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HEPATOPROTECTIVE EFFECT OF THE ETHANOL EXTRACT OF BOERHAVIA REPENS IN ALBINO RATS

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Abstract:

India is a native to many medicinal plants. People in rural areas mainly depend on these native medicinal plants to cure various diseases. Boerhavia repens is one such herbal plant widely used in India for its medicinal virtues. Current study was carried out to explore the hepatoprotective role of this plant. Hepatoprotective activity was carried out in paracetamol induced liver damage in albino rats. Boerhavia repens ethanol extract (200 mg) was found to produce significant hepatoprotective activity as there was marked decrease in serum hepatic markers. The regeneration of hepatocytes was also an evidence for the hepatoproctive activity of extract.

Key word: *Hepatoprotective, Indigofera trifoliata, albino rats, herbal drug, paracetamol.*

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INTRODUCTION:

Liver is one of the important and vital organs in human body and the main site for metabolism and excretion. So it has an impressive role in the upkeep, performance and regulating homeostasis of the body. Liver is also involved with almost all the biochemical pathways related to growth, nutrient supply, maintaining immunity and reproduction [1].

Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to drugs, toxins, infections, and another non-infectious agent. Hepatotoxic agents can react with the basic cellular components and consequently induce cell damage, necrosis which lead to fibrosis [2].

Modern, conventional or synthetic drugs used in the treatment of hepatic disease are inadequate and as there is no reliable liver protective agent, many Ayurvedic preparations, the Indian system of medicine, are recommended for the treatment of liver disorders [3]. Herbal medicines play a major role in the management of various kinds of hepatic disorders. Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity.

Boerhavia repens is an annual to perennial, prostrate or straggling herb, with stems up to 60cm long. The plant has a slender taproot and a stem that is few to much-branched. The plant is widely used, mainly in India, for its medicinal virtues. It is harvested from the wild, mainly for medicinal use, but also for local use as a food. It also has the potential to be used to cover the soil and protect it from erosion. The present investigation evaluates the hepatoprotective role of *Boerhavia repens.*

MATERIALS & METHODS:

Preparation of plant extract:

The whole plant of *Boerhavia repens* was collected from the Campus of Government Vidarbha Institute of Science and Humanities, Amravati (Maharashtra). The plant materials were dried under shade and grinded to a coarse powder. Then the air dried coarsely powdered plant material, were extracted with ethanol in a Soxhlet apparatus, concentrated and dried under reduced pressure in a large petridish. The dried extract was stored in airtight container in refrigerator below 10ºC until experimental testing [4].

Procurement, maintenance and acclimatization of animals:

Wistar albino rats (200-220 gm) used for the experiment were purchased from the animal house of Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra) and maintained in animal house of Government Vidarbha Institute of Science and Humanities, Zoology Department, Amravati (Maharashtra). All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photoperiod (12:12hr dark: light cycle) and temperature (25±2ºC). They were provided with commercial rat feed and water given *ad libitum*. The animals were habituated to laboratory conditions for 15 days prior to the experimental protocols to minimize any non-specific stress.

Selection of animals for experiments:

In each experiment thirty adult, healthy male albino rats of Wistar strain which were three months of age and weighing about 200-220 gm. were selected. The experimental animals were divided into five groups (G_1, G_2, G_3, G_4, and G_5) containing Six animals each. As per the treatment plan first group served as a control and the rest served as experimental groups.

Acute toxicity test:

Toxicity test:

Adult albino male rat were divided into four groups i.e. containing six animals in each group**.** Acute toxicity study was performed as described by Turner (1971) [7]. The rat were fasted for eighteen hours, the prepared drug was administrated orally at three different doses 500,1000 and 1500 mg/kg body weight, respectively to different groups of rat separately. Control rats received the vehicle (distilled water) only. The animals were observed for 72 hrs for behavioral changes and mortality. As there was no mortality seen at this dose level, the procedure was repeated by further increasing the dose (2000 mg/kg) using fresh animals.

Treatment protocol: (Experimental design)

Overnight fasted, healthy rats were randomly divided into five groups (6 rats per group)

Group I: (Control group) / (Normal control) received oral dose of distilled water (1ml each) for15 days.

Group II: (Toxic control) Paracetamol control group, received Paracetamol dissolved in normal saline (NaCl 0.9%) orally for 15 days (Parthsarthy,*et al*., 2007).

Group III: (Standard group) received Silymarin and in addition received Paracetamol dissolved in normal saline orally for 15 days.

Group IV: (Extract 100 group) received 100 mg/kg alcoholic extract of plant parts and in addition Paracetamol dissolved in normal saline orally for 15 days.

Group V: (Extract 200 group) Received 200 mg/kg of alcoholic extract of plant parts and in addition Paracetamol dissolved in normal saline orally for 15 days.

Preparation of samples for biochemical studies:
After administration of the last dose of the treatment, blood samples were collected on 15th day in case of chronic liver damage experiments. All rats were sacrificed by cervical dislocation and blood was collected by intracardiac puncture. The blood was kept for 30 minutes without disturbing. The clot was dispersed with glass rod and then centrifuged for 15-20 minutes at 2000 r.p.m. to separate serum.

Assessment of hepatoprotective activity:

Biochemical investigation:

Biochemical evaluation: The serum of each animal of all experimental groups of rats was used for estimation of various biochemical parameters to determine the functional state of the liver.

Statistical Analysis:

The result was expressed as mean ± SEM (Standard error of mean) for each parameter. The statistical analysis was done by using Student t-test for estimating variation in set of data. Data was analysed to determine the significant difference of result between the treated and control groups. The statistically significant level was taken as described by Khan and Khanum (2008) [5].

	Bili –T mg/dl	Bili –D mg/dl	SGOT Units/ml	SGPT Units/ml	Total Protein mg/dl	Albumin mg/dl	ALP Unit/ml	Cholesterol mg/dl
Control	0.78±0.3	8.23±0.33	215.18±1.1	84.23±0.7	7.77±0.45	3.38±0.1	81.66±0.92	184.13±1.7
Paracetamol	1.43±0.18***	10.5±0.43***	297.8±7.6***	111.7±2.4***	11.85±0.58***	4.7±0.28***	98.41±1.8***	212.7±3.5***
Silymarin	1.18±0.08***	8.37±0.26	251.40±1.99***	84.83±0.68	8.13±0.34	3.60±0.29	85.86±1.46***	190.6±1.6***
Ext. 1 150Mg/Kg	0.99±0.03***	10.00±0.39***	254.06±13.4**	96.06±1.05***	9.2±0.50**	3.4±0.48	86.53±0.95***	203.81±2.79***
Ext. 2 200Mg/Kg	0.95±0.26***	9.50±0.34***	272.8±2.43***	93.80±0.67***	8.24±0.47	3.85±0.3	85.66±0.8***	195.2±1.5***

Observation and Result

Table No. 1 Effect of alcoholic extract of *Boerhavia repens* whole plant on Paracetamol-induced hepatotoxicity in rats.

Value in mean ± S.E (Standard Error), n=6,*P<0.05,**P<0.02, ***P<0.01, when compared between group.

Acute toxicity study of *Boerhavia repens* whole plants extract

No mortality and changes in behaviour were observed in all the treated and control group of rat up to dose of 2000 mg/kg body weight *Boerhavia repens* whole plants extract. Hence 200 mg/kg body weight plant extract was used as the maximum dose for hepatoprotective study.

RESULT AND DISCUSSION:

Table 1 shows the hepatoprotective activity of alcoholic extract of *Boerhavia repens* (whole plant) on Paracetamol induced hepatotoxicity in rats. Paracetamol treatment showed an increased level of Bilirubin T, Bilirubin D, SGOT, SGPT, Total protein, albumin, ALP and Cholesterol as compared to the control group.

However in the group of rats pre-treated with Silymarin the levels of Bili. T, Bili. D, SGOT, SGPT total protein, albumin and ALP were considerably lowered as compared to paracetamol group. Similarly the animals treated with alcoholic extract of *Boerhavia repens* showed statistically significant ($p<0.01$) hepatoprotective activity against paracetamol induced hepatotoxicity in rats, which is comparable to the standard drug Silymarin. These findings suggested that the extract administered has significantly neutralized the toxic effects of paracetamol and helped in regenerating the hepatocytes.

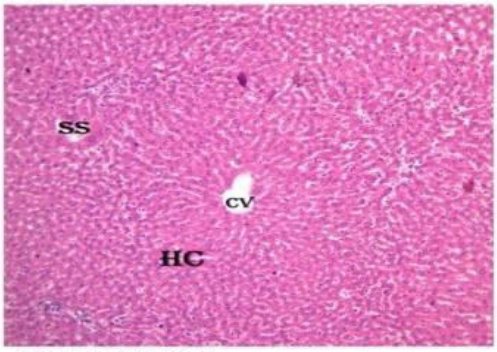
Paracetamol (acetaminophen) produces acute hepatic damage on accidental over dosage. It is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite, N–acetyl–p–benzoquinamine (NAPQI), which is normally conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction [6] and the development of acute hepatic necrosis. In the assessment of liver damage by acetaminophen the determination of enzyme levels such as SGOT, SGPT is largely used. Necrosis or membrane damage releases the enzyme into systemic circulation and hence it can

be measured in the serum. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Droatman, 1978).

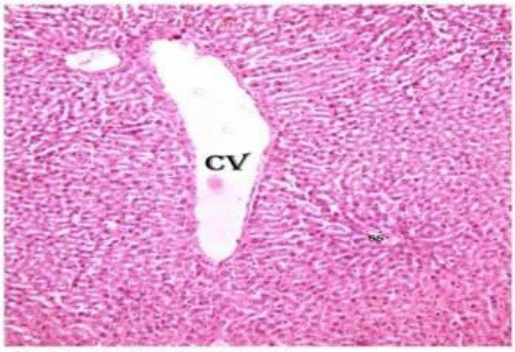
Increase in serum level of ALP is suggested to be due to increased synthesis, in presence of increasing biliary pressure (Muriel, 1992) [8]. The reversal of increased serum enzymes in acetaminophen- induced liver damage, by whole plant extract of *Boerhavia repens* may be due to its membrane stabilizing activity. The plant extract decreased acetaminophen induced elevated enzyme levels in test group, indicating the protection of structural integrity of hepatic cell membrane of damaged liver cells.

Histological Study:

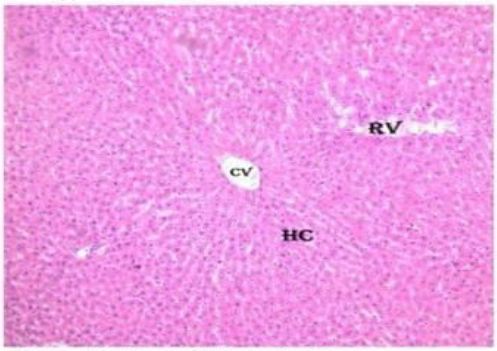
The histopathological study studies also support the hepatoprotective role of *Boerhavia repens* plant extract. Histological section of control group (Group I) of rat liver tissue shows normal hepatic cells (HC) with central vein (CV) and normal sinusoidal spaces (SS) (Fig.I). In paracetamol treated group (Group II) hepatotoxicity was observed by severe necrosis (N), formation of vacuole (VC) and increased sinusoidal spaces (Fig.II). However in the group III (Silymarin treated group) there was mild degree of necrosis with normalization of cells (HC), central vein, restoration of vacuole and reduced sinusoidal dilation (SS)(Fig.III). The Group IV and Group V, tissue section (extract treated group) also showed normalization of liver tissue architecture comparable to group III animals (Fig.IV&V). Thus proving that the *Boerhavia repens* plant extract can significantly prevent the paracetamol induced hepatotoxicity.



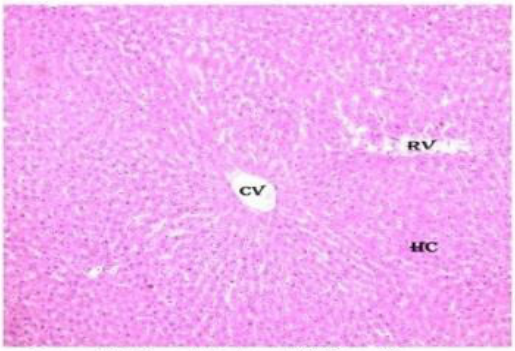
Group IControl
Fig.I



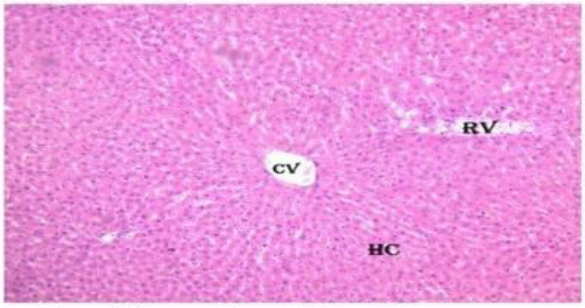
Group II Paracetamol
Fig.II



Group III Para.+ Silymarin
Fig.III



Group IV Para.+ Extract 150m /Kg
Fig.IV



Group V Para.+ Extract 200mg/Kg
Fig.V

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