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ORIGINAL ARTICLE

Effects of *Alternanthera sessilis* on Liver Function in Carbon Tetra Chloride Induced Hepatotoxicity in Wister Rat Model

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Abstract *Alternanthera sessilis* commonly known as 'sessile joy weed' or 'dwarf copperleaf' is found throughout the hotter parts of India up to an altitude of 1200 m^2. In Assam, the plant has been traditionally used in the treatment of jaundice along with other ailments. The study focuses primarily on the evaluation of hepatoprotective activity of the plant with special references to its putative protective role in carbon tetrachloride induced liver injury on Wister albino rat. The in vivo hepatoprotective activity of the methanolic extract at the dose of 250 mg/kg body weight was highly effective in controlling SGPT, SGOT, ALP, serum cholesterol and serum bilirubin level as compared to silymarin. The said extract also significantly lowered the lipid profile caused by CCl_4. The activity shown by methanolic extract of the whole plant of *A. sessilis* is of considered importance and thus justified its use in controlling hepatic diseases in traditional treatment system.

Keywords *Alternanthera sessilis* · Methanolic extract · Hepatoprotective · Lipid profile

Introduction

In the treatment of human diseases and ailments use of natural products from plant, animal and minerals have always been of first preference due to toxicity and side effects of allopathic medicines. Drugs from various sources are still in the traditional medicine for treating a number of diseases [1, 2] and it has been estimated that about 80% of people in developing countries still relays on traditional medicine for their primary health care [3]. Herbal medicines are far safer than allopathic medicines in concern of side effects, acute and severe allergic reactions and liver or kidney diseases. Now a days the use of herbal medicine is becoming popular compared to allopathic medicines leading to sudden increase in the number of herbal formulations in the market [4]. As per the World Health Organization (WHO) list of 21,000 medicinal plants of the world, 2500 species are found in India. Among these plants around 150 species are used commercially in large scale making India one of the largest producers of medicinal herbs in the world [5].

Liver disease is a worldwide problem irrespective of age, sex, region or race. The allopathic system of medicines used in the treatment of liver diseases is sometimes not enough and can have serious adverse effects. As compared to allopathic drugs, herbal drugs are more widely used for the treatment of hepatic disorder because of their better compatibility and acceptability with minimal/no side effects. In India, more than 40 polyherbal formulations are available in the market claiming to have hepatoprotective activity. The scrutiny of literature indicates that 160 phytoconstituents from 101 plants have hepatoprotective activity [6, 7].

The plant is commonly known as 'sessile joyweed' or 'dwarf copperleaf'. This is a perennial herb often found in and near ponds, canals and reservoirs. In Assam, this plant is commonly known as 'Matikanduri'. *Alternanthera sessilis* possibly originates from tropical America but is now widespread in the tropics and subtropics of the world including the whole of tropical Africa. It is a weed in tropical lands and can grow in all soil types. Shoots and

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leaves are often eaten as vegetable. Depending on location, many other common names exist: 'Daun tolod' in Indonesia, 'Keremak' in Malaysia, 'Brede chevrette' in French, 'Lian zi cao' in China, 'bunga-bunga' in Philippines, 'Angelica' in Spain, 'Lupo' in Ilonggo etc. [8]

A decoction of *A. sessilis* is recommended as an herbal remedy to treat wounds, flatulence, nausea, vomiting, cough, bronchitis, diarrhea, dysentery and diabetes. Its root can relieve inflamed wounds [9]. It is used as a local medicine often in mixtures with other medicinal plants, to treat hepatitis, tight chest, bronchitis, asthma and other lung troubles. The leaves and shoots boiled and drunk as antihypertensive remedy [10].

The main objective of this study is to focus on the evaluation of hepatoprotective activity of the whole plant of *A. sessilis* with special references to its putative protective role in carbon tetrachloride induced liver injury on Wister albino rat.

Materials and Methods

Collection and Authentication of Plant Material

The whole plant of *A. sessilis* were collected from Sivasagar district, Assam in the month of July, 2013. The plant is identified and authenticated taxonomically by Dr. A A Mao at Botanical Survey of India, Shillong.

Preparation of Whole Plant Powder

The freshly collected whole plant of *A. sessilis* were air-dried for 25–30 days. Then it was grinded to make fine powder and stored in a desiccator to protect the content from moisture.

Pharmacological Evaluation

Selection of Animals

Male Wistar rats weighing about 100–200 gm were used for the study. All studies were performed in accordance with the guidance for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Approval No. IACE/DU/64 Dated 24/9/2013).

Maintenance and Acclimatization of Animals

The animal house was well ventilated and animals were kept under conditions of 24 ± 20 °C and RH 44–56% and 12 h light: 12 h dark cycles each day for 1 week before and during the experiments. Animals described as fasted were deprived of food for 16 h but allowed free access to water.

Preparation of Plant Extract of Alternanthera sessilis

The crude drug was extracted with methanol using hot continuous process and concentrated to dryness in rotary vacuum evaporator.

Acute Toxicity Studies

Acute toxicity study was performed for methanolic extract according to OECD guidelines. Female albino rats were kept fasting for overnight providing only water before administering the extract orally at dose level of 250, 1000 and 2500 mg/kg body weight [11]. The animals were observed continuously for 2 h and then occasionally for further 4 h and finally any mortality behavior of the animals and any other toxic symptoms also observed for 72 h. The animals were kept under observation up to 14 days.

Hepatoprotective Activity Study

The animals are divided randomly into six groups of six rats as mentioned in Table 1. The hepatoprotective activity of the plant extracts was tested using three days CCl_4 model. The rats of group (I) received double doses of 5% gum acacia mucilage at 24 h intervals. The rats of group (II) received two doses of vehicle at 12 h intervals and a single dose of Carbon tetrachloride diluted in liquid paraffin. The rats of group (III) received double doses of Silymarin followed by CCl_4 after 30 min of last dose received. While the rats of group (IV), (V) and (VI) received double doses of methanolic extract of *A. sessilis* at 0 and 24 h followed by CCl_4 after 30 min of last dose received.

Lipid Peroxidation

Tissue Sample Preparation

Liver tissues were collected from each experimental rat, washed and homogenized in 1.15% w/v solution of KCl and centrifuged at 1200 rpm at 4 °C for 10 min. The same procedure was repeated twice with increasing centrifugal force at 10,000 rpm and at 20,000 at 4 °C for 10 and 60 min respectively. The microsomal fraction were isolated and stored at −20 °C [12].

Procedure

4 ml tissue sample was prepared with the addition of 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8%

Table 1 Animal distribution chart for in vivo hepatoprotective study

Group	No. of animals	Vehicle	Drug/route	Name of group
I	6	2% w/v Gum acacia suspension	1 ml/kg, p.o.	Vehicle control (+ve control)
II	6	-DO-	CCl_4 (1.25 ml/kg i.p.)	CCl_4 induced (−ve control)
III	6	-DO-	Silymarin (100 mg/kg p.o.) + CCl_4 (1.25 ml/kg i.p.)	Reference standard
IV	6	-DO-	Plant extract (50 mg/kg p.o.) + CCl_4 (1.25 ml/kg i.p.)	Test Dose 1
V	6	-DO-	Plant extract (200 mg/kg p.o.) + CCl_4 (1.25 ml/kg i.p.)	Test Dose 2
VI	6	-DO-	Plant extract (250 mg/kg p.o.) + CCl_4 (1.25 ml/kg i.p.)	Test Dose 3

TBA in 0.2 ml of microsomal fraction. The prepared fraction was heated at 95 °C bath for 60 min; incubated, cooled and final volume was adjusted to 5 ml with double distilled water. The sample was again vortexed with 5.0 ml of butanol: pyridine mixture for a couple of minutes followed by centrifugation at 3000 rpm for 10 min. The OD of organic layer was recorded at 532 nm in Shimadzu UV 160. The levels of lipid peroxides were expressed as 'n' moles of thiobarbituric acid substances (TBARS)/mg protein using extinction co-efficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

Glutathione (GSH) Activity

Tissue Sample Preparation

Liver tissues were collected, washed, weighed and stored at −20 °C. Tissue fragments (200 mg) were thawed and homogenized on ice in 1 ml of homogenizing buffer (250 Mm sucrose, 20 mg Tris–HCl, 1 mg dithiothreitol, pH 7.4), using glass-Teflon homogenizer. The homogenates were centrifuged at 75,000 rpm at 40 °C for 2 h and stored at −20 °C [13].

Procedure

Each 0.1 ml of tissue sample was kept on ice bath with 2.4 ml of EDTA solution. After 10 min 2 ml of distilled water and 0.5 ml of 50% TCA were added and centrifuged at 3000 rpm for 15-min at 4 °C. To 1.0 ml of supernatant 2.0 ml of Tris buffer and 0.05 ml of DTNB solution (Ellman's reagent) was added and vortexed thoroughly. OD was read (within 2–3 min after the addition of DTNB) at 412 nm in Shimadzu UV 160. Appropriate standards were run simultaneously.

Catalase Activity

Catalase activity was measured based on the ability of the enzyme to break down H_2O_2. 10 µl samples were taken in tube containing 3.0 ml of H_2O_2 in phosphate buffer pH 4.2. Time required for 0.05 optical density changes was observed at 240 nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer (0.16 ml H_2O_2 is 30% w/v was diluted to 100 ml of phosphate buffer pH 4.2). Reading was taken at every 3 s interval. Catalase activity is expressed as follows [14]:

$$\text{Units/mg protein} = 2.3/\Delta t \times \ln(\text{E initial/E final}) \times 1.63 \times 10^{-3}$$

E = optical density at 240 nm

2.3 = factor to convert into log.

Δt = time required for a decrease in the absorbance.

Histopathology

Isolated liver samples were preserved at 2–3 °C with Bouin's solution. For the histopathological examination samples were prepared using Rapid process. The samples were cut into thin ribbon using rotary microtome and strips placed in glass slides. These slides were then incubated overnight at a temperature of 37 °C. After treatment with xylene and alcohol the slides were finally washed with water for 20 min. The slides were stained with haematoxylin and eosin, after which it was fixed and observed under microscope (Leica Photomicroscope DM1000) [15, 16].

Results and Discussion

Bio-Chemical Parameters Investigation

The effects of different fractions of methanolic extracts of the whole plant on the SGPT, SGOT, ALP, serum cholesterol and serum bilirubin levels were investigated in the carbon tetrachloride induced hepatic albino rats using silymarin as standard drugs. The results are summarized in Table 2.

Table 2 Effects of *A. sessilis* on SGOT, SGPT, ALP, serum cholesterol and serum bilirubin level on experimental animals

Groups	SGOT(U/L)	SGPT (U/L)	ALP (U/L)	Serum cholesterol (mg/dl)	Serum bilirubin (mg/dl)
G-I vehicle control	54.126 ± 5.238	48.306 ± 4.074	102.729 ± 5.067	181.832 ± 7.931	0.613 ± 0.128
G-II hepatic control	108.834 ± 6.402	95.739 ± 3.783	167.683 ± 6.449	175.676 ± 5.529	1.898 ± 0.078
G-III standard drug	61.692 ± 4.656	56.454 ± 4.656	127.930 ± 4.742	153.933 ± 8.131	0.632 ± 0.047
G-IV test 1 (50 mg/kg)	82.644 ± 2.910	78.279 ± 3.783	148.267 ± 5.988	169.809 ± 2.810	1.320 ± 0.049
G-V test 2 (200 mg/kg)	75.660 ± 4.656	66.057 ± 4.947	141.818 ± 4.146	163.276 ± 1.208	0.786 ± 0.097
G-VI test 3 (250 mg/kg)	71.205 ± 4.365	52.586 ± 3.492	131.278 ± 6.449	159.432 ± 6.435	0.730 ± 0.036

Values are statistically significant at ($p < 0.05$)

* Values are mean ± SEM (n = 6)

Table 3 Lipid peroxidase and glutathione reductase activity from liver tissue

S no.	Groups	Lipid peroxidase activity		Glutathione reductase activity	
		Absorption (nm)	nM MDA/mg of protein	Absorption (nm)	nM MDA/mg of protein
1	G-I-vehicle control	2.416	15.487	7.923	50.788
2	G-II hepatic control	6.493	41.621	3.142	20.141
3	G-III standard Drug	4.315	27.660	5.631	36.096
4	G-IV test 1 (50 mg/kg)	5.093	37.647	3.949	27.724
5	G-V test 2 (200 mg/kg)	4.739	33.378	5.271	29.788
6	G-VI test 3 (250 mg/kg)	4.298	29.551	5.747	34.839

Values are statistically significant at ($p < 0.05$)

* Values are mean ± SEM (n = 6)

Discussion

Liver enzymes are liberated into blood whenever liver cells are damaged and enzyme activity in the plasma is increased. The whole plant extracts of *A. sessilis* at doses of 50, 200 and 250 mg/kg body weight showed varied effect on biochemical parameters. Increased serum concentration of enzymes such as SGPT, SGOT and ALP were observed in CCl_4 induced rats indicating an altered liver function and/or liver mitochondrial injury in comparison to normal control rats receiving only the vehicle. Liver injury contributes to increased serum level of transaminase enzymes due to easy availability of amino acids. On treatment with test extract of varied doses it was found that the dose 250 mg/kg body weight significantly reversed the elevated marker enzymes i.e. SGOT, SGPT, ALP indicating its hepatoprotective effect (Table 2).

The lipid profile of CCl_4 induced rats showed important indicator for metabolic disturbance including hepatic disease. The higher concentration of serum cholesterol, serum bilirubin may be attributed to liver disease such as hepatitis, cirrhosis, obstructive jaundice etc. The treatment with methanolic extract of *A. sessilis*, particularly with that of the higher dose showed marked improvement in diminishing the higher levels of these markers with respect to that of hepatic control group (Table 2).

The oxidative stress induced by CCl_4 may lead to imbalance of in vivo antioxidant system which was also evaluated by this study. The oxidative stress in the hepatic animals measured by free radical (CH_3^+) measurement is difficult due to their very short half-life and their low concentration. Moreover oxidative stress markers are lipid peroxidation and total antioxidant status. Elevated lipid-peroxidation was also studied in CCl_4 induced hepatic animal. The 250 mg/kg body weight dose of methanolic extracts of *A. sessilis* and silymarin treated groups significantly reduced the oxidation of lipids in liver (Table 3).

Total antioxidant status was measured by amount of enzymatic GSH. GSH plays the important role in balance the oxidative stress. In hepatic control groups, the decreased GSH may be due to reduction in GSH synthesis or degradation of GSH by oxidation stress in hepatic animal. CCl_4 induced group produce less glutathione reductase activity (20.141) whereas the standard drug showed the activity of 36.093. The *A. sessilis* treated groups; particularly the higher dose groups have showed prominent activity in the increment of reductase power (Table 3).

Catalase is a haem containing ubiquities enzyme which detoxify the H_2O_2 into water and oxygen. The level of catalase in liver was improved significantly by methanolic

Table 4 Catalase activity from liver tissues

Sl. no	Groups	Time (s)	E-initial	E-final	μM of H_2O_2 decomposed/min/mg protein
1	G-I-vehicle control	30	0.553	0.495	13.960
2	G-II hepatic control	84	0.540	0.486	4.958
3	G-III standard drug	45	0.549	0.493	9.277
4	G-IV test 1 (50 mg/kg)	81	0.431	0.378	5.277
5	G-V test 2 (200 mg/kg)	54	0.494	0.439	7.740
7	G-VI test 3 (250 mg/kg)	51	0.487	0.431	8.350

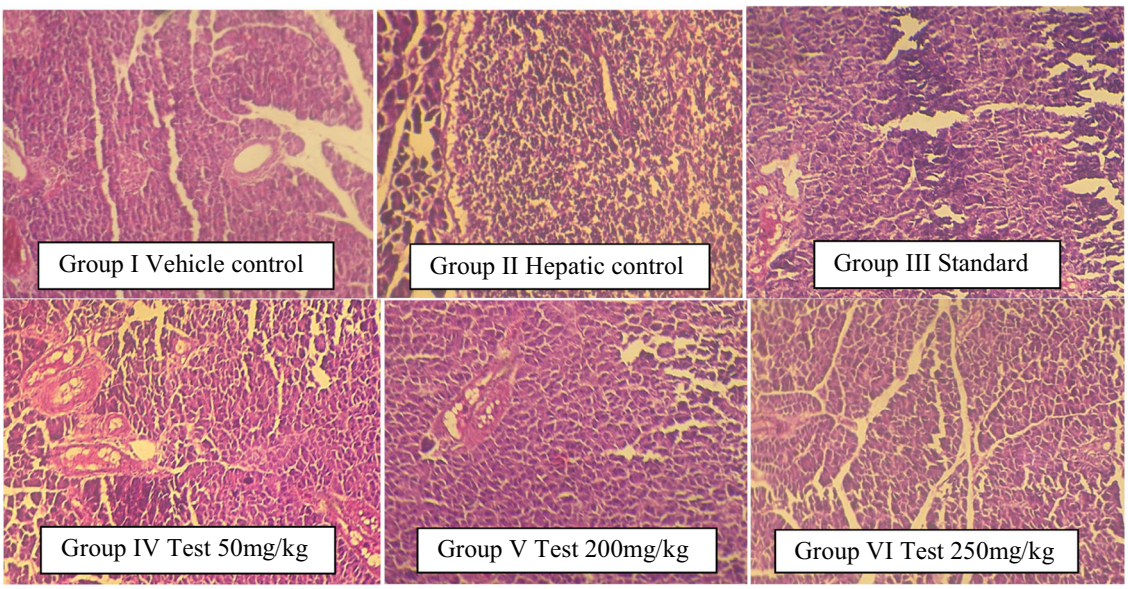

Fig. 1 Liver tissues sections as observed under Leica DM1000 at ×40 magnification

extract of *A. sessilis* (250 mg/kg) and silymarin. Antioxidants may have a role in the prevention of hepatic disease. The methanolic extract of *A. sessilis* fractions has the potential to detoxify the reactive oxidation species and the higher dose fraction has the better ability to do that (Table 4).

Histopathological examination of liver sections from vehicle control group (Fig. 1) showed distinct hepatic cells, sinusoidal spaces and central vein. The duct area is prominent and there is no fatty layer. In case of hepatic control group there is predominant disarrangement of normal hepatic cells with necrosis and vacuolization. Significant fatty layer degradation has occurred. Small blackish, blood clotting spots are visible that may be sign of necrosis. The standard drug treated group (Fig. 1) had well managed cellular integrity. Mild fatty layers degeneration has occurred along with few small ballonic spots also seen but no blood clotting or necrosis has been noticed. The sections of lower dose drug treated groups (dose 50 mg/kg body wt.) (Fig. 1) revealed very little restoration of cellular integrity as observed by scattered blood clotting, fatty layers degeneration and severe necrosis. However the higher doses i.e. 200 and 250 mg/kg body wt. (Fig. 1) showed significant reversal of degeneration marked by prominent decrease of necrosis, cell integrity restoration that can be comparable with that of the standard.

Conclusion

The pharmacological parameters of *A. sessilis* have been evaluated with the objective to validate the traditional hepatoprotective uses. In vivo hepatoprotective activities were carried out and measurement of different biochemical parameters were performed after the 36 h of induction of CCl_4 followed by sacrifice of animals by cervical dislocation. From the results obtained, we can conclude that the dose of 250 mg/kg body weight was more effective than the dose of 50 mg/kg body weight. The methanolic extract

of the whole plant of *A. sessilis* also significantly normalized the lipid profile caused by CCl_4 induction of rats. The activity shown by methanolic extract of the whole plant of *A. sessilis* is of considerable importance and thus justified its use in controlling hepatic diseases in traditional treatment system.

Further studies are required to identify the bio-active chemical constituents present in the crude extracts of this plant and development of herbal drug formulations for the treatment of hepatic diseases.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interests.

Human and Animal Rights All procedures performed in studies involving animals were in accordance with the ethical standards promulgated by the Institutional Animal Care Committee, CPCSEA, India. The institutional registration number is 1576/GO/a/11/CPCSE dated: 17/2/2012. The present study is approved vide Approval No. IACE/DU/64 Dated 24/9/2013.

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