a total of about 70%–80% of the world population relies on non commercial medicine mainly the herbal sources, in the primary health care units[27].

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids and alternative aromatic compounds are secondary metabolites that are created in plants as a response to stress or as a part of their defense reaction against prediction by several microorganisms, insects and alternative herbivores[28]. Plants phenols and flavonoids are necessary teams of natural antioxidants and have a larger tendency to cure several sicknesses. The principle of the DPPH methodology based on the production of free radical[29] and also the impact of antioxidants on DPPH radical scavenging activity is due to their hydrogen donating ability. The results indicate that total phenolic and flavonoids content in the ethanolic *U. longissima* extract were liable for its antioxidant activities.

Oxidative stress resulting from an imbalance in the antioxidant defense system and production of reactive oxygen species (ROS) are including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, arising from aerobic metabolism in free radicals interacting with endogenous macromolecules[30].

The toxic action of CCl₄ is closely joined to its metabolic activation to ephemeral reactive intermediates. Reductive dehalogenation of CCl₄ is catalyzed by cytochrome P450, the terminal enzyme of the hepatic mixed perform oxidase system. The existence of free radicals during CCl₄ metabolism has been tried by spin trapping experiments[31,32]. Tetra-chloromethane biotransformation leads to the production of extremely reactive free radical metabolites, that causes lipid peroxidation and injury to hepatocyte membranes, resulting in centrilobular necrosis of hepatocytes accompanied by lipid degeneration. Neutrophils gather round the broken hepatocytes, and they induce inflammatory reaction through pro-inflammatory cytokines (TNF-α, IL-1 and IL-6)[33].

In the acute toxicity study of the extract, no changes in the behavior and in the sensory nervous system responses were observed. Also no adverse gastrointestinal responses were observed in experimental animals. The extract can be classified as non toxic, since the oral minimum lethal dose (LD₅₀) of the ethanolic *U. longissima* extract was estimated as > 2 000 mg/kg body weight, implying the relative safety of the plant extract when administered acutely. There was a significant increase (*P* < 0.05) in the body weights of the animals treated with the higher doses compared with the control, there was neither decrease nor a significant increase in the body weights of animals (Table 1). The results further revealed that the extract caused an increase in the liver weight and body weight of rats in the test groups, compared to the rats in the control group, though the increase was non-significant. Since no death was recorded in the acute toxicity study, and no changes in animal behavior.

The various hematologic parameters accustomed assess the toxic potential of plant extract in men and animals[34]. Assessment of hematologic parameters will be used to verify the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Such toxicity testing is relevant to risk analysis as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies[35]. It can also be used to justify blood relating functions of plant extract[36]. The present study has revealed that there was no vital difference in Hb, red blood cell, white blood cell, erythrocyte sedimentation rate and PVC in the *U. longissima* treated groups compared with control.
In the present study, there was a significant ($P < 0.001$) increase in the level of MDA and a significant ($P < 0.001$) decrease in SOD, GSH, GST and vitamin C levels in the test groups (200–400 mg/kg) when compared to the control, which are signs of oxidative stress due to excessive formation of free radicals in the experimental animals[37,38]. The liver is the main organ with the highest content of GSH, which provides to respiratory organ and excretory organ by distant GSH transport system[39]. Malondialdehyde (MDA), a breakdown product, is a measure of lipid hydroperoxides, causing lipid peroxidation. MDA assay has been found to be a reliable predictor of oxidative damage and considered to be the most reliable marker of lipid peroxidation[37].

The bread (U. longissima) may be immune-stimulating because of high polysaccharide content. Half of the carbohydrates were found in Iceland longissima where digested. Main polysaccharide is 53%–64% assimilated[40].

ALT and AST are cytoplasmic amino transferases that release extra cellularly and go into the circulation upon hepatocytes damage. They are commonly used biomarkers for measuring hepatic injury in both experimental and clinical studies[41].

Liver injury elicited by CCl$_4$ was confirmed by histopathologic analysis that disclosed cellular correlates of damage 24 h when CCl$_4$ administration. This hepatocellular injury led to an increase in liver weight. Rupture of cellular membranes resulted in intracellular content outflow with concomitant elevation of blood viscous body fluid markers. Silymarin and also the extract prevented the increase of liver weight in rats. U. longissima extract administration induced microscopic anatomy and biochemical changes reflecting liver recovery toward normality. Analogous results have been according beneath similar experimental conditions[42].

In conclusion, as method as a result of the current information worries, in this study is that the investigation to indicate that the presence of chemical constituents from U. longissima, and potent hematological and antioxidant activity against CCl$_4$ iatrogenic acute liver damage in rats. This was disclosed by changes in level of LPO and GSH concentration in liver, additionally to the elevation of SOD, CAT and GPx activity. Whereas, U. longissima extract is ready to ameliorate acute liver damage to a high degree, as proved by the improvement in histopathological changes. The ULE evidenced its activity either through stabilization of cellular membrane or through anti-peroxidase activity. The outcome of this study reveals that, there is a powerful antioxidant and hepatoprotective activity of ULE.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**References**

[1] Lundström J, Jonsson F, Perhans K, Gustafsson L. Lichen species richness on retained aspens increases with time since clear-cutting. *Forest Ecol Manag* 2013; 293: 49-56.

[2] Scheidegger C, Groner U, Keller C, Stofer S. Biodiversity assessment tools–lichens. In: Nimis PL, Scheidegger C, Wolseley PA, editors. Monitoring with lichens monitoring lichens. Dordrecht: Kluwer Academic Publishers; 2002. p. 359-65.

[3] Insarov D, Blagoveshchenskaya EY. Lichen symbiosis: search and recognition of partners. *Biol Bull* 2016; 43: 408-18.

[4] Larsson P, Solhaug KA, Gauslaa Y. Seasonal partitioning of growth into biomass and area expansion in a cephalolichen and a cyanolichen of the old forest genus *Lobaria*. *New Phytol* 2012; 194(4): 991-1000.

[5] Karagoza ID, Ozaslana M, Kilica IH, Guler I. Hepatoprotective effect of diffractaic acid on carbon tetrachloride-induced liver damage in rats. *Biotechnol Biotechnol Equip* 2015; 29(5): 1011-6.

[6] Nagai T, Suzuki N, Kai N, Tanoue Y. Functional properties of autolysate and enzymatic hydrolysates from yam tsukuneime (*Dioscorea opposita* Thunb.) tuber mucilage tororo: antioxidative activity and antihypertensive activity. *J Food Sci Technol* 2014; 51(12): 3838-45.

[7] Marcano V, Alcocer VR, Mendez AM. Occurrence of usnic acid in *Usnea* leaves Nylander (*lichenized ascomycetes*) from the Venezuelan Andes. *J Ethnopharmacol* 1999; 66: 343-6.

[8] Huyhn BLC, Hoang Le D, Takenaka Y, Tanahashi T. New phenolic compounds from the lichen *Parmotrema praesorediosum* (Nyl.) Hale (Parmeliaceae). *Magn Reson Chem* 2016; 54: 81-7.

[9] Yang Yi, Thanh Thi N, Min-Hye J, Florin C. Inhibitory activity of (+)-usnic acid against non-small cell lung cancer cell motility. *PLoS One* 2016; 10: 1771.

[10] Kumar V, Kalita J, Misra UK, Bora HK. A study of dose response and organ susceptibility of copper toxicity in a rat model. *J Trace Elem Med Biol* 2015; 29: 269-74.

[11] Nalbantoglu ILK, Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20(27): 9026-37.

[12] Alqasoumi SI. ‘Okra’ *Hibiscus esculentus* L.: a study of its hepatoprotective activity. *Saudi Pharm J* 2012; 20: 135-41.

[13] Luo MS, Dong L, Li J, Wang Y, Shang B. Protective effects of pentoxifylline on acute liver injury induced by thioacetamide in rats.
Int J Clin Exp Pathol 2015; 8(8): 8990-6.

[14] Tohma HS, Gulcin I. Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (Glycyrrhiza glabra L.). Int J Food Prop 2010; 13: 657-71.

[15] Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. Antioxidant activity of some common plants. Turk J Biol 2008; 32: 51-5.

[16] Al-Nooman SA, Ela-Segaey O, Ab-Allah A. Experimental study of antioxidant and hepatoprotective effects of clove and cardamom in ethanol induced hepatotoxicity. Tanta Med Sci J 2007; 2(1): 27-36.

[17] Anyasor GN, Funmilayo O, Odutola O. Chemical constituents in n-butanol fractions of Costus afer Ker Gawl leaf and stem. J Intercult Ethnopharmacol 2014; 3: 2.

[18] Sani D, Sanni S, Ngulde SI. Phytochemical and antimicrobial screening of the stem aqueous extract of Anisopus manni. J Med Plants Res 2009; 3(3): 112-5.

[19] Mankani KL, Krishna V, Manjunatha BK, Vidya SM, Jagadeesh Singh SD, Manohara YN, et al. Evaluation of hepatoprotective activity of stem bark of Pterocarpus marsupium Roxb. Indian J Pharmacol 2005; 37(3): 165-8.

[20] Nawwar MA, Hussein SA, El-Mousallami AM. Phenolics from Caesalpinia ferrea (Fabaceae) in rodents. Pharmazie 2015; 70: 553-8.

[21] Rosalki SB, Foo AY, Burlina A. Multicenter evaluation of iso-ALP activity of stem bark of Costus afer Ker Gawl and members of the Lamiaceae. Clin Chim Acta 1993; 39: 648-52.

[22] Barros L, Cabrita L, Boas MV, Carvalho AM, Ferreira ICFR. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chem 2011; 127: 1600-8.

[23] Mahecha L, Nuernberg K, Nuernberg G, Martin J, Hubbermann KM, Knoller S, et al. Antioxidant enzyme activities and antioxidant capacity in longissimus muscle from bulls fed diets rich in polyunsaturated fatty acids. Food Chem 2011; 127: 379-86.

[24] Karuna R, Bharrathi VG, Reddy SS, Ramesh B, Saralakumari D. Protective effects of Phyllanthus amarus aqueous extract against renal oxidative stress in streptozotocin-induced diabetic rats. Indian J Pharmacol 2011; 43(4): 414-8.

[25] Kankofer M. Superoxide dismutase and glutathione peroxidase activities in bovine placenta spectrophotometric and electrophoretic analysis. Revue Méd Vét 2002; 153(2): 121-4.

[26] Yogesh CY. Hepatoprotective effect of Ficus religiosa latex on cisplatin induced liver injury in wistar rats. Revista Brasileira de Farmacognosia 2015; 25: 278-83.

[27] Attar AMA, Taisan WA. Preventive effects of black seed (Nigella sativa) extract on Sprague-Dawley rats exposed to diazinon. Aust J Basic Appl Sci 2010; 4(5): 957-68.

[28] Mohammed SG. Comparative study of in vitro antibacterial activity of miswak extracts and different toothpastes. Am J Agric Biol Sci 2013; 8(1): 82-8.

[29] Hossain MA, Kalbani MSAA, Farsi SAJA. Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of Datura metel L. Asian Pac J Trop Dis 2014; 4(5): 378-83.

[30] Kalegari M, Gemin CAB, Ujo-Silva GA. Chemical composition, antioxidant activity and hepatoprotective potential of Rourea induta Planch. (Connaraceae) against CCl4-induced liver injury in female rats. Nutrition 2014; 30: 713-4.

[31] Conner HD, Thurman RG, Galizi MD, Mason RP. The formation of a novel free radical metabolite from CCl4 in the perfused rat liver and in vivo. J Biol Chem 1986; 261: 4542-8.

[32] Noguchi T, Fong KL, Lai EK, Alexander SS, King MM, Olson L, et al. Specificity of a phenobarbital-induced cytochrome P450 for metabolism of carbon tetrachloride to the trichloromethyl radical. Biochem Pharmacol 1982; 31: 615-24.

[33] El Naggar el MB, Chalapová M, Pra anová G, Parák T, Švajdlenka E, Žemlička M, et al. Hepatoprotective and proapoptotic effect of Echallium elaterium on CCl4-induced hepatotoxicity in rats. Asian Pac J Trop Med 2015; 8(7): 526-31.

[34] Awodele O, Amagon KI, John AGBO, Prasad MNV. Toxicological evaluation of the aqueous stem bark extract of Bridelia ferruginea (Euphorbiaceae) in rodents. Interdiscip Toxicol 2015; 8(2): 89-98.

[35] Joshi N, Manoj KD, Dwivedi L, Khilnani GD. Toxicity study of Lauha Bhasma (calcined iron) in albino rats. Anc Sci Life 2016; 35(3): 159-66.

[36] Ashafa AOY, Orekoyo LO, Yakubu MT. Toxicity profile of ethanolic extract of Azadirachta indica stem bark in male Wistar rats. Asian Pac J Trop Biomed 2012; 2(10): 811-7.

[37] Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biol Sci 2004; 4: 405-12.

[38] Srilaxmi P, Sareddy GR, Kishor PBK, Setty OH, Babu PP. Protective efficacy of natsansin, a dibenzyl glycoside from Salvinianatans against CCl4 induced oxidative stress and cellular degeneration in rat liver. BMC Pharmacol 2010; 10: 1471-2210.

[39] Sanchez-Elsner T, Ramirez JR, Rodriguez-Sanz, Varela E, Bernabew C, Botella LM. A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and smads. J Mol Biol 1996; 36(1): 9-24.

[40] Ahmadjian V, Hale M. The Lichens. New York: Academic Press; 1973.

[41] Goorden SM, Buffart TE, Bakker A, Buijs MM. [Liver disorders in adults: ALT and AST]. Ned Tijdschr Geneeskd 2013; 157: A6443. Dutch.

[42] Megahed HA, Zahran HG, Arbid MS, Osman A, Kandil SM. Comparative study on the protective effect of biphenyl dimethyl dicarboxylate (DDB) and silymarin in hepatitis induced by carbon tetrachloride (CCl4) in rats. N Y Sci J 2010; 3: 1-11.