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Hepatoprotective studies of aqueous leaf and root extracts of *Barringtonia acutangula* (L.) Gaertn. against ethanol induced hepatic stress in rats

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The liver is of vital importance in intermediary metabolism and in the elimination of toxic substances. To maintain the liver in a healthy condition is crucial for overall wellbeing of an individual. A number of plants have been the sources of raw materials for various drug formulations used to treat liver ailments. *Barringtonia acutangula* (L.) Gaertn., is one of the medicinal plant used in Ayurveda which is well-known to have hepatoprotective property. In the present study, the hepatoprotection of aqueous leaf (LWBA) and root (RWBA) extracts of *B. acutangula*, at doses 200 mg/kg and 400 mg/kg were evaluated on ethanol induced hepatic damage in Wistar strain, male albino rats (150-200 g body weight). The liver protective efficacy of the extracts was indicated by reduced levels of serum enzymes, bilirubin, lipid peroxidation and improvement in glutathione, superoxide dismutase, catalase and protein content in a dose dependent manner. The results obtained in the enzyme assays were supported by the histopathological observations. The findings shows that the LWBA extract has better hepatoprotection against ethanol induced liver injury than RWBA extract and also justify the Ayurvedic applications of *B. acutangula* in the management of liver diseases.

Keywords: *Barringtonia acutangula*, Ethanol, Hepatoprotective activity, Lipid peroxidation, *Rattus norvegicus*

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Ethanol is recognized as the most prevalent known cause of abnormal liver development. Chronic alcohol intake also brings about severe damage in liver[1]. According to Zimmerman and Seeff, hepatic injury results in disturbed transportation of hepatocytes leading to the leakage of enzymes[2]. In traditional medicine, there is a record of a number of plants with hepatoprotective property[3]. Ethnopharmacological studies on such medicinal plants continue to interest investigators throughout the world nowadays[4].

Barringtonia acutangula (L.) Gaertn. is a key plant used in the preparation of Ayurvedic preparations mentioned in the management of liver complaints, stomach disorders, spleenic disorders and diabetes since many centuries. According to Kumar *et al.*, laghu (light), ruksha (dry) and tikshna (sharp) are the properties of *B. acutangula*, as mentioned in Ayurveda[5]. The fruit is called Dhatriphala or nurses' fruit and is one of the best known domestic remedies. Various plant parts such as roots, leaves, fruits, stem bark and seeds are used for their prospective curative properties[6]. The use value (UV) of this plant was found to be 4.17 and fidelity level (FL) of 32.84 in a survey conducted by Sen and Bhakat[7]. In spite of the widespread use of this plant in traditional therapy, there is scarcity of evidence related to its protective value in treating hepatic diseases. The current research was aimed to get an overview of the effectiveness of crude aqueous extracts of leaf and root of *B. acutangula* (LWBA & RWBA) as a hepatoprotective agent on experimental rats.

Methodology

Collection of materials: Collection of *B. acutangula* plant parts (leaves & roots) was done from Kanwathirtha, Kerala. The plant was authenticated by Alva's Ayurveda Medical College, Moodubidire. After thorough washing in tap water, the leaves and roots of *B. acutangula* were dried and ground mechanically.

Preparation of extracts: The extracts were prepared by boiling the leaf and root powder of *B. acutangula* in distilled water for 15 min[8]. The extracts were then filtered and a semisolid mass obtained after

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concentrating in water bath was further evaporated in laboratory temperature and preserved in refrigerator till use. The percentage yield of the extracts was calculated.

Drugs and chemicals: Chemicals and reagents of analytical grade were used for the present study.

Animal experiments: Wistar strain, male albino rats (*Rattus norvegicus*, 150-200 g body weight) were kept in standard conditions (12 h light/dark cycle; 25±2°C, 45–60% RH) as per CPCSEA regulations[9]. Before initiation of the experiments, acclimatization of the experimental animals to laboratory conditions was done for one week. Ethical clearance was obtained from Mangalore University before the commencement of the experiments (Reg. No. 232/CPCSEA).

Acute toxicity study: Male Wistar albino rats (200 g body weight) were used for the acute toxicity study following OECD guidelines No. 425[10]. The leaf aqueous extract of *B. acutangula* suspended in gum acacia was administered orally to the overnight fasted animals at a dose 2000 mg/kg body weight. The animals were monitored constantly for 3 h for any behavioural changes and every 30 min for next 3 h and then up to 24 h. The animals were further observed for next 14 days for mortality and behavioural changes.

Ethanol induced hepatotoxicity: Ethanol induced hepatotoxicity study was done following the method of Jafri *et al*[11]. The duration of treatment was of 20 days. On the 21st day, 24 h after ethanol treatment, all the animals were sacrificed. Blood samples and liver tissues were collected for the estimations and histopathological observations. The experimental protocol followed is shown in Table 1.

Biochemical Estimations: Separation of serum from the blood samples was carried out by cold centrifugation, which was used for the assessment of biochemical parameters such as aspartate transaminase[12] (AST, Reitman & Frankel, 1957), alanine transaminase[12] (ALT, Reitman & Frankel, 1957), alkaline phosphatase[13] (ALP, King, 1965), protein[14] (Lowry *et al*., 1951) and bilirubin[15] (Malloy & Evelyn, 1937). The rat livers were divided into two parts. One part was homogenized in ice-cold sucrose for the biochemical estimations, while remaining part of the liver was used for histopathological studies. Antioxidant status of liver tissue was assessed from the levels of glutathione[16] (GSH, Ellman, 1979), lipid peroxidation[17] (LPO, Ohkawa *et al*., 1975), catalase[18] (CAT, Aebi, 1974) and superoxide dismutase[19] (SOD, Kakkar *et al*., 1984).

Histopathological studies: 10% formalin was used for the fixation of collected liver samples. Later samples were dehydrated in graded alcohol and embedded in paraffin. 5 µm thick sections were prepared and stained with haematoxylin- eosin (H & E) for the microscopic observations of the liver architecture[20].

Statistical analysis: The data was expressed as mean ± SE, (n=6). One way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc test (SPSS 10.0 for Windows) was used for analysis. Values of $p<0.05$ were considered statistically significant.

Results

Preparation of extracts: Yield of leaf extract of *B. acutangula* was found to be 20.68%, which might be because of the presence of more phytoconstituents namely, phytosterols, terpenoids, triterpenoids, alkaloids, tannins, steroids, resins and saponins, than root extract (Percentage yield- 10.04).

Table 1 — Protocol for testing hepatoprotective efficacy of LWBA and RWBA extracts of *B. acutangula* on Ethanol induced liver damage

Treatment groups	Dose, route and duration of treatment
I. Normal Control	0.5% Tween 80 (30 mL/kg/day) and corn oil (10 mL/kg/day) p o in two divided doses.
II. Toxic (Ethanol) control	Ethanol (36.6%v/v- 30 mL/kg/day) and corn oil (10 mL/kg/day) p o in two divided doses.
III Standard (Silymarin) Control	Silymarin (25 mg/kg/day) + ethanol (36.6% v/v- 30 mL/kg/day) and corn oil (10 mL/kg/day) p o in two divided doses.
IV Treated Groups (LWBA & RWBA extracts)	Extracts (200 mg/kg/day or 400 mg/kg/day)+ ethanol (36.6% v/v- 30 mL/kg/day) and corn oil (10 mL/kg/day) p o in two divided doses.

Animal experiments:

Acute toxicity study: Acute toxicity studies specified the absence of any toxic changes at the dose level of 2000 mg/kg, thus indicating the safety level of the drug used. The extract was thus considered safe to rats and the doses of 200 mg/kg (one tenth of the maximum dose) and 400 mg/kg (twice that of one tenth dose), po were selected for the present work.

Ethanol induced hepatotoxicity: Oral administration of ethanol to rats produced significant rise (p<0.001) in enzyme levels such as SAST (245 U/L), SALT (189 U/L) and SALP (206 U/L). There was also an increase in serum bilirubin (2.96 mg/dL) and decline in protein content (5.07 mg/dL) compared to normal control rats. Treatment with the LWBA and RWBA extracts (200 mg/kg & 400 mg/kg) resulted in significant changes in these parameters comparable to that of Silymarin treated groups as displayed in Table 2. Treatment with the extracts significantly (p<0.001) reduced LPO and also a substantial rise in GSH, SOD and CAT levels was detected as mentioned in Table 3.

Histopathological findings supported the results obtained from biochemical estimations. As shown in Fig. 1a-b, the liver sections of control rats showed regular liver histology whereas rats treated with ethanol showed inflammatory infiltration and severe fatty changes. Rats treated with 400 mg/kg LWBA and RWBA extracts showed mild fatty changes which was almost comparable with that of silymarin treated groups, whereas same extracts at lower dose (200 mg/kg) exhibited fatty changes slightly more than higher dose as shown in Fig. 1d-g.

Discussion

Phytochemicals such as terpenoids, steroids, tannins, alkaloids have received significant attention in past because of their various pharmacological activities such as hepatoprotective and antioxidant properties[21,22,23]. Oxidant mediated liver damages in experimental animals as well as humans can be effectively ameliorated by plant based antioxidants[24]. The present work uncovers the hepatoprotective potential of the extract which may be due to presence

Table 2 — Protective effect of LWBA and RWBA extracts of *B. acutangula* on enzyme and biochemical parameters in Ethanol induced hepatic damage in rats

Group	Dose (mg/kg po)	SAST (U/L)	SALT (U/L)	SALP (U/L)	Total protein (mg/dl)	Total bilirubin (mg/dl)
I Normal control	5 mL	67.33±2.54	56.67±2.23	68.83±2.26	7.45±0.05	0.54±0.02
II Eth control	0.7 mL	245±2.16^a	189±3.01^a	206±2.19^a	5.07±0.02^a	2.96±0.03^a
III Eth + Silymarin	25	75.67±2.60^b	67±2.67^b	72±2.67^b	7.15±0.05^b	0.71±0.03^b
IV Eth + LWBA	200	131.67±2.95^b	118.8±2.5^b	128.5±2.40^b	6.17±0.03^b	1.11±0.02^b
Eth+ RWBA	400	88.67±3.14^b	85±2.54^b	96.17±2.89^b	6.95±0.05^b	0.82±0.03^b
	200	199.83±3.0^b	157.33±3.17^b	169.17±2.91^b	5.68±0.04^b	1.57±0.04^b
	400	118±2.86^b	105.66±2.87^b	112±5.09^b	6.29±0.02^b	1.12±0.03^b

Values are mean ± SE (n=6)

Units are as given in the text. One way ANOVA followed by Tukey's multiple comparison post hoc test. $^a p$<0.001 when compared with normal group, $^b p$<0.001 when compared with ethanol treated group

Table 3 — Protective efficacy of LWBA and RWBA extracts of *B. acutangula* on LPO, GSH, SOD and CAT levels in Ethanol induced liver injury in rats

Group	Dose (mg/kg po)	LPO (nM MDA/mg P)	GSH (µg/mg P)	SOD (U/mg P)	CAT (U/mg P)
I Normal control	5 mL	0.99±0.03	5.39±0.05	89.3±3.80	360.17±3.55
II Eth control	0.7 mL	5.88±0.03^a	1.57±0.03^a	32.5±2.14^a	236.1±2.81^a
III Eth + Silymarin	25	1.18±0.03^b	5.20±0.04^b	81.3±3.70^b	350.5±3.80^b
IV Eth + LWBA	200	2.50±0.03^b	3.69±0.03^b	63.5±3.30^b	291.33±6.27^b
Eth+ RWBA	400	1.33±0.03^b	5.13±0.04^b	74.67±4.14^b	341.5±4.61^b
	200	2.93±0.03^b	3.11±0.03^b	57.67±2.33^b	275.5±3.55^b
	400	1.71±0.03^b	4.71±0.03^b	71.67±2.76^b	320.66±4.53^b

Values are mean ± SE (n=6)

Units are as given in the text. One way ANOVA followed by Tukey's multiple comparison post hoc test. $^a p$<0.001 when compared with normal group, $^b p$<0.001 when compared with ethanol treated group

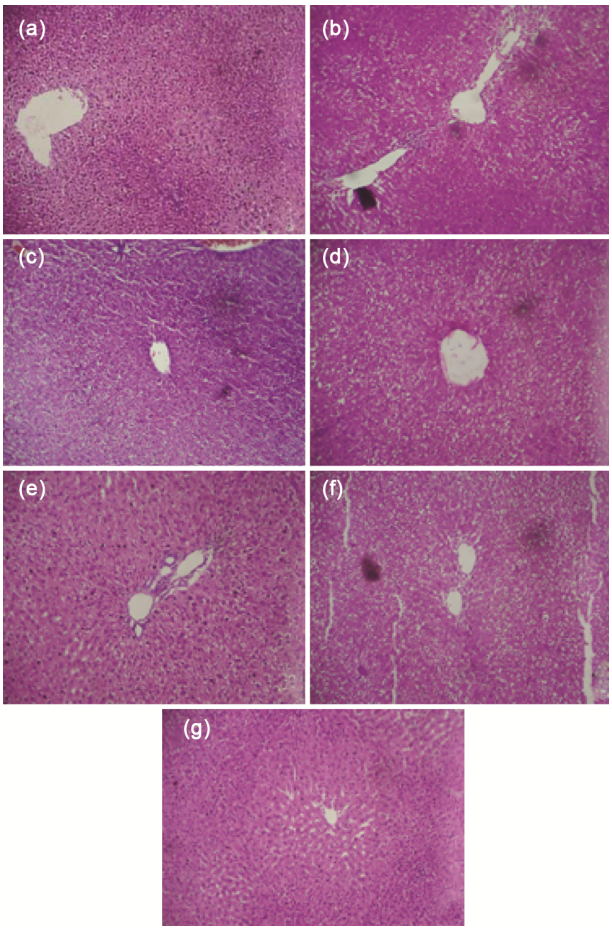

Fig. 1 — Protective effect of LWBA and RWBA extracts of *B. acutangula* on Ethanol induced hepatotoxicity. (a) Normal control- shows typical architecture of hepatocytes; (b) Ethanol treated- shows inflammatory infiltration and severe fatty changes; (c) Silymarin treated prior to Ethanol administration- shows normal architecture with less fatty changes; (d) LWBA (200 mg/kg) treated prior to Ethanol administration- shows a pattern of moderate fatty change; (e) LWBA (400 mg/kg) treated prior to Ethanol administration- shows mild fatty change; (f) RWBA (200 mg/kg) treated prior to Ethanol administration- shows a moderate fatty change; (g) RWBA (400 mg/kg) treated prior to Ethanol administration- shows mild fatty change; (H & E, x 150).

of the plant derived metabolites such as phytosterols, terpenoids, triterpenoids, alkaloids, tannins, steroids, resins and saponins. Previous work by the authors demonstrated the presence of these secondary metabolites in the LWBA extract[25] and methanolic leaf (LMBA) extract[26]. Some of these might also be responsible for the pharmacological activities of *B. acutangula.*

According to Shah *et al.*, ethanol induced hepatotoxicity is one of the frequently used models for the selection of liver protective remedies and the degree of hepatic injury is evaluated by the levels of leaked serum marker enzymes, protein, bilirubin and liver antioxidant enzymes[27]. Administration of ethanol results in elevated levels of SAST, SALT, SALP and also bilirubin and lipid peroxidation. Along with these, decreased level of total serum protein, GSH content, SOD and CAT activities are indicators of cellular damage in liver. These parameters were studied in the present work as markers of hepatotoxicity.

The alcoholic liver injury seems to be caused due to ethanolic metabolism and the consequence of the immune reaction to alcohol or acetaldehyde altered proteins[28]. Continued intake of ethanol boosts the activities of the liver microsomal ethanol oxidizing system which leads to the formation of peroxidase and reduced NADP. Hepatoprotective activity of herbal formulations containing different proportion of *Andrographis paniculata* was evaluated by Vetriselvan *et al.* using ethanol induced toxicity model in which water extracts showed considerable (p<0.05) hepatoprotective effect[29]. CCl_4 induced hepatic oxidative stress was lessened by the oral administration of *B. acutangula* methanolic leaf extract in a study conducted by Aldhafiri *et al*[30]. In the present study there was significant (p<0.001) rise in SAST, SALT, SALP and bilirubin levels and also reduction in protein indicating that considerable hepatocellular injury occurred in ethanol intoxicated rats. Suppression of heightened serum enzymes with depletion of elevated bilirubin level and an enhancement in the total protein content recommends the stability of biliary malfunction in rat liver during hepatic damage with toxicants[31]. Treatment with LWBA and RWBA extracts (200 mg/kg & 400 mg/kg) ameliorated the above said serum parameters to near normalcy (Table 2).

The amount of thiobarbituric acid reactive substances (TBARS) was measured to estimate the level of LPO in liver tissue. Our study indicated higher levels of TBARS in ethanol administered rats, which may be due to disproportionate free radical formation resulting in hepatic and other cellular impairment by ethanol[32]. Oral pretreatment of LWBA and RWBA extracts resulted in decreased lipid peroxidation (1.33 nM malondialdehyde (MDA)/mg protein & 1.71 nM MDA/mg protein, respectively at a dose of 400 mg/kg) indicating their anti-lipid peroxidation activities as shown in Table 3. It is well known that, Ayurvedic remedies could enhance free

radical scavenging mechanism. In order to treat a number of diseases, elimination of superoxide ion and hydroxyl radicals is possibly one of the most operative defense mechanisms. Depressed levels of SOD, catalase and GSH will result in the accumulation of the free radicals leading to harmful effects such as loss of cell membrane integrity and membrane function[33]. Catalase activity was lessened in ethanol treated rats, which could probably be due to loss of NADPH or generation of superoxide or increased activity of LPO or combination of all. A study conducted by Patel *et al.* on *Saccharum officinarum* showed that the administration of the extract significantly decreased antioxidant enzymes and bilirubin in the ethanol treated animals[34]. It also significantly decreased MDA and protein levels and increased GSH levels confirming its antioxidant activity in the animals treated with ethanol. A review carried out by Singh *et al*. highlighted on the hepatoprotective efficacy of 30 medicinal plants against ethanol induced liver damage[35]. Similarly, treatment with LWBA and RWBA extracts in the present study significantly (p<0.001) increased the level of antioxidant enzymes. This might be because of the protective efficacy of these extracts on the liver cells, which reduced the destruction of hepatocytes. According to Verma, liver microsomal metabolism of ethanol leads to increased LPO resulting in hepatitis and cirrhosis[36].

Histopathological results in the present study basically supported the results obtained from biochemical estimations. The liver sections of extract treated rats showed a marked degree of safety against ethanol induced alterations and they were nearly analogous to those from untreated control rats as shown in Fig. 1a-g. Related observations were made in the earlier works of the authors in which the leaf and root extracts showed considerable hepatoprotection against paracetamol and CCl_4 induced hepatic stress respectively[37,38].

Oral administration of LWBA and RWBA extracts amended the changed levels of biochemical parameters to near normalcy. The reclamation towards regularization enzyme levels and histological architecture produced by LWBA extract was found to be comparable with that of silymarin, whereas RWBA extract showed slightly reduced level of hepatoprotection compared to that of LWBA.

The present study elucidates the hepatoprotective property of aqueous leaf and root extracts of *B. acutangula* against ethanol induced hepatotoxicity. Results obtained in the present study reveal that of the two extracts tested, the leaf extract was having slightly better hepatoprotective activity than the root extract. Findings of the study highlights the plant`s ability to prevent the activation of toxicants by free radical scavenging.

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