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Hepatoprotective Activity of Root Extracts of *Boerhaavia erecta* L. and *B. rependa* L.

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Administration of alcoholic extracts of roots of *Boerhaavia erecta* L. and *B. rependa* L protect the liver from the toxic effect of CCl_4 by restoring the levels of serum bilirubin, serum total protein, albumin and subsequent decrease in the levels of serum globulin in experimental rats. The serum alanine transaminase, aspartate transaminase and alkaline phosphatase activities were also restored as compared to the normal rats. The hepatoprotective activity was evaluated to be more in *B. erecta* root extract treated groups.

The indigenous drug *Punarnava* obtained from the genus *Boerhaavia* L. (Nyctaginaceae) is known to be used for the treatment of inflammatory oedema[1,2], liver cirrhosis, diuretic and asthma[3]. *Boerhaavia erecta* L., (*B. punarnava* Shah.) is an erect, glabrescent and white flowered herb and *B. rependa* L., is a scandant climber with tubular purplish flowers and exerted stamens. Phytochemical analysis of the roots and leaves of these species showed the presence of alkaloid Punarnavine and reduced sugars[4]. The tribal community Soligas of the Biligiri Rangana Hill ranges, Karnataka, used the roots of *B. erecta* with goat's milk to cure infective hepatitis as a tribal medicinal practice. The traditional practitioners of Yalandur, Chamarajanagar district of Karnataka used the roots of *B. rependa* with butter milk to heal jaundice[5].

In the genus *Boerhaavia* L. clinical evaluation of hepatoprotective activity of the roots was reported only for *B. diffusa*[6,7]. In the present communication we report the comparative hepatoprotective efficacy of the alcoholic extracts of the roots of *B erecta* and *B. rependa* against carbon tetrachloride (CCl_4)-induced toxic hepatitis in Wistar rats.

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Roots of *Boerhaavia erecta* were collected from the forests of Biligiri Rangana Hill range and the roots of *B. rependa* collected from the agricultural waste lands of in and around Yalandur, Chamarajanagara district, Karnataka. The roots were thoroughly washed, cut into small pieces, dried in an oven at 70° for 4 d and powdered mechanically. One hundred grams each of the powdered roots of *B. erecta* and *B. rependa* were taken separately in a Soxhlet apparatus and refluxed with 50% ethanol for 4 d. The extracts were concentrated in vacuum using a rotary flash evaporator and the solvent was removed completely over a water bath and dried in a desiccator.

Male Wistar rats, weighing 175-200 g were obtained from the Central animal house, Department of Zoology, University of Mysore, Mysore. The experimental animals were divided into four groups of 16 each.. The group-I animals were treated as control. Hepatotoxicity was induced in the animals of group-II, III and IV by oral administration of CCl_4 (0.2 ml/100 g body weight). Feeding was done biweekly for four weeks. From third day onwards, the group-III and group-IV animals received an oral dose (100 mg/100 g b w for 25 d) of root extract of *B. erecta* and of *B .rependa*, respectively. All the animals were fed on commercial standard pellet diet (Hindustan Lever Ltd., Mumbai), water *ad libitum* and were maintained in the animal house (Zoology Department,

University of Mysore) at 25±2°, 12 h light/dark cycle and 60±5% relative humidity. All the animal experimental protocols have been approved by the Institutional Animal Ethics Committee, PG Department of Zoology, University of Mysore, Mysore.

Every week, four animals in each group were sacrificed and blood samples collected by direct heart puncture in to a sterilized dried centrifuge tube. Clear serum was collected and used for the assay of total bilirubin[8], total protein and albumin/globulin ratio[9], serum alanine and aspartate amino transaminase[10] and serum alkaline phosphatase activity[11].

The injury and disfunction of liver caused by the toxic effect of CCl_4 in experimental animals simulated the human viral hepatitis model[12]. In CCl_4-induced toxic hepatitis, a toxic reactive metabolite, trichloromethyle (CCl_3) radical was produced by the microsomaloxidase system. This activated radical binds covalently to the macromolecules of the lipid membranes of endoplasmic reticulum and causes peroxidative degradation of lipids. As a result fats from the adipose tissue were translocated and accumulated in the liver[13]. In most studies this toxic chemical has been used as a tool to induce hepatotoxicity in experimental animals[14,15]. In the present study also at the end of each week of treatment, blood samples of CCl_4 treated groups showed significant elevation in the levels of serum total bilirubin (1.84 to 3.68 mg/100 ml), serum globulin (2.50 to 2.82 mg/100 ml), aspartate aminotrasaminase (16.7 to 32.2 IU/100 mg of protein), alanine aminotransaminase (18.5 to 34.7 Int. units/100 mg of protein) and alkaline phosphatase (15.5 to 33.4 IU/100 mg of protein) activities as compared to the controls. These elevations are indicative of cellular leakage and loss of functional integrity of the cell membrane[16]. On the contrary, serum total protein and albumin levels decreased from 5.60 to 4.15 and 3.48 to 1.30 mg/100 ml, respectively, as shown in Table 1.

The estimation of serum total bilirubin confirms the intensity of jaundice. In normal population the serum bilirubin is in the range of 0.2 to 1 mg/100 ml of serum. In viral or toxic hepatitis the degree of excretion of bilirubin from the intestine is very less and bilirubin present in the liver is excreted into the canaliculi and then regurgitated into the blood stream. Hence hyperbilirubinemia is more common in hepatitis patients[17-19]. At the end of each week of treatment, *B. erecta* and *B. rependa* root extract-treatment significantly reduced levels of serum total bilirubin in the blood samples even with the toxic effect of CCl_4. However, the percentage of bilirubin restoration was found to be more in *B. erecta* treated groups and similar type of result was reported by Murthy and Srinivasulu[20] in Wistar rats treated with the leaf extracts of *Tephrosia purpuea* L.

It is known that liver synthesizes a number of serum proteins. The change in the serum protein levels forms the basis for important laboratory aids to diagnose the depth of jaundice[21]. Further, there is a correlation between the degree of serum hypoalbuminemia and hyperglobulinemia. In normal population the albumin/globulin ratio is in the range of 2:1. The Table-1, depicts that in CCl_4-treated animals, serum total protein and albumin levels decreased with a moderate elevation in the levels of globulin. Even with the toxic effect of CCl_4 the root extracts of *B. erecta* and *B. rependa were effective* in restoring the decreased levels of serum total protein from 6.68 to 6.10 and 6.53 to 6.08 mg/100 ml, respectively, and increased levels of serum globulin from 2.45 0 to 2.55 and 3.53 to 2.75 mg/100 ml, respectively. The percentage of serum protein level restore was found to be more with *B. erecta* treated groups. Similar type of antihepatotoxic effect of herbal extract of *Ricinus communis* was noticed by Visen *et al*[22] against CCl_4-induced toxic hepatitis.

The estimation of serum enzymes such as aspartate aminotrasaminase (ALT), alanine aminotransaminase (AST) and alkaline phosphatase (ALP) individually are helpful in the differential diagnosis of hepatic disease[23]. The normal value of ALT, AST and SALP ranges from 5 to 20, 5 to 15 and 7 to 9 IU/mg of protein, respectively. The concentration of these enzymes increase in serum whenever the liver tissue was damaged. It is presumably due to release of enzymes from the damaged cells. In acute hepatic necrosis the level of AST and ALT are expected to increase by 2 to 20 folds over that of controls. On the contrary, in obstructive and post hepatic jaundice elevation of ALP was more[24]. In the present investigation, in CCl_4 treated group the activities of these enzymes increased parallelly from 1 to 4 weeks of treatments (Table 1). Administration of root extracts of *B. erecta* and *B. rependa* prevented the increase in the levels of these enzymes, showed the pattern of recovery from the toxic effects.

In the Indian system of medicine many plants and their products were known to act as potential hepatoprotective principles but unless corroborated by clinical experimental evidence, the therapeutic effect of these plants cannot be confirmed. The results of this investigation showed that at the end of each week of treatment the root extracts of *B. erecta* and *B. rependa* were most effective in restoring the

TABLE 1: HEPATOPROTECTIVE ACTIVITY OF *B. ERECTA* AND *B. REPENDA*

Groups of Animals	Times in Weeks				F' value	C.D. at 5%
	I	II	III	IV		
Total Bilirubin						
Control	0.83±0.10	0.86±0.79	0.73±0.06	0.86±0.69	2.72	0.016
CCl_4	1.84±0.00	2.28±0.59	2.77±0.15	3.68±0.41	50.1	0.310
B. erecta	1.35±0.71	1.46±0.34	1.53±0.41	1.59±0.00	14.6	0.066
B.rependa	1.45±0.44	1.51±0.02	1.71±0.03	1.77±0.06	46.3	0.062
Total Protein						
Control	7.06±0.23	6.88±0.21	6.93±0.21	7.08±0.46	0.46	0.422
CCl_4	5.98±0.23	5.18±0.29	4.68±0.29	4.15±0.17	38.5	0.347
B. erecta	6.68±0.29	6.45±0.17	6.30±0.24	6.10±0.17	4.05	0.334
B.rependa	6.53±0.15	6.38±0.29	6.30±0.24	6.08±0.15	3.00	0.300
Albumin						
Control	4.68±0.29	4.53±0.24	4.60±0.24	4.73±0.33	0.43	0.372
CCl_4	3.48±0.24	2.60±0.28	1.875±0.39	1.33±0.15	40.7	0.405
B. erecta	4.18±0.39	3.93±0.15	3.70±0.24	3.55±0.17	4.44	0.359
B.rependa	3.75±0.24	3.63±0.38	3.55±0.17	3.40±0.29	1.00	0.406
Globulin						
Control	2.40±0.81	2.35±0.10	2.325±0.13	2.35±0.17	0.18	0.207
CCl_4	2.50±0.18	2.58±0.05	2.80±0.16	2.83±0.15	5.86	0.186
B. erecta	2.45±0.10	2.53±0.10	2.60±0.00	2.55±0.10	1.40	0.143
B.rependa	2.53±0.15	2.75±0.17	2.75±0.30	2.75±0.17	1.17	0.288
Aspartate aminotransaminase						
Control	8.43±0.22	7.52±0.24	7.78±0.75	9.19± 0.30	12.5	0.584
CCl_4	16.71±0.42	22.19±0.87	26.76±0.70	32.20±1.18	243.9	1.170
B. erecta	11.53±0.39	13.21±0.35	13.82±0.54	15.03±0.30	50.67	0.570
B.rependa	12.74±0.38	13.82±0.21	14.27±0.19	16.38±0.71	51.68	0.596
Alanineaminotransaminase						
Control	11.21±0.34	11.83±0.25	11.00±0.29	11.38±0.36	5.22	0.427
CCl_4	18.49±0.50	22.61±0.62	27.54±1.07	34.67±0.67	432.9	0.927
B. erecta	13.82±0.28	14.85±0.44	15.54±0.40	16.17±0.91	12.28	0.790
B.rependa	14.31±0.19	15.14±0.30	16.17±0.51	17.05±0.47	37.7	0.541
Alkaline phosphatase						
Control	9.67±0.64	9.85±0.51	8.63±0.25	8.40±0.64	7.78	0.725
CCl_4	15.25±0.34	21.25±0.24	26.00±0.43	33.36±1.44	263.5	1.289
B. erecta	12.73±0.60	13.27±0.47	14.02±0.50	14.47±0.28	5.16	0.940
B.rependa	13.31±0.67	14.46±0.63	14.81±0.77	15.60±0.43	8.93	0.885

The values of total bilirubin, total protein, albumin and globulin are expressed as mg/100 ml of serum. The values of ALT, AST and SALP are expressed as IU/100 mg of protein. The mean value ± S.D. of four animals in each group and the F' value at 5% above 3.49 are significantly different.

altered liver function. However, gradual increase in the levels of serum total bilirubin, globulin, and serum enzymes noticed even in the drug administered groups from first to fourth week of treatment. These animals received CCl_4 biweekly for four weeks. Though the levels of these marker enzymes were slightly more than the control, they were significantly lower when compared to CCl_4 treated groups.

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Patient Compliant Dosage Form for Roxithromycin

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Roxithromycin, a macrolide antibiotic, is extremely bitter in taste. The present study deals with various techniques utilized for taste masking of roxithromycin viz granulation with Eudragit E 100 and complexation with ion exchange resins. Of these, complexation with ion exchange resins yielded complete taste masking. The drug resin complexation procedure was optimized with respect to parameters like taste of the resinate, drug to resin ratio and volume of medium. The complexation between roxithromycin and ion exchange resin was confirmed by differential scanning calorimetry. The taste-masked complex was then formulated into palatable mouth-dissolve tablets. The tablets were evaluated for various quality control parameters. Taste evaluation of the

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