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HEPATOPROTECTIVE ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN. AGAINST D-GALACTOSAMINE INDUCED HEPATOTOXICITY IN MICE

G. Nalini ^{*1}, Ajithadasaruna ², N. Chidambaranathan ¹ and N. Jegan ¹

Department of Pharmacology ¹, K. M. College of Pharmacy, Uthangudi, Madurai - 625017, Tamil Nadu, India.

Joint Directorate of Medical Education ², Kilpauk, Chennai - 600010, Tamil Nadu, India.

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Correspondence to Author:

Mrs. G. Nalini

Associate Professor,
Department of Pharmacology,
K. M. College of Pharmacy,
Uthangudi, Madurai - 625017,
Tamil Nadu, India.

E-mail: 16nalini@gmail.com

ABSTRACT: *Boerhaavia diffusa* is a natural herb possesses potent diuretic, anticancer and antioxidation properties. This work was designed to investigate the effect of hydro alcoholic extract of *Boerhaavia diffusa* Linn. on hepatoprotective efficacy in D-galactosamine-intoxication in mice. Albino Mice divided into 5 groups each consisting of 6 animals. Group 1 served as vehicle control, Group 2 served as hepatotoxin (D-Galactosamine treated) group, Group 3 served as positive control (Vitamin-E) treated group, Group 4, 5 served as treatment control (200 and 400 mg/kg b.w p.o.) HAEBD. Treatment given for 8 days and D-Gal on last day for all the groups except normal control. D-galactosamine-induced toxicity was manifested by the elevation of serum hepatic marker enzyme activities (Aspartate amino transferase, Alanine amino transferase, alkaline phosphatase and gamma-glutamyl transpeptidase). It also caused significant lipid peroxidation and reduced the levels of antioxidant defense mechanisms. Treatment with HAEBD (200 and 400 mg/kg body weight) orally for 8 days decreased hepatic marker enzyme activities and lipid peroxidation products, lipid hydroperoxides and conjugated dienes, increased the activities of free-radical scavenging enzymes superoxide dismutase, catalase and glutathione peroxidase and the levels of non-enzymatic antioxidants reduced glutathione, Vitamin C and Vitamin E. These findings demonstrate that HAEBD acts as a hepatoprotective and antioxidant agent against D-Galactosamine induced hepatotoxicity.

INTRODUCTION: Liver diseases remain one of the serious health problems. Acute hepatic failure (AHF) has become the focus of attention for researchers because of its extremely high mortality rate and the lack of ideal treatment in clinical medicine. Management of liver diseases is still a great challenge to the modern medicine.

In the absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal plants in Ayurveda are recommended for the treatment of liver disorders.

Natural treatments from medicinal plants are considered to be effective and safe medicaments for hepatotoxicity. The effectiveness of these plant products must be proved so as to identify newer medicaments acting against hepatic injury. Hepatic injury may be caused by different agents such as viruses, chemicals, alcohol, autoimmune diseases and D-Galactosamine. D-Galactosamine (D-GalN) is a well known hepatotoxicant. It induces liver injury closely resembling human viral - hepatitis

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with necrosis, inflammation and regeneration. The toxicity of D-GalN is associated with the depletion of uridine pools, limited ribonucleic acid (RNA) and protein synthesis thus overall affecting hepatocellular function. *Boerhaavia diffusa* L. (Nyctaginaceae), a perennial herbaceous plant has chosen due to the numerous phytoconstituents present in it.

Considering its use in Indian folk medicine and the large number of chemical substances isolated from *B. diffusa* it seemed justified evaluating the potential existence of a hepatoprotective effect of this plant. Plant voucher specimen no. is (KMCP/GN/BD-0296).

Experimental Animals: In house laboratory bred albino mice were selected for the study. Animals were maintained under controlled temperature at 20 ± 2 °C and relative humidity of 50 - 60% with an alternating 12 h light / dark cycle. The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed and water *ad libitum*. The research work was approved by Institutional animal Ethical Committee. (IAEC/KMCP/42/2012-2013)

Methodology: Swiss Albino mice with body weight 25 - 30 g were selected and used for the study and divided into five groups of six animals each.

Group 1- Normal Control: Received normal diet and water for 8 days.

Group 2 - Tumor Control: Received distilled water 5 ml/kg b.w. p.o. for 8 days and D-galactosamine in distilled water at 200 mg/kg b.w intra-peritoneally after one hour of vehicle on the 8th day¹.

Group 3 - Standard: Received Vitamin-E Pre-treatment for 8 days and D-galactosamine after 1 h of drug on the 8th day.

Group 4: Received 200 mg/kg b.w. p.o. hydro alcoholic extract of *Boerhaavia diffusa* orally daily for 8 days. A single dose of D-galactosamine in distilled water given intra peritoneally after one hour of drug on the 8th day.

Group 5: Received 400 mg/kg b.w. p.o hydro alcoholic extract of *Boerhaavia diffusa* orally daily for 8 days². A single dose of D-galactosamine in

distilled water given intra peritoneally after one hour of drug on the 8th day.

Biochemical Estimation: On the 9th day after overnight fast all animals were sacrificed using Ketamine HCl and blood was withdrawn by cardiac puncture and allowed to clot for 30 min at room temperature. The serum was separated by using cooling centrifuge and used for the assay of marker enzymes *viz.* AST, ALT, ALP, TP, TB, GGPT and total albumin. Different biochemical parameter like Alanine transaminase [ALT], Aspartate transaminase [AST],^{3,4} alkaline phosphatase [ALP],⁵ γ -glutamyl transferase [γ GTP], Bilurubin,⁶ Total protein were estimated using serum.

Evaluation of Biochemical Parameters: The livers were dissected out immediately, washed with ice-cold saline and 10% homogenates in phosphate buffer solution (pH 7.4) were prepared. The following biochemical measurements were carried out in the liver tissues. Liver homogenate was used for the assay of MOA while some fraction of homogenates were centrifuged at 2500 rpm for 10 min at 4 °C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)^{7, 8, 9} by standard methods using enzyme assay kits. The enzyme assays were performed on a semi-auto analyser ERBA Chem⁷.

Histopathological Observation: The liver from each group was aseptically excused stored separately for analysis of oxidative stress-related biomarkers and in phosphate - buffered formalin (10%) for histopathological evaluation.

Statistical Analysis: The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Newmann Keul's multiple range tests. The values are represented as Mean \pm SEM. Probability value at $p < 0.01$ was considered as statistically significant.

RESULTS:

Biochemical Observations: Significant increase in ($p < 0.01$) serum Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Total bilirubin (TB) and Gamma-glutamyl transpeptidase (GGTP) and significant decrease in ($p < 0.01$) Total protein (TP) and Total albumin (TA) levels were observed in animals treated with

D-Galactosamine (Group II) as compared to normal control group (Group I). Pretreatment with hydro alcoholic extract of *Boerhaavia diffusa* Linn. (HAEBD) at a dose 200 and 400 mg/kg orally for 8 days decreased the levels of above indices like AST, ALT, ALP, TB, GGTP and increased the

levels of TP and TA significantly ($p < 0.01$) in Group IV and V. Vitamin-E pretreatment produced significant decrease in ($p < 0.01$) serum AST, ALT, ALP, TB, GGTP and significant increase in TP and TA at ($p < 0.01$) in Group 3 **Table 1**.

TABLE 1: EFFECT OF HAEBD AND VITAMIN-E PRETREATMENT ON BIOCHEMICAL PARAMETERS IN MICE INTOXICATED WITH D-GALACTOSAMINE

Group	AST (IU/ml)	ALT (IU/ml)	ALP (IU/ml)	TP (gm/dl)	TB (mg/dl)	GGTP (mg/dl)	Total Albumin (g/dl)
G1	44.40 ± 1.45	29.15 ± 1.05	22.65 ± 1.30	5.15 ± 0.12	1.90 ± 0.10	95.80 ± 2.65	3.75 ± 0.16
G2	106.90 ± 3.40*a	92.55 ± 3.25*a	140.15 ± 5.30*a	3.15 ± 0.22*a	4.30 ± 0.20*a	170.25 ± 5.85*a	2.15 ± 0.10*a
G3	58.05 ± 1.80*b	40.60 ± 2.10*b	53.40 ± 2.40*b	3.90 ± 0.25*b	2.70 ± 0.15*b	120.20 ± 3.10*b	2.85 ± 0.15*b
G4	66.60 ± 2.10*b	52.75 ± 2.80*b	66.40 ± 2.95*b	4.50 ± 0.30*b	3.22 ± 0.18*b	138.15 ± 3.65*b	2.50 ± 0.14*b
G5	61.90 ± 1.95*b	45.85 ± 2.60*b	58.50 ± 2.65*b	4.05 ± 0.27*b	2.90 ± 0.16*b	128.90 ± 3.25*b	2.30 ± 0.12*b

Note: G1 – Normal Control, G2 – Toxic Control, G3 – Positive control,

G4 – Treatment (HAEBD 200 mg/kg), G5 – Treatment (HAEBD 400 mg/kg)

Values are expressed as Mean ± SEM. Values are found out by using one way ANOVA followed by Newmann Keul's multiple range tests.

*a – values are significantly different from Normal control at $p < 0.01$.

*b – values are significantly different from Toxic control (G2) at $p < 0.01$.

Biochemical Observation in Liver Homogenate

Tissue: In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with D-Galactosamine (Group 2) as compared to normal control group (Group 1) **Table 2**.

TABLE 2: EFFECT OF HAEBD AND VITAMIN E PRE-TREATMENT ON BIOCHEMICAL LIVER PARAMETERS IN D-GALACTOSAMINE INDUCED HEPATOTOXICITY

Group	SOD (U/mg) Protein	CATALASE (U/mg) Protein	GPX (U/mg) Protein	MOA (U/mg) Protein
G1	130.30 ±2.45	288.40 ±4.40	1.15 ±0.09	3.90 ±0.22
G2	66.20 ±1.30*a	190.80 ±2.75*a	0.35 ±0.02*a	7.40 ±0.45*a
G3	86.05 ±1.60*b	258.40 ±3.90*b	0.88 ±0.06*b	4.50 ±0.30*b
G4	95.45 ±1.95*b	235.15 ±3.50*b	0.55 ±0.04*b	5.25 ±0.38*b
G5	90.65 ±1.75*b	242.75 ±3.65*b	0.70 ±0.07*b	4.80 ±0.32*b

Note: G1 – Normal Control, G2 – Toxic Control, G3 – Positive control, G4 – Treatment (HAEBD 200mg/kg), G5 – Treatment (HAEBD 400 mg/kg). Values are expressed as Mean ± SEM. Values are found out by using one way ANOVA followed by Newmann Keul's multiple range tests. *a – values are significantly different from Normal control at $p < 0.01$. *b – values are significantly different from Toxic control (G2) at $p < 0.01$.

Pretreatment with hydro alcoholic extract of *Boerhaavia diffusa* Linn. (HAEBD) at a dose of 200 and 400 mg/kg orally for 8 days increased the levels of SOD, CAT and GPx levels and decreased

the levels of LPO significantly ($p < 0.01$) in Group 4 and 5. Vitamin-E pretreatment also produced significant increase in ($p < 0.01$) liver homogenate enzymes such as SOD, CAT, GPx levels and decreased the levels of LPO significantly ($p < 0.01$) in Group 3.

The levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C and Vitamin E in the liver homogenate of D-Galactosamine induced hepatotoxic mice showed significant decrease ($p < 0.01$) when compared to normal control animals **Table 3**. The levels of non-enzymatic antioxidants in D-Galactosamine hepatotoxic mice significantly increased on pretreatment with *Boerhaavia diffusa* Linn. (HAEBD) at both doses tested when compared with untreated hepatotoxic mice.

TABLE 3: EFFECT OF HAEBD ON NON-ENZYMATIC ANTIOXIDANT LEVELS IN THE LIVER TISSUE OF D-GALACTOSAMINE INDUCED HEPATOTOXICITY

Group	Glutathione mg/100g tissue	Vitamin C mg/100g tissue	Vitamin E mg/100g tissue
G1	130.60±3.60	0.80±0.10	5.95±0.70
G2	73.40±1.75*a	0.30±0.03*a	2.30±0.30*a
G3	110.25±2.70*b	0.70±0.07*b	5.60±0.55*b
G4	97.30±2.15*b	0.62±0.04*b	4.95±0.40*b
G5	90.60±1.95*b	0.66±0.06*b	5.20±0.60*b

Note: G1 - Normal Control, G2 - Toxic Control, G3 - Positive control, G4 - Treatment (HAEBD 200 mg/kg), G5 - Treatment (HAEBD 400 mg/kg). Values are expressed as Mean ± SEM. Values are found out by using one way ANOVA followed by Newmann Keul's multiple range tests. *a - values are significantly different from Normal control at $p < 0.01$. *b - values are significantly different from Toxic control (G2) at $p < 0.01$.

Histopathological Observations: Histology of liver sections of normal control animals (Group 1) showed normal liver architecture with central vein, well preserved cytoplasm and prominent nucleus and nucleolus **Fig. 1A** while the liver sections of galactosamine treated animals (Group 2) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells **Fig. 1B**.

The Vitamin-E (Group 3) exhibited protection from galactosamine induced changes in the liver **Fig. 1C**. While hydro alcoholic extract of *Boerhaavia diffusa* Linn. (HAEBD) pretreatment at a dose of 200 and 400 mg/kg (Group 4 and 5) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with well-preserved cytoplasm **Fig. 1D, 1E**. HAEBD pre-treatment also caused marked decrease in inflammatory cells.

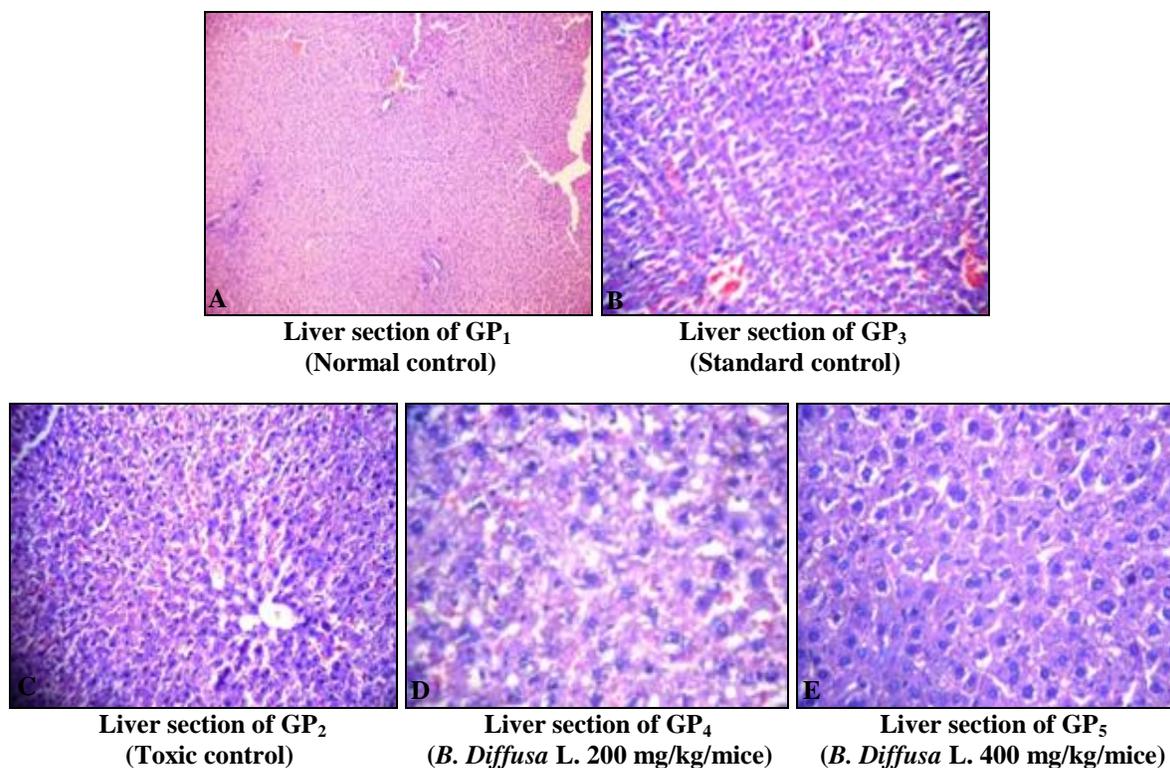


FIG. 1: HISTOPATHOLOGICAL STUDIES OF LIVER TISSUE

DISCUSSION: Liver damage induced by D-Galactosamine, reflects disturbances of liver cell metabolism, which lead to characteristic changes in the serum enzyme activities. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the hepatocyte¹⁰. Their estimation in the serum is useful as a quantitative marker of the extent and type of hepatocellular damage. D-Galactosamine - induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH·), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane¹¹.

D-Galactosamine toxicity enhanced lipid peroxidation and reduced antioxidants were reported in the kidney¹². Increased lipid peroxidation in various tissues cause functional

degradation increase oxidative stress. Treatment with HAEBD and Vitamin E showed a significant reduction in lipid peroxidation which might be due to the antioxidant ability of these compounds and free-radical scavenging effects. Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage, and the inactivation of many enzymes¹³.

The enzymatic antioxidant defense system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase. Reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H₂O₂. Superoxide dismutase protects against the superoxide radical

($O_2^{\cdot-}$), which damages the membrane and its biological structure. Catalase primarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues¹⁴. Significant increases in the activities of these enzymes were observed on HAEBD administration.

The second line of defense consists of the non-enzymic scavenger's glutathione, ascorbic acid, and α -tocopherol, which scavenge residual free radicals escaping from decomposition by the antioxidant enzymes. Glutathione a major non-protein thiol in living organisms plays a central role in coordinating the antioxidant defense process. Glutathione reacts directly with reactive oxygen species and electrophilic metabolites, protects the essential thiol group from oxidation, and serves as a substrate for several enzymes including glutathione peroxidase. Apart from glutathione, α -tocopherol and ascorbic acids are important free-radical scavengers which protect cell membrane against toxic agents. Vitamin-C functions as a free-radical scavenger of oxygen radicals and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid appears to trap the peroxy radical in the aqueous phase with a rate large enough to lipids and dehydroascorbate is produced in this reaction. Both Vitamins C and E have a synergistic action in scavenging oxygen-derived free radicals¹⁵.

Vitamin C functions as a free-radical scavenger of oxygen radicals and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid appears to trap the peroxy radical in the aqueous phase with a rate large enough to lipids¹⁶ and dehydroascorbate is produced in this reaction. A thiol cycle converts the dehydroascorbate into ascorbate. The thiol cycle consists of a GSSG / GSH couple¹⁷. Thus glutathione in blood keeps up the cellular levels of the active form of Vitamin C. When there is a reduction in glutathione, the cellular level of ascorbic acid is also lowered. The observed decrease in the levels of α -tocopherol and ascorbic acid in the D-galactosamine mice might be due to an antioxidant defense against increased ROS or

due to a decrease in glutathione levels in D-galactosamine - hepatotoxic mice. Our study observed increase the levels of these antioxidants in HAEBD and Vitamin-E administered mice.

The ability of HAEBD to enhance the levels of antioxidants along with its anti-lipid peroxidative activity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepatotoxicity. Studies on the anti-oxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the anti-oxidative properties of polyphenols depend on hydroxylation of ring B.

The present results corroborate the protective action of HAEBD in D-Galactosamine intoxication of mice, particularly noticeable with the high dose used by us (400 mg/kg body weight). Supplementation with this flavonoid ameliorated the hepatoprotective and antioxidant activity in D-Galactosamine-induced hepatitis in mice.

CONCLUSION: In conclusion, our findings demonstrated that *Boerhaavia diffusa* linn. possesses hepatoprotective and antioxidant activity, which is evidenced by lowered serum hepatic marker enzyme activities, increased antioxidant enzymes levels which is also supported by histopathological studies. Therefore Hydro alcoholic extract of *Boerhaavia diffusa* Linn. showed promising hepatoprotective and antioxidant activity against D-Galactosamine induced Hepatotoxicity.

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CONFLICT OF INTEREST: We declare that we have no conflicts of interest.

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