

HEPATOPROTECTIVE PROPERTIES OF *BOERHAAVIA DIFFUSA* AND *AERVA LANATA* AGAINST CARBON TETRA CHLORIDE INDUCED HEPATIC DAMAGE IN RATS.

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Summary

The petroleum ether extract, methanolic extracts and isolated compounds of *B. diffusa* and *A. lanata* were subjected to pharmacological screening on mice models. All the extracts obtained showed hepatoprotective activity in albino rats where the hepatotoxicity was induced by administering CCl₄. Animals treated with doses of 100, 200 and 300mg/kg, of plant extracts, it showed a significant protection of liver histologically and serum tests after the last doses of CCl₄. Thus the current study reveals that plant extracts from the plants *B. diffusa* and *A. lanata* have significant hepatoprotective property.

Key words: Plant extract, Herbal medicine, Liver toxicity

Introduction

Boerhaavia diffusa (Spreading Hogweed in English), belonging to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (known also under its traditional name as *Punarnava*) and of Brazil (known as *Erva tostão*). The root is mainly used to treat gonorrhea, internal inflammation of all kinds, dyspepsia, edema, jaundice, menstrual disorders, anemia, liver, gall bladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumors, and cancers [1], then as a diuretic [2]. The plant is used in Brazilian herbal medicine for all liver problems. It is used to treat eye diseases [3]. Potent antidote for snake and rat bites [4], in the treatment of nephrotic syndrome [5], hepatitis, gall bladder abnormalities, and urinary disorders [6,7], glycoprotein from *B. diffusa* exhibited strong antimicrobial activity against RNA bacteriophages [8]. The chloroform extract of *B. diffusa* has significant antidiabetic activity and this supports the traditional usage of the plant by Ayurvedic physicians for the control of diabetes [9].

Aerva lanata belongs to the Family Amaranthaceae, known as Kurandaka (Sanskrit). It is a bushy, prostrate tomentose herb. The plant is used by Ayurvedic practitioners for a number of ailments. It is used for the treatment of gonorrhoea, kidney disorders, cutaneous infections, sugar in urine, in a snake-bite treatment and eye complaints. Root and flower decoction is given to treat headache. Root decoction is used as an antidote for snakebite. Root powder is used as tooth paste to treat toothache. Whole plant is used to treat cough, boils, lithiasis (calculus formed by inorganic salts) and pus [10]. Benzene and alcoholic extracts of *A. lanata* was investigated in the rat to evaluate the antiinflammatory activity [11]. The alcoholic extract of *A. lanata* was tested for Anti-diabetic activity [12]. Studies have shown hydroalcoholic extract of *A. lanata* possesses hepatoprotective activity against paracetamol-induced hepatotoxicity in rats [13].

To validate folkloric claim the scientific data is mere. So the study was undertaken to evaluate the hepatoprotective activity of the plants, *B. diffusa* and *A. lanata* studying the effect of their crude extracts and obtained compounds.

Methods

Plant material

The leaves, stem bark and roots of *Boerhaavia diffusa* and *Aerva lanata* were collected from Davanagere and Shivamogga campuses of Kuvempu University. The plants were authenticated by comparing with the herbarium voucher specimen deposited in Kuvempu University herbaria. The plant materials of both the species were shade dried, powdered mechanically (sieve no.10/44) and stored in airtight containers for extraction.

Extraction

The powdered materials of both the plants were subjected to successive solvent extraction using Soxhlet apparatus and refluxed successively with petroleum ether, chloroform and methanol. The extracts were filtered and concentrated in vacuum using rotary flash evaporator (Buchi, flawil, Switzerland). The crude extract was administered to the animals as aqueous solutions. The bio active compounds were got by subjecting the petroleum ether fractions to chromatographic columns.

Animals

Sprague Dawley Albino rats (180 to 200g) were used for the present studies. They were housed in clean polypropylene cages (38 x 23 x 10cm) with not more than six animals per cage and maintained under standard laboratory condition (temperature 25±2 °C) with dark and light cycle (12/12h). They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India). The animals were acclimatized to laboratory condition for seven days before commencement of experiments. Ethical clearance was obtained from National College of Pharmacy, Shivamogga.

Chemicals and Drugs

Carbon tetrachloride (CCl₄), Diazo-reagent, Standard bilirubin, Phosphate standard, Sodium pyruvate standard, DL-aspartic acid, α-ketoglutaric acid, DL-alanine 2, 4-dinitro phenyl hydrazine, amino naphthol sulphonic acid (ANSA), Barbitone buffer, Phosphate buffer and Silymarin.

Hepatoprotective study

The albino rats weighing 180-200gms were divided into nine groups of six animals in each. Animals in Group I served as a vehicle (normal) control, which received 0.025% Carboxy methyl cellulose solution (CMC) at the dose of 3.0ml/kg. The group II animals received CCl₄ in liquid paraffin (1:2) at the dose of 1.0ml/kg intraperitoneally once in every 72h for 10 days and served as standard group. Mortality was observed after 72h. The animal group III is treated with standard drug silymarin at the dose of 30mg/kg (positive control). The animal group Group-IV, V, VI, VII, VIII and IX received the petroleum ether, methanol crude extracts and isolated compounds from *B. diffusa* and *A. lanata* whole plant, animals were treated with daily doses of 100, 200 and 300mg/kg, p.o., respectively for 10 days. The animals of Group III, IV, V, VI, VII, VIII and IX were given single dose of CCl₄, 1.5ml/kg, i.p., 6h after the last treatment. On 11th day all the animals subjected to the last dose were sacrificed, blood was collected from retro-orbital plexus under ether anesthesia. The blood samples were collected separately into dry centrifuge tubes by carotid bleeding and allowed to coagulate for 30min. at 37 °C. The clear serum was separated by centrifugation at 2500rpm for 10min and subjected to liver function biochemical investigations. The toxicity was seen by estimating markers like AST, ALT, ALP and bilirubin.

Estimation of total Bilirubin

In this study bilirubin was estimated using Diazo reagent. The optical density was measured at 540 nm to get a quantitative measure of bilirubin present in the serum of the test animals [14].

Estimation of AST and ALT

Aspartate transaminase converts L-Aspartate and α -Ketoglutaric acid to oxaloacetate and L-Glutamate. Oxaloacetate under basic conditions react with 2,4 dinitro phenyl hydrazine to give 2, 4 dinitro phenyl hydrazone, brown coloured complex which has been colorimetrically estimated at 505nm. Similarly, Alkaline transaminase converts L-Alanine and α -Ketoglutaric acid to pyruvate and glutamate. Pyruvate then reacts with 2,4 DNPH to give a brown colour complex. The serum obtained from the test animals were used to convert L-Aspartic acid and L-Glutamate and α -Ketoglutaric acid to give brown coloured complex on treatment with 2, 4 DNPH. The optical densities of the reaction mixtures were noted at 505nm. These optical readings gave the quantitative measure of ASP and ALP [15].

Estimation of ALP

Sodium β glycerophosphate was used as the substrate to estimate the alkaline phosphatase present in the serum of the test animals. This reaction was colorimetrically assayed using ANSA reagent [16].

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple pair wise comparison test to assess the statistical significance. $P \leq 0.05$ was considered statistically significant, using software ez ANOVA ver. 0.98. The data are presented in Tables 1 and 2.

Results

The effect of Petroleum ether and methanol crude extracts and phytoconstituents from *B. diffusa* and *A. lanata* on serum transaminases, alkaline phosphatase and bilirubin in CCl₄

intoxicated rats are summarized in Table 1 and 2. There was a significant ($p < 0.05$) increase in serum AST, ALT, ALP and bilirubin levels in CCl_4 intoxicated group compared to the normal control group. Administration of crude extracts, GM-1, GM-2, GAL-1 and GAL-2 at the dose of 200 and 300mg/kg orally significantly decreased the elevated serum marker enzymes and reversed the altered the markers to almost normal level (Table 1 and 2). The extract at the dose 200 and 300 mg/kg also reduced the level of bilirubin. The results are well comparable with silymarin (standard drug) treated group.

The biochemical estimation of blood serum of CCl_4 treated animal groups showed elevated levels of serum total bilirubin ($2.29 \pm 0.14 \text{ mg/dL}$) indicating the liver damage due to CCl_4 absorption. Phytoconstituents GM-02 ($0.61 \pm 0.19 \text{ mg/dL}$) showed highly significant decrease in the bilirubin level. Moderate decrease in the total bilirubin was noticed in the blood of the animal groups treated with GM-1 ($0.58 \pm 0.12 \text{ mg/dL}$). The animal groups treated with extracts of plants showed significant reduction in the bilirubin level indicating the potency of tested drugs in suppressing the hepatic damage caused by CCl_4 and the results are presented in Table 1 and 2.

Table 1: Hepatoprotective effect of crude extracts and isolated phytoconstituents of *B. diffusa*

Samples	Total bilirubin (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal control	0.53 ± 0.03	194.81 ± 16.49	77.08 ± 10.24	121.35 ± 7.96
Negative control CCl_4	2.29 ± 0.14	449.53 ± 79.00	282.89 ± 7.27	405.27 ± 11.00
Positive control	0.73 ± 0.04	200.14 ± 8.55	86.10 ± 5.96	162.91 ± 4.83
Pet. ether crude + CCl_4	$0.60 \pm 0.06^*$	$253.57 \pm 19.48^*$	$87.07 \pm 16.09^*$	$182.31 \pm 14.03^*$
Methanol crude + CCl_4	$0.78 \pm 0.09^*$	$240.13 \pm 9.41^{**}$	$79.00 \pm 3.29^*$	$188.45 \pm 5.30^*$
GM-1	$0.58 \pm 0.12^*$	$231.25 \pm 27.76^*$	$81.99 \pm 7.40^*$	$146.81 \pm 22.51^*$
GM-2	$0.61 \pm 0.19^{**}$	$226.24 \pm 25.65^*$	$81.82 \pm 8.64^*$	$138.08 \pm 19.56^*$

Values are mean \pm S.E.; $n = 6$ in each group. $*P < 0.05$ is compared to control.

The concentration of AST and ALT increases in serum whenever the tissues are damaged. It is presumably due to release of enzyme from the destroyed cells. In hepatic necrosis the serum levels of AST and ALT could be expected to increase from 2-20 folds of the upper

limit of normal depending upon intensity of liver damage. In the present study, it was observed that in CCl₄ treated group of animals, the AST and ALT enzyme levels were increased significantly due to severe hepatotoxicity (149.53±79.00IU/L, 282.89±7.27IU/L) respectively. Animals treated with crude extracts and isolated compounds GM-1 and GM-2 from plant *B. diffusa* showed significant reduction in the levels of ALT (253.57±19.48IU/L, 240.13±9.41IU/L, 231.25±27.76IU/L, 226.24±25.65IU/L) respectively and AST (87.07±16.09IU/L, 77.00±3.29IU/L, 81.99±7.40IU/L, 81.82±8.64IU/L) respectively. In the animal groups treated with GAL-01 of the plant *A. lanata* showed less significant changes in the ALT (254.37±17.29 IU/L, 258.48±8.33 IU/L, 230.42±27.66 IU/L) and AST (83.99±15.79 IU/L, 81.12±15.18 IU/L) respectively. The other extracts and compounds showed moderate effect on the enzyme markers.

In CCl₄ treated group due to necrosis of hepatobiliary tract the level of ALP significantly increased when compared to normal animals. In the present study the CCl₄ treated animal groups showed significant rise in the ALP (405.27±11.00IU/L). However, in the animal groups treated with standard drug Silymarin, GM-1 and GM-2 extracts of *B. diffusa* (162.91±4.83IU/L, 146.81±22.51IU/L, 138.08±19.56IU/L) respectively showed significant reduction in the levels of ALP and the petroleum ether, chloroform, methanol crude extracts *A. lanata* showed significant reduction in the levels of ALP (178.15±14.18IU/L, 176.95±15.41IU/L, 185.96±6.39IU/L) respectively. In the animal groups treated with GAL-01 and GM-1 showed moderate reduction in the ALP (141.96±20.15IU/L, 146.81±22.51IU/L) respectively.

Table 2: Hepatoprotective effect of crude extracts and isolated phytoconstituents of *A. lanata*

Samples	Total bilirubin (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal control	0.53± 0.03	194.81±16.49	77.08±10.24	121.35±7.96
Negative control CCl ₄	2.29±0.14	449.53±79.00	282.89±7.27	405.27±11.00
Positive control	0.73±0.04	200.14±8.55	86.10±5.96	162.91±4.83
Pet. ether crude+CCl ₄	0.62±0.04*	254.37±17.29	83.99±15.79*	178.15±14.18
Methanol crude+CCl ₄	0.58±0.08*	258.48±8.33*	79.33±3.51*	185.96±6.39**
GAL-01	0.64±0.17*	230.42±27.66*	81.16±7.77*	141.96±20.15*
GAL-02	0.58±0.15*	228.07±27.98	81.44±7.86**	138.54±19.81*

Values are mean±S.E.; n = 6 in each group. *P < 0.05 is compared to control.

Discussion

Liver diseases, especially viral hepatitis occurs predominantly in the developing world [17] with an enormous impact on public health and economy. Liver can be damaged by drugs like tetracyclines, sulphonamides and antihypertensive drug methyldopa [18]. The liver disorders can be marked by the presence of Transaminases, Alkaline Phosphatases and Bilirubin in high levels. The liver injury due to toxins can result in defective excretion of bile by hepatocytes which are reflected as their increased levels in serum [19].

One of the best models of injury produced in liver is by CCl₄. CCl₄ is used as hepatotoxic agent in animals research work to study the hepato-curative action of plants and other compounds [20,21]. The study done showed significant (p<0.05) increase in serum AST, ALT, ALP and bilirubin levels in CCl₄ intoxicated group compared to the normal control group. The ratio of AST to ALT is calculated by dividing the AST value by the ALT value. It helps to determine whether the liver or other organ has been damaged [22,23]. The extract at the dose 200 and 300 mg/kg also reduced the level of bilirubin to 0.78 mg/dL and 0.58 mg/dL respectively from the level of 1.53 mg/dL in the untreated group. The results are well comparable with silymarin (standard drug) treated group. The effect of the crude extracts and isolated compounds on all markers was seen to be significant at some cases and moderately significant in the others. Thus the study proves that the extracts from plants *B. diffusa* and *A. lanata* have hepatoprotective activity. But the extracts need to be formulated and tested over other models before they can be used as drugs.

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