HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF OXALIS DEBILIS KUNTH AGAINST CCl₄ - INDUCED LIVER DAMAGE

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ABSTRACT

Objective: To evaluate the hepatoprotective activity of aqueous extract of Oxalis debilis Kunth in carbon tetrachloride (CCl₄)-induced hepatotoxicity in Swiss albino mice.

Methods: Hepatotoxicity was induced by CCl₄ 30% in olive oil (1 ml/kg intraperitoneally). Mice were treated with aqueous extract of O. debilis at doses of 250 and 500 mg/kg body weight orally for 14 days. There were two groups, pre-treatment (once daily for 14 days before CCl₄ intoxication) and post-treatment (2, 6, 24, and 48 hrs after CCl₄ intoxication). The observed effects were compared with a known hepatoprotective agent, silymarin.

Results: Pre-treatment and post-treatment groups of aqueous extract of O. debilis significantly reduced elevated serum levels of serum transaminases, alkaline phosphatase, and bilirubin and increased the level of total protein as compared to CCl₄-treated group. The histopathological study also confirms the hepatoprotection. Preliminary qualitative phytochemical analysis of the plant revealed the presence of phenolic compounds, tannins, flavonoids, and saponins.

Conclusion: The results of this study suggest that O. debilis can be used as safe, cheap, and alternative preventive and protective drugs against liver injury. The protective effect observed could be attributed to the presence of various phytochemicals which are responsible for the restoration of liver function.

Keywords: Oxalis debilis, Hepatoprotective, Carbon tetrachloride, Liver, Transaminases.

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INTRODUCTION

Plants are the chief source of many useful compounds and possess the broadest spectrum of synthetic activities. In traditional practice, the therapeutic value of medicinal plants has been exploited for the management of various disease conditions. And as a result, a growing interest has emerged around the globe in rediscovering medicinal plants as useful therapeutic agents. Synthetic drugs available in the market come with severe side effects which led to focus in demand of ethnomedicinal drugs for the treatment of various diseases. And as a result, a growing interest has emerged around the globe in rediscovering medicinal plants as useful therapeutic agents.

The liver is an important and primary target organ for nearly all toxic chemicals due to its unique metabolism and relationship to the gastrointestinal tract [3]. Worldwide, liver disorders have become a serious health problem and a cause of morbidity and mortality due to its limited prevention and treatment options. Liver injury is initiated by the various toxic agents produced by chemicals, alcohol, and viruses [4]. The most common liver diseases are jaundice, hepatitis, cirrhosis, and fatty liver.

Oxalis debilis is tristylos species and it is a member of bulb-forming section Ionoxalis, it is an aggressive weed easily propagated from bulbils [5]. Leaves along with petiole of this plant are eaten as a vegetable and it is also used as a souring agent in curry. Medicinally, it is useful for treating appetite loss and antidote to toxicity [6-9].

Carbon tetrachloride (CCl₄) is a well-known and most widely used hepatotoxin to induced liver injury in a large range of laboratory animals. Fatty liver, cirrhosis, and necrosis are the most remarkable pathological characteristics in CCl₄-induced hepatotoxicity and shown to be superficially similar to the human cirrhosis of the liver [10]. In the present study, we aimed to determine the hepatoprotective activity of aqueous extract of O. debilis Kunth in CCl₄-induced hepatotoxicity.

METHODS

Plant collection and authentication

The whole plant of O. debilis was collected from vegetable growing area of Imphal district, Manipur, India. The material was identified and authenticated in Botanical survey of India (BSI), Eastern Regional Centre, Shillong, India. A voucher specimen was deposited in the Department of Life Science and Bioinformatics, Assam University, Silchar, India.

Preparation of extract

The plant parts were shade dried, powdered with a mechanical grinder and passed through a sieve and were extracted with distilled water in the ratio of 1:10 w/v (weight/volume). The extract thus obtained was concentrated and dried in a vacuum desiccator. The aqueous extract of O. debilis was prepared fresh each time after triturating with distilled water immediately before the administration.

Preliminary phytochemical screening

The crude aqueous extract was subjected to preliminary phytochemical analysis to test for the presence of various chemical constituents such as alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phytosterols, fixed oils, fats, phenolic compounds, and flavonoids [11-18].

Experimental animals

The Swiss albino male mice, 8-12 weeks old (weighing between 22 and 28 g) were procured from Pasteur Institute, Shillong, Meghalaya. The animals were housed in large, clean polypropylene cages in a...
temperature-controlled room (27±3°C) with 12 hrs light and dark cycle, free access to water ad libitum and fed with standard pellet diet. All the experiments and protocols described in the present study were approved by the Institutional Ethical Committee (IEC) of Assam University, Silchar, (Reg. No. IEC/AUS/2013-045 dt-20/3/13).

**Dose selection**
The dosage of the extract was determined after toxicity test (LD<sub>50</sub>) median lethal dose described by Lorke [19]. The 250 mg/kg b.wt and 500 mg/kg b.wt were taken as the low and medium doses.

**Experimental design**
The experiment was designed following the method of Vuda et al. [20]. The mice were divided into eight groups of six mice each group. Group I served as a normal control for both pre-treatment and post-treatment and received distilled water orally for 14 days. Group II served as a toxic control and received distilled water orally for 14 days and on the 14<sup>th</sup> day, they received 30% CCl<sub>4</sub> in olive oil (1 ml/kg b. wt, i.p.). Groups III and IV served as pre-treatment groups. They received an aqueous extract of <i>O. debilis</i> orally at a dose of 250 and 500 mg/kg b. wt. for 14 days, respectively, and on the 14<sup>th</sup> day, they received 30% CCl<sub>4</sub> in olive oil (1 ml/kg b. wt, i.p.). 2 h after administration of the past dose of the plant extract.

Group V served as the standard for the pre-treatment group and they received standard drug silymarin 100 mg/kg b. wt. orally for 14 days and on the 14<sup>th</sup> day they received 30% CCl<sub>4</sub> in olive oil (1 ml/kg b. wt, i.p.), 2 hrs after administration of the past dose of silymarin. Group VI and VII served as post-treatment groups. They received distilled water orally for 14 days and on the 14<sup>th</sup> day received 30% CCl<sub>4</sub> in olive oil (1 ml/kg b. wt, i.p.) followed by the aqueous extract of <i>O. debilis</i> orally at a dose of 250 and 500 mg/kg b. wt, respectively, at 2, 6, 24, and 48 hrs after CCl<sub>4</sub> intoxication. Group VIII served as the standard for the post-treatment group and received distilled water orally for 14 days and on the 14<sup>th</sup> day received 30% CCl<sub>4</sub> in olive oil (1 ml/kg b. wt, i.p.) followed by silymarin 100 mg/kg b. wt. orally at 2, 6, 24, and 48 hrs after CCl<sub>4</sub> intoxication.

All the mice were sacrificed 50 hrs after CCl<sub>4</sub> intoxication and blood were collected and allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 minutes and used for biochemical estimations.

**Measurement of serum biochemical parameters**
The activities of serum aspartate transaminase, alanine transaminase, alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), and total protein (TP) were estimated using standard methods [21-24].

**Histopathology**
The liver was collected and fixed in 10% formalin, cleared in xylene, and embedded in paraffin. Section of 4-5 µm thickness was prepared and stained with hematoxylin and eosin (H-E) dye and observed under a microscope to examined histopathological changes in the liver.

**Statistical analysis**
The data are expressed as mean ± S.E.M one-way analysis of variance followed by multiple comparisons with the Tukey post hoc test to compare different parameters between the groups. Statistical analysis was performed using the SPSS statistical software package, version 21.0 for windows. The results were considered to be statistically significant at p<0.05.

**RESULTS AND DISCUSSION**
The qualitative phytochemical analysis of an aqueous extract of <i>O. debilis</i> showed the presence of carbohydrates, flavonoids, saponins, phenolic compounds, and tannins (Table 1). CCl<sub>4</sub>-induced animals group showed a significant increase in the levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), ALP, TB, and DB (p<0.001) as compared to normal (Figs. 1-5). The animal treated with the standard drug silymarin reduces the serum levels of all above-mentioned parameters significantly when compared to CCl<sub>4</sub> (p<0.001). The pre-treatment and the post-treatment groups at
the doses 250mg/kg and 500mg/kg of O. debilis showed a significant decrease in the serum levels (p<0.05, p<0.01, p<0.001) when compared to the animals treated with CCl₄. However, when these groups were compared with those of silymarin-treated animal groups, the pre-treatment group of both doses 250 mg/kg and 500mg/kg showed a significant difference at SGOT, SGPT (p<0.01), and ALP levels (p<0.01, p<0.05), respectively. There were no such significant differences of all the animals groups treated with O. debilis at the serum levels of total and direct bilirubin when compared with the silymarin-treated groups.

The serum protein level of the animal groups except the CCl₄-treated group measured toward normalization (Fig. 6). Comparative analysis showed that the post-treatment groups found to be more effective than pre-treatment groups and both doses of the post-treatment groups showed no significance at the various serum levels when compared with the silymarin (standard)-treated groups. The results obtained were supported by the histological studies (Fig. 7). The normal control group showed cells with distinct hepatic cells and sinusoidal spaces (Fig. 7a). Liver sections of the animals treated with CCl₄ showed disarrangement and degeneration of hepatocytes with intense centrilobular necrosis and vacuolization (Fig. 7b). The animals treated with the aqueous extract of O. debilis at doses 250 and 500 mg/kg in both pre-treatment and post-treatment groups showed portal vein congestion, less disarrangement, and degeneration of hepatocytes with less vacuolization and an absence of necrosis (Fig. 7c,d). The liver sections of the silymarin-treated animal groups at a dose 100 mg/kg showed a cell damage protection (Fig. 7e and h). These histopathological studies confirmed the hepatoprotective effect of aqueous extract of O. debilis against CCl₄-induced hepatotoxicity.

In this experiment, it is found that post-treatment groups showed more hepatoprotective activity than pre-treatment groups for both doses. Protective efficacies of the post-treatment groups were found to be comparable to that of the silymarin-treated groups. CCl₄ is the most common and extensively used hepatotoxin in the experimental study of liver diseases. Administration of CCl₄ causes acute liver damage that mimics the damage done to the liver due to natural causes. CCl₄ is biotransformed by cytochrome P⁴⁵⁰ to free radicals (trichloromethyl, Cl₃C-CCl₃ (hexachloroethane), COCl₂ (phosgene) which are to known to involve in the pathogenesis of liver. This result in the necrosis of liver due to peroxidation of lipids, covalent binding of macromolecules, disruption of metabolic mechanisms in mitochondria, decrease in the levels of phospholipids, increase in triglycerides levels, inhibition of calcium pumps of microsomes [3]. Excessive generation of reactive oxygen species results in the damage of plasma membrane making it unable to resist leakage of cytosolic proteins into the bloodstream.
The extent of liver damage, in general, is assessed by histopathological evaluation and serum levels of SGOT, SGPT, ALP, TB, and TP release in circulation [25]. The elevated levels of these serum enzymes interpreted as a result of the liver cell destruction or changes in the membrane permeability indicated the severity of hepatocellular cell damage caused by CCl₄ administration [26].

The enzymes SGOT and SGPT are important metabolic enzymes of the liver which normally exist in the cytoplasm but these enzymes enter into the circulatory system due to toxicity mediated altered permeability of the cellular membrane upon liver injury [27]. CCl₄-induced elevation of ALP is in line with the high levels of serum bilirubin and the depletion of increased ALP with simultaneous suppression of raised bilirubin level indicates the stabilization of biliary dysfunction in the liver during the hepatic injury [4]. The higher concentration of bilirubin and lower concentration of TP confirms the depth and intensity of liver necrosis [28]. CCl₄-induced elevation of ALP is in line with the high levels of serum bilirubin and the depletion of increased ALP with simultaneous suppression of raised bilirubin level indicates the stabilization of biliary dysfunction in the liver during the hepatic injury [4]. The higher concentration of bilirubin and lower concentration of TP confirms the depth and intensity of liver necrosis [28]. CCl₄-induced elevation of ALP is in line with the high levels of serum bilirubin and the depletion of increased ALP with simultaneous suppression of raised bilirubin level indicates the stabilization of biliary dysfunction in the liver during the hepatic injury [4]. The higher concentration of bilirubin and lower concentration of TP confirms the depth and intensity of liver necrosis [28].

CONCLUSION

Our findings clearly revealed that the histopathological alterations produced by CCl₄ in tissue were significantly reserved by the aqueous extract of O. debilis and silymarin correlating with its ability to reduce the activity of serum enzymes. In conclusion, the results of this study suggest that O. debilis can be used as safe, cheap, and alternative preventive and protective drugs against liver injury. The protective effect observed could be attributed to the presence of various phytochemicals which are responsible for the restoration of liver damage.

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