ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 9, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *OXALIS DEBILIS* KUNTH AGAINST CCL_4 - INDUCED LIVER DAMAGE

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Received: 12 April 2017, Revised and Accepted: 29 May 2017

ABSTRACT

Objective: To evaluate the hepatoprotective activity of aqueous extract of *Oxalis debilis* Kunth in carbon tetrachloride (CCl_4)-induced hepatotoxicity in Swiss albino mice.

Methods: Hepatotoxicity was induced by CCl_4 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 CCl_4 intoxication) and post-treatment (2, 6, 24, and 48 hrs after CCl_4 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 CCl_4-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 damage.

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

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i9.19100

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 ethnopharmacological drug discovery [1]. Herbal medicine has gained more attention and popularity because of their safety and efficacy and it is considered to be of great importance among different rural or indigenous communities in developing countries [2].

The liver is an important and primary target organ for nearly all toxic chemicals because of its unique metabolism and relationship to the gastrointestinal tract [3]. Worldwide, liver disorders have become 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 tristylous species and it is a member of bulb-forming section Ionoxalis, it is an aggressive weed easily propagated from bubils [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 (CCl_4) 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 CCl_4-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 CCl_4-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 qualitative 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_{50}) 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 14th day, they received 30% CCl_4 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 *O. debils* orally at a dose of 250 and 500 mg/kg b. wt. for 14 days, respectively, and on the 14th day, they received 30% CCl_4 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 14th day they received 30% CCl_4 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 14th day received 30% CCl_4 in olive oil (1 ml/kg b. wt, i.p.) followed by the aqueous extract of *O. debilis* orally at a dose of 250 and 500 mg/kg b. wt., respectively, at 2, 6, 24, and 48 hrs after CCl_4 intoxication. Group VIII served as the standard for the post-treatment group and received distilled water orally for 14 days and on the 14th day received 30% CCl_4 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_4 intoxication.

All the mice were sacrificed 50 hrs after CCl_4 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 ± SEM 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 *O. debilis* showed the presence of carbohydrates, flavonoids, saponins, phenolic compounds, and tannins (Table 1). CCl_4-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

Table 1: Phytochemical screening of aqueous extract of *Oxalis debilis*

Components	Aqueous extract
Alkaloid	-
Carbohydrate	-
Glycoside	-
Protein	-
Amino acids	-
Phytosterol	-
Fixed oil and fats	-
Phenolic compound	+
Tannin	+
Flavonoid	+
Saponin	+

-: Absence, +: Presence, *O. debilis: Oxalis debilis*

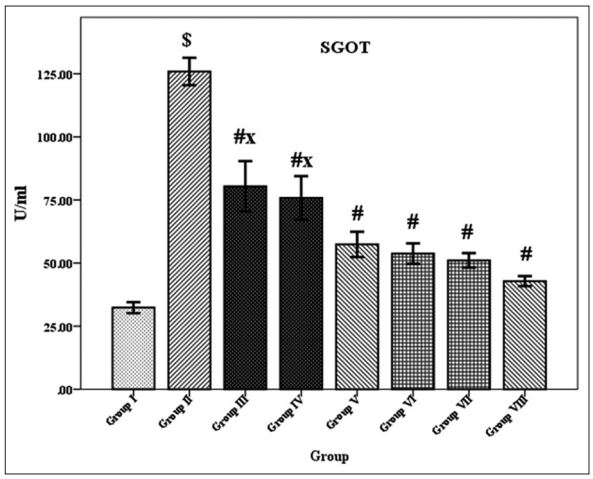

Fig. 1: Effects of aqueous extract of *Oxalis debilis* on serum glutamic oxaloacetic transaminase in experimental animals. Values are expressed as mean ± S.E.M (n=6). $^{\$}p<0.001$ when compared to Group I, $^{\#}p<0.001$ when compared to Group II, $^{x}p<0.01$ when compared to Group VIII

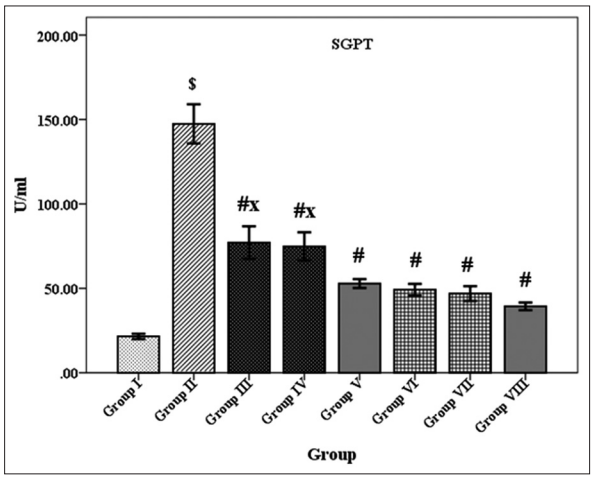

Fig. 2: Effects of aqueous extract of *Oxalis debilis* on serum glutamic pyruvic transaminase in experimental animals. Values are expressed as mean ± S.E.M (n=6). $^{\$}p<0.001$ when compared to Group I, $^{\#}p<0.001$ when compared to Group II, $^{x}p<0.01$ when compared to Group VIII

animal treated with the standard drug silymarin reduces the serum levels of all above-mentioned parameters significantly when compared to CCl_4 ($p<0.001$). The pre-treatment and the post-treatment groups at

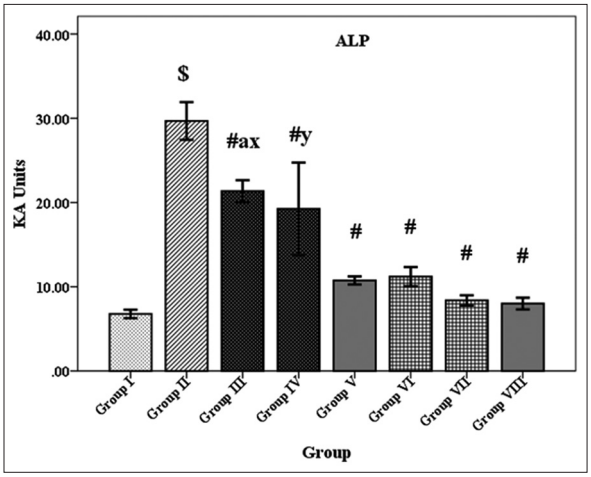

Fig. 3: Effects of aqueous extract of *Oxalis debilis* on alkaline phosphatase in experimental animals. Values are expressed as mean ± S.E.M (n=6). $p<0.001 when compared to Group I, #p<0.001 when compared to Group II, ap<0.05 when compared to Group V, xp<0.01 and yp<0.05 when compared to Group VIII

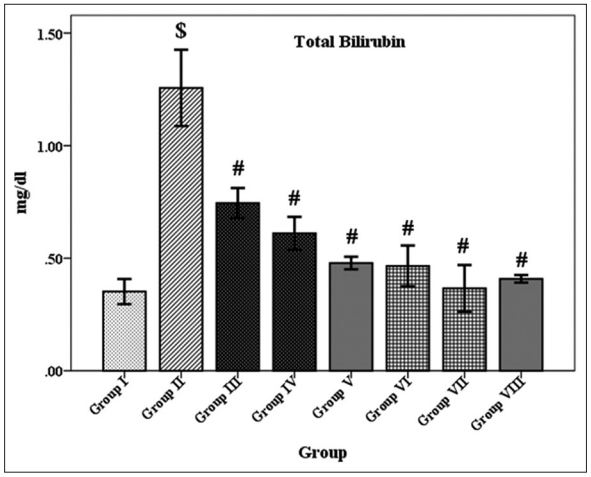

Fig. 4: Effects of aqueous extract of *Oxalis debilis* on serum total bilirubin in experimental animals. Values are expressed as mean ± S.E.M (n=6). $p<0.001 when compared to Group I, #p<0.001 when compared to Group II

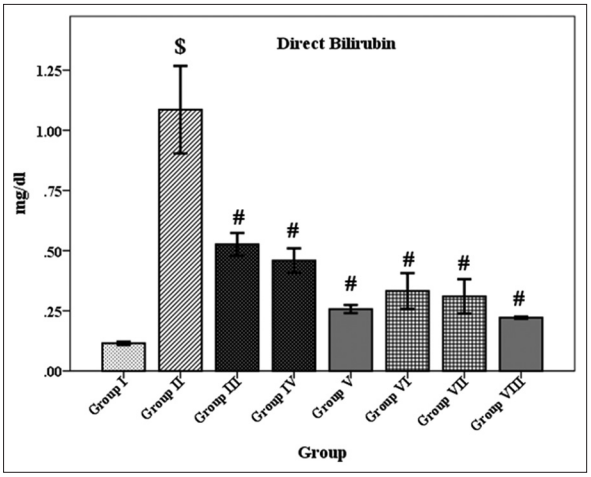

Fig. 5: Effects of aqueous extract of *Oxalis debilis* on serum direct bilirubin in experimental animals. Values are expressed as mean ± S.E.M (n=6). $p<0.001 when compared to Group I, #p<0.001 when compared to Group II

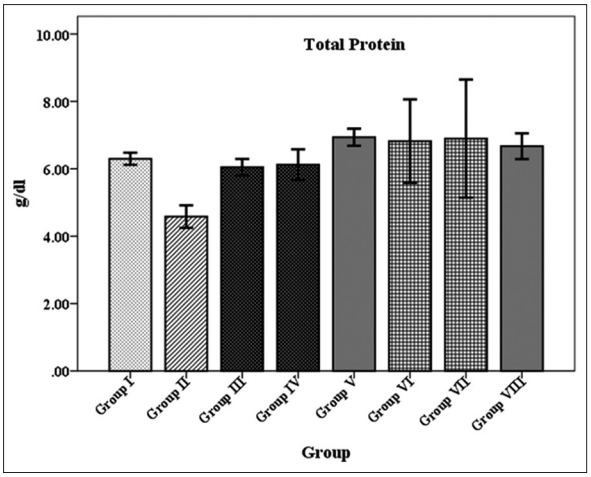

Fig. 6: Effects of aqueous extract of *Oxalis debilis* on serum protein in experimental animals

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 CCl_4. 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 CCl_4-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 CCl_4 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,f,g). 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. debils* against CCl_4-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. CCl_4 is the most common and extensively used hepatotoxin in the experimental study of liver diseases. Administration of CCl_4 causes acute liver damage that mimics the damage done to the liver due to natural causes. CCl_4 is biotransformed by cytochrome P_{450} to free radicals (trichloromethyl, Cl_3C-CCl_3 (hexachloroethane), $COCl_2$ (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.

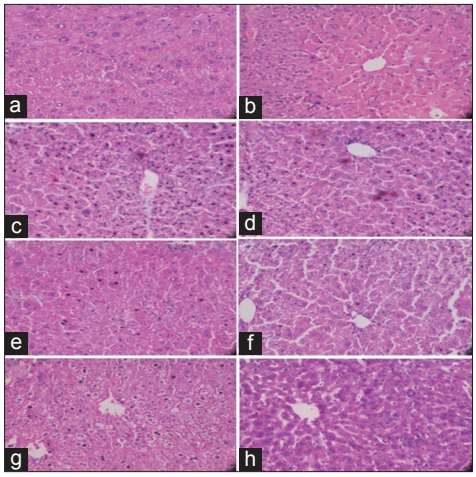

Fig. 7: Effects of *Oxalis debilis* on the liver histopathological photomicrographs of the experimental animal groups. (a) normal control group, (b) toxic control group (CCl_4-treated group), (c and d) pre-treatment group (*O. debilis* 250 mg/kg + CCl_4 and *O. debilis* 500 mg/kg + CCl_4), (e) standard for pre-treatment group (silymarin 100 mg/kg + CCl_4), (f and g) post-treatment group (CCl_4 + *O. debilis* 250 mg/kg and CCl_4 + *O. debilis* 500 mg/kg) and (h) standard for post-treatment group (CCl_4 + silymarin 100 mg/kg)

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 CCl_4 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]. CCl_4-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 and the ability of the hepatoprotective drugs to reduce the injurious effects showed the index of its protectivity. Although serum enzymes levels are not a direct measure of hepatic injury, they showed the functional status of the liver. Hence, the lowering of enzyme level is a definite indication of hepatoprotection [28]. The treatment with the aqueous extract of *O. debilis* attenuated the increased in the levels of the serum enzymes suggesting that extract causes parenchymal cell regeneration in the liver, thus protecting membrane fragility and decreasing enzyme leakage [26]. The aqueous extract of *O. debilis* possesses various phytochemicals including flavonoids, saponins, tannins, and a polyphenolic compound which are natural antioxidants and can scavenge free radicals. Hence, the presence of these components may be attributed to the hepatoprotective activity in the plant [29].

CONCLUSION

Our findings clearly revealed that the histopathological alterations produced by CCl_4 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.

ACKNOWLEDGMENT

We express our sincere thanks to Silchar Medical College and Hospital, Silchar for the help and advice in histopathological examination. We are also thankful to the Biotech Hub, Department of Life Science and Bioinformatics, Assam University, Silchar for the photomicrograph. A special acknowledgment is owed to the Director, Botanical Survey of India, Shillong for identification of the plant *O. debilis* Kunth.

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