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EXPLORING THE HEPATOPROTECTIVE POTENTIAL OF ETHANOL FRACTION OF ETHANOL EXTRACT FROM HIPTAGE BENGHALENSIS AGAINST THIOACETAMIDE-INDUCED HEPATOTOXICITY

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Abstract

Liver diseases pose a significant global health burden, necessitating the search for effective hepatoprotective agents. In this study, we investigated the hepatoprotective properties of the Ethanol Fraction of Ethanol Extract (EFEE) from Hiptage benghalensis using thioacetamide (TAA)-induced hepatotoxicity models. Two distinct approaches were employed, one simulating pre-treatment and the other post-treatment scenarios, to comprehensively evaluate EFEE's potential in preventing and ameliorating liver damage. In the pre-treatment model, EFEE administration significantly mitigated TAA-induced hepatotoxicity, as evidenced by the marked reduction in elevated biochemical markers of liver damage, including ALKP, GOT, CHOL, TBL, SODIUM, and POTASSIUM, along with the restoration of depleted TPTN levels. Histological examination confirmed these findings, revealing preserved hepatic architecture and reduced fatty changes and centrilobular necrosis in rats treated with EFEE. The post-treatment model yielded parallel results, demonstrating EFEE's effectiveness in therapeutically addressing TAA-induced liver damage. EFEE significantly reduced elevated biochemical markers and enhanced depleted TPTN levels, indicating its potential as a hepatoprotective intervention even after hepatotoxic insult. Histological analysis corroborated these outcomes, highlighting reduced centrilobular necrosis and fatty changes in rats treated with EFEE. The underlying mechanisms of EFEE's hepatoprotective effects may involve interference with the metabolic pathway of TAA, including its conversion into a toxic S-oxide metabolite and subsequent generation of reactive oxygen species by cytochrome P450 enzymes. EFEE may inhibit these processes or act as a membrane stabilizer, preventing TAA-induced damage and promoting hepatocyte regeneration. Moreover, qualitative phytochemical analysis revealed the presence of phenolic compounds, flavonoids, terpenoids, and steroids in EFEE, known for their antioxidant and hepatoprotective properties. An increase in total phenolic and flavonoid content suggests their

potential contribution to EFEE's hepatoprotective activity. In conclusion, this study unveils the remarkable hepatoprotective potential of EFEE from Hiptage benghalensis, both in preventing and alleviating hepatotoxicity induced by TAA. Further investigations are warranted to elucidate the precise mechanisms involved and identify bioactive compounds responsible for this activity.

Keywords: Hepatoprotective, Hiptage benghalensis, thioacetamide, liver damage, hepatotoxicity

Introduction

The liver, a vital organ central to various metabolic processes, can incur damage from multiple sources, including drug abuse, viral infections, excessive alcohol consumption, exposure to biological and chemical agents, and autoimmune attacks on hepatocytes. Failure to reverse this damage can result in severe disruption of hepatic structure and function. Long-term damage may lead to conditions such as hepatic cirrhosis, fibrosis, fatty liver disease, and even liver carcinoma, posing a significant epidemiological challenge [1]. Amidst these concerns, the exploration of alternative treatment methods employing phytochemicals derived from natural sources gains prominence. Notably, the persistent scarcity of effective hepatoprotective drugs remains a concern despite significant advances in modern medicine. The issue is further exacerbated by the withdrawal of many drugs from the market due to drug-induced liver injury (DILI), with severe cases necessitating liver transplantation and risking organ failure. Consequently, hepatic diseases often find their primary treatment in herbal medicines, as modern pharmaceuticals offer limited relief for hepatic ailments. However, there remains a shortage of medications specifically targeting liver diseases, making herbal remedies increasingly relevant due to their potency, purity, and cost-effectiveness [2]. Plants encompass a rich array of bioactive substances, known as secondary metabolites, which include alkaloids, polysaccharides, phenylpropanoids, flavonoids, resins, tannins, steroids, essential oils, organic acids, anthraquinones, polyphenols, and saponins. These compounds have evolved due to coevolutionary processes, resulting in their abundance [3]. Among these secondary metabolites, flavonoids stand out with their prominence. This extensive family comprises over five thousand polyphenolic compounds, typically featuring two phenyl rings (A and B) and a heterocyclic C ring with an attached oxygen atom. The term "flavonoid" originates from the Latin word "flavus," meaning "yellow," alluding to their role in safeguarding plants against oxidative damage, contributing to male fertility, and serving as visual signals [4]. Flavonoids, alternatively referred to as vitamin P for humans (although the classification as vitamins is debated), have garnered significant attention due to their diverse influence on various inflammatory diseases [5, 6]. Harnessing the therapeutic potential of plant-derived phytoconstituents and their synthetic and semisynthetic derivatives has been a cornerstone of pharmaceutical innovation since the advent of modern medicine [7]. To effectively address the pressing challenge of hepatic diseases, the development of safe and cost-effective treatment alternatives is imperative. Historically, natural secondary metabolites derived from plants have been employed for centuries as sources of therapeutic agents. Notably, between 1981 and 2010, the FDA approved approximately 35% of medicines and their compounds that were derived from natural origins. These natural substances exhibit robust medicinal and anti-inflammatory properties. Numerous bioactive compounds, particularly flavonoids, demonstrate the capacity to mitigate inflammation by modulating mediators such as prostaglandins, reactive oxygen species, cyclo-oxygenase-2, and various cytokines like interleukin-6, interleukin-1, and TNF-α. Given their wide array of bioactivities, including antimutagenic, antioxidant, antiviral, and anti-inflammatory effects, flavonoids present a favorable avenue for dietary and therapeutic interventions [8]. Hiptage benghalensis, belonging to the Malpighiaceae family, is indigenous to regions including India, Srilanka, Bangladesh, the Andaman Islands, Myanmar, and southern China. Within the realm of Ayurveda, this herb finds distinction for its leaves and bark, recognized for their vulnerary properties and particularly esteemed for their efficacy in addressing various skin ailments. Hiptage benghalensis, a versatile plant, exhibits diverse pharmacological activities. It demonstrates potent anthelmintic activity, with its aqueous leaf extract showing effectiveness [10, 15]. Additionally, various extracts

from this plant display antibacterial properties against a range of pathogens [11, 16]. The methanolic leaf extract demonstrates analgesic and anti-inflammatory effects, with flavonoids being associated with its analgesic potential [12, 13, 17, 14]. Moreover, Hiptage benghalensis serves as a promising larvicide against mosquito vectors, offering potential for mosquito-borne disease control [18]. It also exhibits hepatoprotective qualities and acts as an antioxidant by raising glutathione levels [19]. Furthermore, it shows promise in managing diabetes, particularly in controlling glucose absorption [20, 21, 22]. This plant's anti-asthmatic properties in both early and late stages of asthma make it a noteworthy candidate for further study [23]. Additionally, it displays anticancer activity against various cancer cell lines [24]. Beyond these pharmacological attributes, traditional medicinal use of different parts of Hiptage benghalensis encompasses its role in treating skin diseases, digestive problems, worm infections, itching, ulcers, obesity, and more. These findings underscore the plant's potential significance in natural medicine [9].

Material and Methods

Collection and Identification of Plant

The plant source chosen for this study was Hiptage benghalensis L.Kurz, and its aerial components were procured from the foothills of Tirumala. The authenticity of these plant samples was officially confirmed by the Botanical Survey of India, Southern Circle, Coimbatore, India. A voucher specimen has been meticulously preserved in the Herbarium of our Institute.

Macroscopic Evaluation

To gain insights into the morphology of the aerial parts of Hiptage benghalensis, a detailed macroscopic examination was conducted, employing established methods.

Extraction

In the preparation of extracts for this study, the aerial parts of the collected Hiptage benghalensis plant were shade dried and powdered. A total of 600 gm of shade-dried, coarsely powdered leaf material was subsequently subjected to ethanol extraction using a Soxhlet apparatus. The solvent was distilled to concentrate the extract, and the obtained extract was dried in desiccators to determine the percentage yield of extracts.

Fractionation of Ethanol extract

For the fractionation of the ethanol extract, the total ethanol extract was dissolved in ethanol and subjected to fractionation using column chromatography with a mixture of chloroform and ethanol. The solvents (chloroform and ethanol extracts) were distilled to concentrate the fractions, which were then dried in desiccators, and the percentage yields of the resulting extracts, referred to as CFEE and EFEE, were recorded.

Phytochemical Screening

Phytochemical studies involved qualitative evaluation of the extracts and fractions. Various phytoconstituents were identified through qualitative tests. Alkaloids were tested using Dragendorff, Wagner, and Mayer tests; cardenolides were examined through Kedde, Legal, and Raymond tests; while phenolics and flavonoids were detected using Ferric chloride, Schinoda, Alkali, and Lead acetate tests. Salkowski test was employed for the detection of terpenoidal and steroidal saponins, and terpenoids and steroids were identified through vanillin sulphuric acid and Liebermann-Buchard tests. Detection of phenols and flavonoids involved Shinoda, Ferric chloride, and Lead acetate tests. Alkaloids were detected using Dragendroff's, Mayer's, and Wagner's tests. Terpenoids and steroids were identified through Salkowski's and Liebermann Burchard's tests, while the detection of carbohydrates was done via Fehling's, Molisch's, and Benedict's tests. The presence of coumarins was assessed under UV light, and anthracene glycosides were identified using a modified

Borntrager's test. Cardenolides were detected through Legal's test, and proteins and amino acids were identified using Xanthoproteic and Ninhydrin tests [25].

Estimation of Phenolic Content

The assessment of the total phenolic content in the ethanol extract of H. benghalensis*was carried out utilizing the Folin-Ciocalteu method [26]. The procedure included the preparation of a stock solution (1mg/1ml) of the extract in respective solvents. Subsequently, 1ml of the extract stock solution was placed into a 25 ml volumetric flask, followed by the addition of 10 ml of H2O and 1.5 ml of Folin-Ciocalteu reagent. After a 5-minute incubation period, 4 ml of 20% sodium carbonate solution was mixed in, and the volume was adjusted to 25 ml with double-distilled water. The solution was then allowed to rest for 30 minutes, and the absorbance of the developed blue color was measured at 765 nm. Calibration curves for Gallic acid were prepared using standard solutions with concentrations ranging from 2 to 10 µg/ml.

Estimation of Total Flavonoid Content

The total flavonoid content of the ethanol extract of H. benghalensis was estimated through established methods by Chang et al., 2002 [27].

Aluminium Chloride Method

A standard solution was prepared by dissolving 1 mg of quercetin in 10 ml of 95% ethanol. The procedure involved the preparation of 0.1, 0.2, 0.3, 0.4, and 0.5 ml of standard solutions, which corresponded to concentrations of 10, 20, 30, 40, and 50 µg, respectively. These solutions were mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% AlCl3, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled H2O. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. A similar procedure was followed using 0.5 ml of ethanol extract of H. benghalensis (2 mg/ml) [28].

2, 4-Dinitro Phenyl Hydrazine Method

A standard solution was prepared by dissolving 10 mg of naringenin in 10 ml of methanol. The procedure involved the preparation of calibration curves for naringenin with concentrations ranging from 250 to 2000 µg/ml. Each diluted standard solution (0.5 ml) was mixed with 2 ml of 1% 2, 4-dinitro phenyl hydrazine reagent and 2 ml of methanol at 50ºC for 50 minutes. After cooling to room temperature, the reaction mixture was mixed with 5 ml of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2 minutes. The absorbance of the supernatant was measured at 495 nm. A similar procedure was followed using 5 ml of ethanol extract of H. benghalensis (5 mg/ml) [29].

Biological Studies

Acute Toxicity Studies

Acute toxicity assessments were conducted on the ethanolic extract in accordance with OECD guidelines 423 (OECD, 1996). Female albino rats were utilized for this study, with the animals fasting overnight but having access to water. The rats were divided into groups, each comprising three animals. Subsequently, each group received an oral dose of 300 mg/kg of the ethanol extract (EE). Continuous monitoring was conducted during the first 30 minutes post-dosing, followed by periodic observations over the initial 24 hours. Special attention was given within the first 4 hours, with daily observations continued for a total of 14 days. Various parameters, including sedation, convulsions, tremors, salivation, lethargy, and mortality, were systematically recorded. As no mortality was observed at the 300 mg/kg dose, the procedure was repeated with a higher dose of 2000 mg/kg p.o. using fresh animals.

In Vivo Assessment of Hepatotoxicity Activity Using TAA Models

The hepatoprotective potential of the ethanolic fraction of the ethanol extract was evaluated using an acute hepatotoxicity model induced by thioacetamide (TAA). The plant *H. benghalensis* was found to contain flavonoids and phenolics, which are known for their antioxidant properties. Hence, the hepatoprotective properties of the ethanolic fraction were systematically screened.

Animals

Wistar rats of either sex, weighing between 175-225 g, were selected for this study. The animals were maintained under standard husbandry conditions, including a controlled temperature of 23 ± 2ºC, relative humidity of 55 ± 10%, and a 12-hour light-dark cycle. They were provided with standard laboratory feed and tap water. All experimental protocols were conducted in accordance with the guidelines approved by the institutional animal ethics committee. The rats were divided into groups, each consisting of 6 animals.

Preparation of Solutions for Administration

Thioacetamide Suspension: Thioacetamide powder was dissolved in distilled water to create a suspension, which was administered at a dose of 100 mg/kg s.c.

Suspension of Test Substances

All selected extracts were suspended in 1% sodium CMC mucilage and administered at doses of 150 and 250 mg/kg. Silymarin, used as a positive control, was also suspended in 1% sodium CMC mucilage and administered orally.

Model I: Pretreatment of EFEE of H.benghalensis on TAA induced Hepatotoxicity

In this study, the experiment was divided into groups, including control, toxicant, standard, and test groups. The protocol followed for thioacetamide (TAA)induced hepatotoxicity [31]. Control (Group I): Received a daily dose of 2% Sodium CMC mucilage (1 ml/kg, p.o.). Toxicant (Group II): Received a daily dose of vehicle (1 ml/kg, p.o.) for seven days and a single subcutaneous injection of thioacetamide (TAA, 100 mg/kg) in water for injection on the sixth day. Standard (Group III): Received silymarin (100 mg/kg p.o.) seven times at 24hour intervals. TAA was administered 30 minutes after the sixth dose of silymarin. Test Groups (IV and V): Given orally a single daily dose of EFEE (Ethanol fraction of ethanol extract) in vehicle for seven days and a single dose of TAA (100 mg/kg s.c.) on the sixth day, 30 minutes after the administration of the respective test suspensions.

Model-II: Post-treatment of EFEE of H.benghalensis on TAA-induced Hepatotoxicity

In this study, the experiment was again divided into groups, including control, toxicant, standard, and test groups. The protocol for TAA-induced hepatotoxicity [31] Control (Group I) Received saline. Toxicant (Group II) I received TAA (thioacetamide). Standard (Group III) I received TAA and silymarin. Test Groups (IV and V) Received TAA and EFEE at different doses (150 and 200 mg/kg).

D. Assessment of Liver Function

Blood was collected from all groups through retro-orbital plexus puncture. The serum was separated by centrifugation for the estimation of various biochemical parameters related to liver function, including Glutamic Oxaloacetic Transaminase (GOT), Gamma-GT (GGT), Alkaline Phosphate (ALP), Total Bilirubin (TBL), Total Cholesterol (CHL), and Total Protein (TPTN). These estimations were performed using standard kits on an auto analyzer [32].

Estimation of Enzymes and Biochemical Parameters

For the estimation of crucial enzymes and biochemical parameters:

GOT (Glutamic Oxaloacetic Transaminase): Utilized an enzyme-coupled system based on IFCC reference method with standard kits [33].

Gamma-GT (Gamma-Glutamyl Transferase): Employed the Szasz methodology with standard kits [34].
Alkaline Phosphatase: Utilized a modified method following Bessey-Lowery-Brock with standard kits [35].
Total Bilirubin: Employed Jendrassik and Grof's method [36].
Cholesterol: Determined using the CHOD-PAP method by Richmond [37].
Total Protein: Estimated using the Biuret method [38].

Histological Studies:
To confirm the hepatoprotective effects of the various extracts, we conducted histopathological examinations of the treated livers. Following animal sacrifice, liver samples were collected, sliced into 5 mm thick sections, and carefully cleansed of blood. These liver pieces were then fixed in Bouin's solution for 12 hours, followed by rinsing until free from Bouin's fluid. Standard procedures were employed for paraffin embedding, and 5 mm thick sections were obtained using a rocking microtome. These sections were subsequently stained with haematoxylin and eosin and mounted in diphenyl xylene (DPX). Under light microscopy, these stained sections were examined for any histopathological alterations in liver architecture, with photomicrographs captured to illustrate cellular structural changes in the test groups, control, and toxicant groups [39].

In-vivo Antioxidant Activity - Tissue Analysis
Liver samples were excised, meticulously cleansed with ice-cold saline to remove any residual blood, and then finely sliced using a surgical scalpel. The tissue was further minced using ice-cold 10% KCl in a homogenizer operating at 2500rpm for 10 minutes. Afterward, it underwent centrifugation at 3000rpm for 5 minutes, resulting in a clear supernatant used for the estimation of catalase (CAT) activity [40].

Estimation of Catalase
Catalase activity was determined following the method established by Hugo E. Aebi. In this UV-based assay, the consumption of H2O2, indicated by the decrease in absorbance (O.D 240) over time, was measured to assess catalase activity [41].

In-vitro Antioxidant Activity - ABTS Scavenging Assay
For screening antioxidant activity, we employed the ABTS scavenging assay, a decolorizing assay suitable for assessing both lipophilic and hydrophilic antioxidants. This assay involved the generation of the ABTS monocation by oxidizing ABTS with potassium persulfate. It was then reduced in the presence of antioxidants capable of donating hydrogen. The influence of antioxidant concentration and reaction duration on the inhibition of radical cation absorbance was considered during the determination of antioxidant activity [42].

Calculation of IC50 Values
The Half maximal inhibitory concentration (IC50) quantifies the effectiveness of a compound in inhibiting a biological or biochemical process by half. In our ABTS method, IC50 signifies the antioxidant concentration at which a 50% reduction in ABTS absorbance occurs. To calculate IC50 values, we utilized GraphPad Prism software, creating an XY data table with the logarithm of the inhibitor concentration (X) and the corresponding response (Y). Employing a nonlinear regression model, we extracted IC50 values from the data [42].

Statistical Analysis
For the assessment of intergroup differences in each parameter, mean values ± SEM were calculated. One-way analysis of variance (ANOVA) was performed for individual parameter analysis. Subsequently, Dunnet's test was applied to perform pairwise comparisons of group mean values.

Results

Preparation of Selective Extracts

The ethanol extract from the aerial parts of H. benghalensis yielded 7.5% w/w.

Fractionation of Selective Extract

The chloroform fraction of the ethanol extract from H. benghalensis aerial parts had a yield of 22.22% w/w. The ethanol fraction of the ethanol extract from the same parts yielded 77.77% w/w.

Phytochemical Studies

Qualitative Evaluation of Ethanol Extract & Fractions

Qualitative analysis of the successive extracts from Hiptage benghalensis L. kurz leaves was conducted and the results are summarized in Table 6. The analysis indicates the presence of phenolic compounds, flavonoids, anthracene glycosides, cardenolides, terpenoids, tannins, steroids, carbohydrates, and amino acids.

Table 1: Qualitative Evaluation of Extracts and Fractions from H. benghalensis

Phytochemical constituents	Ethanol Extract (EE)	Chloroform Fraction of Ethanol Extract (CFEE)	Ethanol Fraction of Ethanol Extract (EFEE)
Phenols	+	-	+
Flavonoids	+	-	+
Anthracene glycosides	+	+	-
Cardenolides	+	+	-
Alkaloids	-	-	-
Terpenoids	+	+	-
Tannins	+	-	+
Steroids	+	-	+
Carbohydrates	+	-	+
Amino acids	+	-	+

Phenolic Content

Phenolic content was found to be 8.23% w/w in the ethanol extract and 12.97% w/w in the EFEE extract, indicating the presence of various phenolic constituents including phenolic acids, polyphenols, and flavonoids.

Flavonoid Content

The flavonoid content in EE and EFEE of H. benghalensis was found to be 2.053% w/w and 9.552% w/w, respectively.

Biological Screening of Extracts

Acute Toxicity Studies

The acute toxicity of the ethanol extract (EE) was assessed following OECD guidelines. No mortality occurred even at a dose level of 2000 mg/kg, confirming its safety.

Hepatoprotective Activity

EFEE (Ethanol Fraction of Ethanol Extract) of Hiptage benghalensis was evaluated for its hepatoprotective activities using thioacetamide (TAA) models, both in pre- and post-treatment.

Thioacetamide-induced Hepatotoxicity

Model I: Extract Pre-treatment

In this model, TAA (100 mg/kg s.c) administration significantly increased serum levels of ALKP, GOT, CHOL, TBL, SODIUM, and POTASSIUM, except for GAMMA-GT. This increase indicated parenchymal cell necrosis. Significant reductions ($p<0.05$) in all elevated biochemical parameters and significant increases ($p<0.05$) in depleted TPTN levels were observed in the groups of rats that received EFEE at dose levels of 250 mg/kg, similar to the silymarin-treated group. Results of EFEE pre-treatment on TAA-induced toxicity are shown in Table 2 and Figure 1.

Normal cellular structural design with distinct hepatic cells, sinusoidal spaces, and central veins was observed in histological examinations of liver sections from control groups. Liver sections from rats intoxicated with thioacetamide displayed hepatic cells with severe toxicity characterized by centrilobular necrosis, along with various grades of fatty changes, comprising tiny to large-sized vacuoles and disarrangement of hepatic cells. In contrast, liver sections from rats administered EFEE at 150 mg/kg followed by thioacetamide intoxication showed fewer visible changes and regeneration of hepatic cells. Liver sections from rats administered EFEE at 250 mg/kg followed by thioacetamide intoxication showed an even lesser degree of visible changes, suggesting the protective effect of the extract.

Table 2: Effect of EFEE Pre-treatment on TAA-induced Hepatotoxicity in Rats

GROUPS	GAMMA-GT	ALKP	SGOT	CHOL	TP	TB	SODIUM	POTASSIUM
CONTROL	2.127±0.66	224.48±28.5	170.91±14.6	53.63±03.49	7.127±0.561	0.061±0.021	0.522±0.071	0.711±0.041
TAA	2.861±0.51	893.61±58.1	453.92±37.1	272.84±25.2	3.790±0.422	0.793±0.125	5.723±0.490	3.197±0.312
SILYMARIN	2.403±0.93	231.31±22.3 *	210.33±18.7 *	42.15±05.26*	7.579±0.581**	0.188±0.015*	1.300±0.189*	1.189±0.088*
EFEE 150 mg/kg	2.176±0.50	394.10±38.2 *	268.66±22.1 *	95.09±06.02*	7.020±0.328**	0.251±0.056*	1.822±0.132*	1.654±0.114*
EFEE 250 mg/kg	2.112±0.34	285.26±47.8 *+	185.81±39.9 *+	70.13±07.50*+	8.192±0.341** +	0.160±0.030*+	1.210±0.093*+	1.192±0.124*+

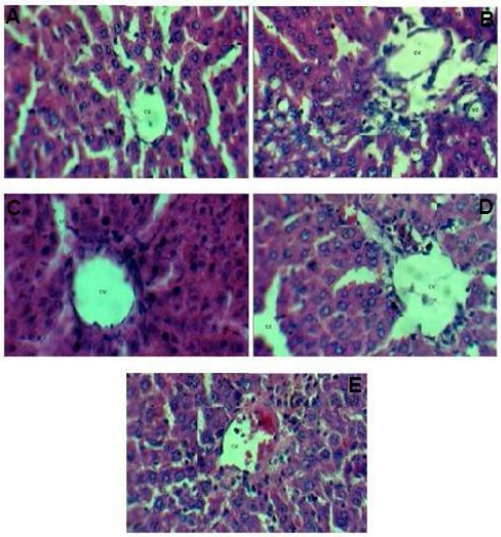
Data represents the mean ± SEM of six animals

Significant reduction compared to TAA ($p<0.05$).

Significant increase compared to TAA ($p<0.05$).

Non-significant compared to Silymarin

Figure-1: Photomicrographs representing the effect of EFEE of *H.benghalensis* against TAA-induced Hepatotoxicity in rats (Extract Pre-treatment).



A: Control rat liver section; **B**: Liver section of the rat intoxicated with thioacetamide; **C**: Liver section of the rat treated with silymarin and intoxicated with Thioacetamide; **D**: Liver section of the rat treated with EFEE 150 mg/kg and intoxicated with Thioacetamide; **E**: Liver sections of the rat treated with EFEE 250 mg/kg and intoxicated with Thioacetamide.

Model II: Extract Post-treatment.

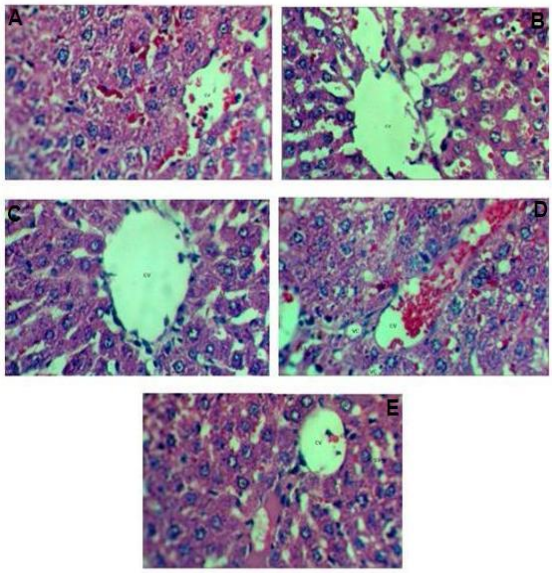
In this model, TAA (100 mg/kg s.c) administration also increased serum levels of GAMMA-GT, ALKP, SGOT, CHOL, TBL, SODIUM, and POTASSIUM while decreasing TPTN levels, indicating parenchymal cell necrosis. Significant reductions ($p<0.05$) in all elevated biochemical parameters and significant increases ($p<0.05$) in depleted TPTN levels were observed in the groups of rats that received EFEE at dose levels of 250 mg/kg, similar to the silymarin-treated group. Results of EFEE post-treatment on TAA-induced toxicity are shown in Table 3 and Figure 2. Normal cellular structural design with distinct hepatic cells, sinusoidal spaces, and central veins was observed in histological examinations of liver sections from the control group. Liver sections from rats intoxicated with thioacetamide displayed hepatic cells with severe toxicity, characterized by centrilobular necrosis along with various grades of fatty changes and disarrangement of hepatic cells. Liver sections from rats administered EFEE at 150 mg/kg followed by thioacetamide intoxication showed vacuole formation and infiltration of red blood cells in the central vein. Liver sections from rats administered EFEE at 250 mg/kg followed by thioacetamide intoxication showed an even lesser degree of visible changes, similar to that observed in the silymarin-treated rat liver sections, suggesting the protective effect of the extract.

Table 3: Effect of EFEE Post-treatment on TAA-induced Hepatotoxicity in Rats

GROUPS	GAMMA-GT	ALKP	SGOT	CHOL	TP	TB	SODIUM	POTASSIUM
CONTROL	4.974±1.129	257.2±17.24	153.2±5.213	48.81±4.750	7.234±0.326	0.102±0.027	0.466±0.025	0.782±0.043
TAA	22.87±2.472	742.5±43.01	251.4±14.31	174.9±9.248	4.719±0.316	1.119±0.229	4.384±0.273	2.257±0.150
SILYMARIN	7.843±1.731*	239.0±16.53 *	172.3±6.347 *	66.14±13.35*	7.225±0.432**	0.137±0.038 *	0.949±0.158*	0.935±0.046*
EFEE 150 mg/kg	9.572±1.502*	320.4±20.21 *	191.5±7.766 *	138.8±3.135*	6.915±0.107**	0.213±0.031 *	2.030±0.332*	1.544±0.157*
EFEE 250 mg/kg	4.942±1.035*+	221.3±10.31 *+	179.2±3.555 *+	75.41±11.72*+	8.103±0.248**+	0.127±0.022 *+	1.091±0.140*+	0.897±0.076*+

Data represents the mean ± SEM of six animals.
TAA: Thioacetamide
EFEE: Ethanol fraction of ethanol extract followed by TAA intoxication.
* Significant reduction compared to TAA ($p<0.05$).
** Significant increase compared to TAA ($p<0.05$).
+ Non-significant compared to Silymarin

Figure-3: Photomicrographs representing the effect of EFEE of *H.benghalensis* against TAA-induced hepatotoxicity in rats (Extract Post-treatment).



A: Control rat liver section; B: Liver section of the rat intoxicated with thioacetamide; C: Liver section of the rat intoxicated with Thioacetamide and treated with silymarin; D: Liver section of the rat

intoxicated with Thioacetamide and treated with EFEE 150 mg/kg; E: Liver sections of the rat intoxicated with Thioacetamide and treated with EFEE 250 mg/kg.

Discussion

The hepato-protective potential of EFEE (Ethanol Fraction of Ethanol Extract) from Hiptage benghalensis was thoroughly investigated in this study using thioacetamide (TAA) models. The liver plays a crucial role in metabolic processes, detoxification, and the maintenance of overall homeostasis within the body. Any insult to the liver, such as that induced by TAA, can lead to severe hepatotoxicity and compromise these vital functions. Thus, identifying hepatoprotective agents is of significant interest, especially when considering the global burden of liver diseases. In our study, we employed both pre and post-treatment models to comprehensively assess the hepatoprotective effects of EFEE. These models simulate scenarios where EFEE is administered before or after the onset of hepatotoxicity. The results from both models consistently demonstrated that EFEE exerts potent hepatoprotective effects. In the pre-treatment model, EFEE significantly reduced the elevated levels of various biochemical markers of liver damage induced by TAA, including ALKP, GOT, CHOL, TBL, SODIUM, and POTASSIUM. Moreover, EFEE increased depleted TPTN levels, indicating the prevention of parenchymal cell necrosis. Histological examinations of liver sections further confirmed these findings, showing the preservation of normal hepatic architecture, reduced fatty changes, and centrilobular necrosis in rats treated with EFEE. The post-treatment model yielded similar results, with EFEE significantly mitigating TAA-induced hepatotoxicity by decreasing elevated biochemical markers and increasing depleted TPTN levels. This demonstrates EFEE's effectiveness not only as a preventive agent but also as a therapeutic intervention after hepatotoxic insult. Histological analysis in this model further substantiated these findings, revealing a reduction in centrilobular necrosis and fatty changes in rats treated with EFEE. The mechanisms underlying EFEE's hepatoprotective effects could be multifactorial. TAA is known to induce hepatotoxicity by metabolizing into a toxic S-oxide metabolite, which directly damages hepatocytes. This metabolite is further metabolized by cytochrome P450 enzymes, which can generate reactive oxygen species (ROS) leading to cellular damage. EFEE may interfere with this metabolic pathway, possibly by inhibiting cytochrome P450 enzymes or acting as a membrane stabilizer, thus preventing TAA's interaction with hepatocyte machinery and allowing for hepatocyte regeneration. Furthermore, the qualitative phytochemical analysis of EFEE revealed the presence of phenolic compounds, flavonoids, terpenoids, and steroids, which are known for their antioxidant and hepatoprotective properties. The increase in total phenolic and flavonoid content in EFEE suggests that these compounds could contribute to its hepatoprotective activity.

Conclusion

In summary, our study provides strong evidence that EFEE from Hiptage benghalensis possesses potent hepatoprotective properties, both in a preventive and therapeutic context. These findings warrant further investigation into the specific mechanisms involved and the isolation of bioactive compounds responsible for this hepatoprotective activity.

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