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Mitigating of Paracetamol-induced Hepatotoxicity in Albino Mice Using *Boerhaavia diffusa* Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author PG did conceptualization of the manuscript. Author UK and SD wrote and prepared the original draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Boerhaavia diffusa, renowned for its therapeutic efficacy in chronic liver diseases, operates through multifaceted mechanisms to uphold internal homeostasis. Its pivotal roles encompass modulation of intermediary metabolism of macromolecules, synthesis of crucial plasma proteins, enzymatic activities, and bile excretion.In this investigation, we aimed to evaluate the hepatoprotective potential of *Boerhaavia diffusa* against paracetamol-induced hepatotoxicity in rats. Our study revealed promising outcomes as the extracts obtained from *Boerhaavia diffusa* demonstrated a significant hepatoprotective role in albino rats subjected to hepatotoxicity induced by paracetamol

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administration. During the study, the animals were administered doses of 1g/kg b.wt and 2 g/kg b.wt of the plant extracts. Following paracetamol administration, conspicuous changes were observed in hepatocyte biochemical markers, indicative of hepatotoxicity. However, treatment with *Boerhaavia diffusa* extracts ameliorated these alterations, underscoring their potential to mitigate paracetamol-induced liver injury. These findings underscore the significant hepatoprotective properties inherent in the plant extracts derived from *Boerhaavia diffusa*. which can be utilized as a potential therapeutic avenue for the management of paracetamol-induced liver damage. These findings hold promise for the development of novel therapeutic interventions aimed at safeguarding liver health and mitigating the adverse effects of hepatotoxic insults.

Keywords: Boerhaavia diffusa; hepatotoxicity; bilirubin; bioactive compound.

1. INTRODUCTION

Paracetamol-induced hepatotoxicity represents a significant clinical challenge due to its widespread use as an over-the-counter medication and its potential to cause severe liver damage if ingested in excessive amounts. Hepatocellular necrosis, oxidative stress, and inflammatory responses are among the hallmark features of paracetamol overdose-induced liver injury, culminating in acute liver failure if not promptly addressed [1,2]. Consequently, the exploration of effective therapeutic strategies to counteract paracetamol-induced hepatotoxicity is of paramount importance in safeguarding public health.

Boerhaavia diffusa, commonly known as "punarnava," has emerged as a promising candidate for hepatoprotection, drawing upon its extensive traditional use and rich pharmacological profile. As a member of the Nyctaginaceae family, this perennial herbaceous plant is indigenous to regions of India and Brazil, where it has been revered for its medicinal properties for centuries. In Indian traditional medicine systems such as Ayurveda, *Boerhaavia diffusa* is revered for its versatility in addressing various ailments, including gonorrhea, internal inflammation, liver and gallbladder disorders, and serving as a diuretic. Similarly, Brazilian herbal medicine has long recognized the therapeutic potential of *Boerhaavia diffusa* in treating liver-related ailments and eye diseases, as well as its efficacy as an antidote for snake and rat bites [3-7].

The pharmacological activities of *Boerhaavia diffusa* are attributed to its diverse array of bioactive constituents, including glycoproteins, alkaloids, flavonoids, and terpenoids [8]. Studies have demonstrated the antimicrobial activity of glycoproteins derived from *Boerhaavia diffusa*, particularly against bacteriophages (RNA), highlighting its potential in combating infectious diseases [9]. Furthermore, chloroform-based extracts of *Boerhaavia diffusa* have exhibited significant activity against diabetes, aligning with its traditional use in Ayurvedic medicine for diabetes management [10]. The isolation and characterization of various metabolites from *Boerhaavia diffusa* roots, such as Punarnavine, hentriacontane, β-Sitosterol, and β-D-glucoside tetracosanoic acid, underscore its pharmacological complexity and therapeutic potential [11, 12].

Given the traditional use and pharmacological promise of *Boerhaavia diffusa*, this research endeavors to explore its effectiveness in mitigating paracetamol-induced hepatotoxicity using an experimental model with albino mice. By bridging traditional knowledge with contemporary scientific inquiry, this research holds the potential to contribute to the development of novel therapeutic interventions for paracetamol overdose-induced liver injury. The findings of this study may pave the way for the advancement of hepatoprotective strategies in clinical settings, offering new avenues for the prevention and management of drug-induced liver damage. By harnessing the therapeutic potential of *Boerhaavia diffusa*, we can aspire to enhance patient outcomes and alleviate the burden of liver diseases on global health systems. Thus, this research represents a significant step forward in the exploration of natural remedies for hepatoprotection and underscores the importance of integrating traditional wisdom with modern scientific methodologies.

2. MATERIALS AND METHODS

Plant material: the roots were first air-dried in shade to preserve their biochemical composition. Subsequently, they were mechanically powdered to facilitate the extraction process and stored in

airtight containers to prevent degradation of active constituents [13].

Extraction: Extraction of metabolites from the powdered plant material was achieved using a Soxhlet apparatus. Petroleum ether, chloroform, and methanol were utilized as solvents for extraction. This method ensures efficient extraction of a wide range of compounds with varying polarities, thereby maximizing the yield of bioactive constituents from the plant material [14].

Preparation of extraction: 100 grams of *Boerhaavia diffusa* root powder were added to continuously stirred 100 ml of dw overnight (12h) at 37°C on magnetic stirrer. This process was repeated thrice consecutively to ensure thorough extraction of the desired metabolites. After filtration to remove the pellet, the extract was dried at a temperature below 40 degrees Celsius using a rotary evaporator. This gentle drying process helps preserve the integrity of the extracted compounds, ensuring their biological activity remains intact.

Animals: For the experimental studies, 30 male albino rats weighing approximately 130-160g were utilized. These rats were divided randomly in five groups. These rats were housed in clean polypropylene cages and maintained at a temperature of 25 ± 2°C with a 12/12-hour light/dark cycle. Prior to the commencement of experiments, the animals underwent an acclimatization period to minimize stress and ensure consistent baseline conditions for the study [15].

Induction of liver damage: Induction of liver damage in the experimental animals was achieved by orally administering paracetamol at doses of 4g/kg and 3g/kg of body weight. Paracetamol overdose is a well-established model for inducing hepatotoxicity in animal studies, mimicking the liver damage observed in cases of human overdose. This standardized protocol allows for the evaluation of the hepatoprotective effects of *Boerhaavia diffusa* extract under controlled experimental conditions [16].

Experimental design: The animals were grouped as follows, with each group consisting of six rats:

Group I: Control and received distilled water (1 ml/kg b.w.) for 12 days along with the normal diet (n = 6).

Group II: Animals were administered paracetamol at a dose of 3g/kg body weight once daily for 12 days.

Group III: were treated with paracetamol (3g/kg bw) once daily and *Boerhaavia diffusa* (1g/kg body weight) for 12 days daily.

Group IV: Animals were administered paracetamol at a dose of 4g/kg body weight once daily for 12 days.

Group V: were treated with paracetamol (4g/kg bw) once daily and *Boerhaavia diffusa* (2g/kg body weight) for 12 days daily.

All doses were administered orally. After completing the experimental time frame, the rats were fasted overnight. The animals were euthanized, sacrificed and the blood was collected on 12th day into dry centrifuge tubes by carotid bleeding. The blood was allowed to coagulate for 30 minutes at 37°C. The clear serum was separated by centrifugation at 2500 rpm for 10 minutes and subjected to biochemical investigation.

The toxicity was assessed by estimating markers such as AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase), and bilirubin. Elevated levels of these enzymes in the serum are indicative of liver damage and serve as biomarkers for hepatotoxicity. In this study, the levels of these enzymes were measured to determine the extent of liver injury induced by paracetamol and to evaluate the potential protective effect of *Boerhaavia diffusa*.

In addition to biochemical parameters, the rats were observed for any physical signs of toxicity, such as changes in behavior, physical appearance, and weight loss. This comprehensive approach allowed for a thorough evaluation of the hepatoprotective effects of *Boerhaavia diffusa* against paracetamol-induced liver damage. The data obtained were statistically analyzed to determine the significance of the observed differences among the groups.

Biochemical Investigation: In this study, several biochemical assays were employed to assess the hepatic function and evaluate the efficacy of *Boerhaavia diffusa* in protecting against paracetamol-induced hepatotoxicity.

Estimation of Total Bilirubin: Bilirubin levels were estimated using Diazo reagent. The absorbance was measured at 540 nm to quantify the amount of bilirubin in the treated

animals. Bilirubin, a product of heme metabolism, serves as a marker for liver function and damage.

AST and ALT Analysis: Serum extracted from the blood of test animals was converted to L-aspartate and L-glutamate, and L-ketoglutaric acid, respectively. Treatment with 2,4-dinitrophenylhydrazine (2,4-DNPH) produced a brown-colored complex, which was measured spectrophotometrically at 505 nm. AST and ALT, enzymes predominantly found in hepatocytes, increase in response to liver injury, making them useful markers of hepatocellular damage.

Estimation of ALP: ALP levels were estimated using a calorimetric assay with sodium β-glycerophosphate as the substrate. ANSA reagent facilitated the reaction, and absorbance was measured at a specific wavelength. Elevated ALP levels indicate cholestasis or biliary obstruction, among other conditions.

Statistical Analysis: Statistical analysis was performed using the Student's t-test, with data presented as mean ± standard error (SE). A p-value below 0.05 was considered statistically significant, indicating a meaningful difference between treatment and control groups.

3. RESULTS

The hepatoprotective effects of *Boerhaavia diffusa* were evaluated by measuring various biochemical parameters in different groups of rats (Table 1). The parameters assessed included Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and total bilirubin.

AST Levels: The control group (Group I) showed an AST level of 39.32±0.4332 U/L. Rats in Group II, which were administered paracetamol at a dose of 3g/kg, exhibited significantly elevated AST levels (56.40±0.447 U/L, P<0.05), indicating liver damage. However, Group III, treated with both paracetamol (3g/kg) and *Boerhaavia diffusa* (1g/kg), had AST levels reduced to 36.19±0.290 U/L, significantly lower than the paracetamol-only group (P<0.05). Group IV, which received a higher dose of paracetamol (4g/kg), showed even higher AST levels (57.30±0.373 U/L, P<0.05). Interestingly, Group V, treated with the higher paracetamol dose and *Boerhaavia diffusa* (2g/kg), had AST levels of 38.34±0.135 U/L, demonstrating a significant reduction (P<0.05).

ALT Levels: Similarly, ALT levels in the control group were 39.95±0.2163 U/L. Group II rats showed a significant increase to 58.56±0.206 U/L (P<0.05). In contrast, Group III rats treated with *Boerhaavia diffusa* showed a marked decrease in ALT levels to 36.30±0.278 U/L (P<0.05). Group IV displayed elevated ALT levels (59.60±0.288 U/L, P<0.05), while Group V showed significantly reduced levels (37.72±0.151 U/L, P<0.05).

ALP Levels: ALP levels followed a similar trend. The control group had ALP levels of 119.83±0.7367 U/L. Group II showed elevated levels of 130.88±1.024 U/L (P<0.05), while Group III, treated with *Boerhaavia diffusa*, showed a significant reduction to 121.17±1.118 U/L (P<0.05). Group IV rats had ALP levels of 130.63±0.490 U/L (P<0.05), but Group V rats showed a reduction to 120.46±0.150 U/L (P<0.05).

Table 1. Hepatoprotective effects of *Boerhaavia diffusa* in various biochemical Parameters

S.N.	Groups	AST	ALT	ALP	Total Bilirubin
1	**Control Group I**	39.32±0.4332	39.95±0.2163	119.83±0.7367	0.59166±0.014
2	**Group II Paracetamol(3g/kg) induced mice**	56.40±0.447*	58.56±0.206*	130.88±1.024*	0.908±0.044
3	**Group III Paracetamol (3g/kg) Induced + *Boerhaavia diffusa* 1g/kg Treated mice**	36.19±0.290*	36.30±0.278*	12I.17±1.118*	0.586± .002*
4	**Group IV Paracetamol(4g/kg)Induced mice**	57.30±0.373*	59.60±0.288*	130.63±0.490*	1.011±0.007*
5	**Group V Paracetamol(4g/kg) induced + *Boerhaavia diffusa* (2g/kg) treated mice**	38.34±0.135*	37.72±0.151*	120.46±0.150*	0.601±0.005

N= Six animal in each Group. Values are expressed as Mean±SE
**=Significant different at P<0.05*

Total Bilirubin Levels: Total bilirubin levels in the control group were 0.59166±0.01400 mg/dL. Group II had significantly elevated levels (0.908±0.044 mg/dL, P<0.05). In Group III, the bilirubin levels were significantly reduced to 0.586±0.002 mg/dL (P<0.05). Group IV showed a further increase in bilirubin levels to 1.011±0.007 mg/dL (P<0.05), whereas Group V rats showed a significant reduction to 0.601±0.005 mg/dL (P<0.05).

4. DISCUSSION

The results clearly indicate that *Boerhaavia diffusa* has a protective effect against paracetamol-induced hepatotoxicity. Elevated levels of AST, ALT, ALP, and total bilirubin in the paracetamol-treated groups (Group II and Group IV) confirm liver damage. However, the concurrent treatment with *Boerhaavia diffusa* (Groups III and V) significantly mitigated these elevations, demonstrating its hepatoprotective potential.

Boerhaavia diffusa's ability to reduce these biomarkers suggests its role in stabilizing the plasma membrane and regenerating liver cells, possibly due to its antioxidative properties. This aligns with previous studies highlighting the hepatoprotective and antioxidative effects of *Boerhaavia diffusa* [17,15]. The significant differences observed in biochemical parameters between the paracetamol-only and *Boerhaavia diffusa*-treated groups (P<0.05) underscore the herb's potential therapeutic benefits. The hepatoprotective mechanisms of *Boerhaavia diffusa* involve its antioxidant, anti-inflammatory, and detoxifying properties. Its bioactive compounds such as punarnavine, punarnavoside, and rotenoids have been implicated in scavenging free radicals, reducing oxidative stress, and modulating inflammatory responses, thereby safeguarding hepatocytes from paracetamol-induced injury [15,18].

The findings of our study support the potential therapeutic role of *Boerhaavia diffusa* in protecting against paracetamol-induced hepatotoxicity, highlighting its importance as a natural remedy for liver health [19,20]. These results are in line with previous research indicating the hepatotoxic effects of paracetamol overdose and the protective effects of *Boerhaavia diffusa* against liver damage [15,21].

The observed hepatoprotective effects of *Boerhaavia diffusa* are consistent with its traditional use in folk medicine for liver ailments. Our findings suggest that *Boerhaavia diffusa* holds promise as a natural remedy for protecting against paracetamol-induced hepatotoxicity [15, 18]. Incorporating *Boerhaavia diffusa* into therapeutic regimens may offer a complementary approach to mitigating liver damage associated with paracetamol overdose. However, additional preclinical and clinical studies are necessary to validate its efficacy and safety for human use.

Incorporating *Boerhaavia diffusa* into therapeutic regimens could offer a promising adjunctive approach to conventional treatments for paracetamol overdose. Its natural origin and relatively low incidence of adverse effects compared to synthetic drugs make it an attractive candidate for hepatoprotection. Moreover, the potential synergy of *Boerhaavia diffusa* with existing therapies could enhance therapeutic outcomes and reduce the burden of liver-related complications.

However, despite promising preclinical evidence, the translation of these findings into clinical practice requires rigorous evaluation through well-designed human trials. Comprehensive studies are essential to determine the optimal dosage, duration, and safety profile of *Boerhaavia diffusa* supplementation in different populations. Additionally, investigating potential drug interactions and long-term effects is crucial for establishing its efficacy and safety as a therapeutic agent for paracetamol-induced hepatotoxicity. Only through robust clinical research can the full therapeutic potential of *Boerhaavia diffusa* be realized, providing clinicians with evidence-based recommendations for its incorporation into clinical practice.

5. CONCLUSION

Boerhaavia diffusa exhibits substantial hepatoprotective properties against paracetamol-induced liver damage, as evidenced by its ability to significantly reduce elevated levels of AST, ALT, ALP, and total bilirubin in experimental rat models. These biochemical markers serve as critical indicators of liver function and injury, and their modulation by *Boerhaavia diffusa* highlights its potential therapeutic efficacy in liver health.

The observed effects are likely attributable to *Boerhaavia diffusa's* rich phytochemical composition, which includes punarnavine, punarnavoside, and rotenoids known for their antioxidant, anti-inflammatory, and detoxifying

properties. These compounds are believed to scavenge free radicals, mitigate oxidative stress, and modulate inflammatory responses, thereby protecting hepatocytes from damage induced by paracetamol overdose. Such mechanisms align with traditional uses of *Boerhaavia diffusa* in folk medicine for treating liver ailments, underscoring its historical and pharmacological relevance.

While these preclinical findings are promising, translating them into clinical applications requires rigorous human trials to establish optimal dosage, safety profiles, and efficacy across different populations. Moreover, assessing potential drug interactions and long-term effects is crucial for comprehensive therapeutic recommendations.

Incorporating *Boerhaavia diffusa* into therapeutic regimens could offer a complementary approach to conventional treatments for liver damage, potentially enhancing therapeutic outcomes while reducing the burden of liver-related complications. However, to fully realize its therapeutic potential and ensure evidence-based clinical use, further research is imperative. Robust clinical studies will provide the necessary foundation to integrate *Boerhaavia diffusa* effectively into clinical practice, offering clinicians and patients a natural and potentially safer alternative for managing paracetamol-induced hepatotoxicity and other liver disorders.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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