ETHANOLIC EXTRACT OF TEPHROSIA PURPUREA FLOWERS AMELIORATES PHENYTOIN-INDUCED HEPATOTOXICITY IN WISTAR RATS

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Abstract

The present research endeavor aims to assess the impact of ethanolic extract of *Tephrosia purpurea* flowers (TPF) on phenytoin-induced hepatotoxicity. The study involved the division of rats into 4 groups, each consisting of six animals. Group 1 received 0.2% carboxymethyl cellulose (CMC) orally (p.o), while Group 2 administered 20 mg/kg of phenytoin orally (p.o). Groups 3 and 4 were administered 100 and 200 mg/kg of TPF in 0.2% CMC, respectively, one hour prior to phenytoin treatment for a duration of 45 days. On the 45th day, blood samples were collected and subjected to a liver function test. Following this, the animals were euthanized, and liver samples were analyzed for antioxidant status and lipid peroxidation levels, in addition to undergoing histopathological examinations. Phenytoin treatment was observed to induce liver injury, as evidenced by elevated serum levels of transaminases, alkaline phosphatase (ALP), and bilirubin in the bloodstream, as well as increased lipid peroxidation within the liver tissue. Phenytoin also led to a decrease in the levels of albumin, endogenous antioxidants and total protein with a reduction in animal body weight. Histopathological investigations further confirmed phenytoin-induced hepatic necrosis with periportal congestion. However, the administration of TPF at higher dose of 200 mg/kg significantly ($p < 0.001$) alleviated the elevated serum enzyme levels, bilirubin, ALP, and lipid peroxidation caused by phenytoin. TPF also effectively reversed the decrease in albumin and total protein levels, restored antioxidant levels, increased body weight, and mitigated the phenytoin-induced histopathological changes in the liver. In conclusion, TPF demonstrated its effectiveness against phenytoin-induced hepatotoxicity, offering a potential therapeutic approach to mitigate the adverse effects of phenytoin on the liver.

Keywords: Tephrosia purpurea, Phenytoin, hepatotoxicity, oxidative stress, antiepileptics.

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1. Introduction

Tephrosia purpurea (L.) Pers. (family: Fabaceae), commonly known as "Sarphonk" or "Wild Indigo," is a medicinal plant renowned for its diverse pharmacological properties, including fever, skin diseases, digestive disorders, and respiratory conditions [1]. It has also been utilized as a potent insecticide and piscicide [1,2]. Traditional healers and herbal medicine practitioners have long employed various parts of *T. purpurea*, including its flowers, in the treatment of various ailments [1]. The plant's ethanolic extract has gained recognition for its antioxidant, anti-inflammatory, and hepatoprotective activities, making it a promising candidate for mitigating drug-induced liver damage [3,4].

Phenytoin, a well-established antiepileptic drug, has revolutionized the management of epilepsy and related neurological disorders by effectively controlling seizures and improving the quality of life for countless individuals [5,6]. However, the therapeutic benefits of phenytoin are not without certain liabilities, one of the most prominent being its potential to induce hepatotoxicity [7]. Hepatotoxicity, characterized by liver injury and dysfunction, represents a serious concern in the clinical administration of phenytoin, necessitating a comprehensive understanding of its underlying mechanisms and potential mitigating strategies [5,8].

Hepatotoxicity refers to the adverse effects on the liver resulting from exposure to drugs, toxins, or other harmful agents [9]. In the context of phenytoin, hepatotoxicity manifests as a complex interplay of biochemical, histological, and molecular alterations within the liver tissue [10,11]. The liver plays a pivotal role in drug metabolism and detoxification, making it particularly vulnerable to the toxic effects of various pharmaceutical agents, including phenytoin [7,10]. Animal models, particularly Wistar rats, have proven invaluable in investigating the hepatotoxic effects of phenytoin. These models allow for controlled experimentation and the assessment of various biomarkers, histopathological changes, and molecular pathways involved in drug-induced liver injury [5,6]. Understanding the mechanisms and severity of phenytoin-inducedhepatotoxicity in such models is crucial for advancing our knowledge of drug-induced liver damage and developing strategies to minimize its clinical impact [5,6,11,12].

In light of the potential hepatotoxicity associated with phenytoin usage and the established hepatoprotective properties of *T. purpurea*, this research article explores the therapeutic potential of the ethanolic extract of *T. purpurea* flowers in ameliorating phenytoin-induced hepatotoxicity. We aim to investigate the biochemical and histopathological changes in the liver of Wistar rats exposed to phenytoin, both with and without concurrent administration of *T. purpurea* flower extract. By elucidating the underlying mechanisms and assessing the extent of liver protection offered by this natural remedy, we aspire to contribute valuable insights into the development of safer and more effective therapeutic strategies for epilepsy patients. This study not only sheds light on the potential synergy between traditional herbal medicine and modern pharmacology but also underscores the importance of exploring natural remedies for countering drug-induced side effects, thereby enhancing the overall quality of patient care.

2. Materials and Methods

Collection and extraction of flowers

In December 2019, we collected the flowers of *T. purpurea* from the scenic campus of Andhra University in Visakhapatnam. To ensure proper documentation, a voucher specimen has been meticulously preserved and deposited in the Herbarium of the Botany Department at Andhra University, Visakhapatnam.

Approximately 200 g of flowers of *T. purpurea* were pulverized into a fine powder and subjected to extraction (300 mL × 14 days × 3) with ethanol (95% ethanol, Sigma, India) using the maceration method [13]. This extraction process was carried out at room temperature. Subsequently, the combined extracts were concentrated using a rotavapor (Shimadzu Rotation evaporator QR, 2005-S, Japan), resulting in the extraction of the ethanol extract of *T. purpurea* (TPF) as an 18.4 g brown solid.

Animals

Male albino rats, aged adult and weighing between 150 to 200 grams, were carefully chosen for this study. They were housed in propylene cages, maintaining a room temperature of 25 ± 3°C. Throughout the duration of the study, the rats had unrestricted access to a standard pellet diet and were provided with ample drinking water. The study protocol received ethical approval from the Institutional Animal Ethical Committee at the University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India, with reference number 516/PO/c/01/IAEC.

Acute toxicity study

An acute toxicity study was conducted using standardized, healthy male albino rats. These rats were orally administered a single dose of 2000 mg/kg body weight (b.w) of the test substance, TPF. Following the administration of this test dose, the rats were closely observed and monitored for a period of 14 days. During this observation period, various behavioral parameters and any signs of toxicity were diligently recorded. Additionally, the

mortality rate, if any, was carefully documented [14]. The purpose of this acute toxicity study was to determine the LD_{50}, which represents the median lethal dose, or the dose at which 50% of the test subjects would succumb to the toxic effects of the substance [15,16].

Study Protocol

In this study, a total of twenty-four adult male albino rats were divided into four groups, each consisting of six animals. The experimental groups were structured as follows:

Control Group (Group 1): Rats in this group were administered 0.2% carboxymethyl cellulose (CMC) orally for a duration of 45 days. This group served as the control, representing the baseline measurements.

Phenytoin Group (Group 2): Rats in this group were orally administered a dose of 20 mg/kg of phenytoin daily for 45 days [6]. This group served as the model for phenytoin-induced hepatotoxicity.

TPF 100 mg/kg Group (Group 3): Rats in this group received 100 mg/kg of *T. purpurea* flower extract (TPF) orally, in a 0.2% CMC solution, 1 h prior to the daily dose of 20 mg/kg phenytoin. This group aimed to assess the protective effects of a lower TPF dose against phenytoin-induced hepatotoxicity.

TPF 200 mg/kg Group (Group 4): Rats in this group were administered 200 mg/kg of *T. purpurea* flower extract (TPF) orally, in a 0.2% CMC solution, 1 hour before the daily 20 mg/kg phenytoin dose. This group sought to evaluate the protective effects of a higher TPF dose against phenytoin-induced hepatotoxicity.

After 45 days of drug administration, the rats were anesthetized using ether anesthesia, and blood samples were collected from the retro-orbital plexus. These blood samples were then subjected to a series of serum biochemical analyses, which included measurements of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, total protein, and total bilirubin (TBL) [17,18]. These parameters were analyzed using enzymatic kits (AGAPPE, India) and an autoanalyzer (Chemistry Analyser CA 2005, B4B Diagnostic Division, China).

Following blood collection, the animals were humanely sacrificed, and their liver tissues were carefully dissected, washed in 100 mM cold phosphate buffer (pH 7.4), weighed, and then sliced for subsequent histopathological examinations. Additionally, liver tissues were stored at -40°C for further analysis.

The stored liver tissues were homogenized, and the resulting homogenate was subjected to centrifugation at 10,000 × g for 10 minutes at 4°C. The supernatant obtained was assessed for the measurement of lipid peroxidation using the malondialdehyde method, as well as the evaluation of antioxidants such as superoxide dismutase (SOD), catalase and glutathione (GSH) using the pyrogallol auto-oxidation [19], hydrogen peroxide [20], and Ellman's [21] methods, respectively.

Histopathological Examinations

Upon reaching the endpoint of the study, rats were anesthetized using ether anesthesia and subsequently euthanized. The liver specimens were then meticulously removed and immediately fixed in a 4% paraformaldehyde solution, where they remained overnight for optimal preservation. To prepare tissue blocks for sectioning, a block preparation unit, specifically the Shandon Histocenter-2, was employed. Using a microtome, specifically the Leica RM 2255 from Lab India, tissue sections of 10 μm thickness were precisely cut. These sections were then carefully transferred onto slides that had been pre-coated with poly-l-lysine. Following sectioning, the tissue specimens were subjected to staining with hematoxylin and eosin. This staining process allowed for the visualization of cellular and tissue structures within the liver samples, enabling detailed histopathological examination. The stained sections were then ready for further analysis to assess any potential histological changes or abnormalities induced by the experimental conditions.

Statistical analysis

The data obtained from the experiments were presented as the mean ± standard error of the mean (SEM) for each group, with a sample size of n=6 for each group using Graphpad Prism. To assess the significance of differences among the various experimental groups, a statistical analysis was carried out employing analysis of variance. Subsequently, post hoc analysis was performed using Tukey-Kramer's Multiple Comparison Test to identify specific group differences.

3. Results and Discussion

Impact of TPF on liver parameter changes induced by phenytoin

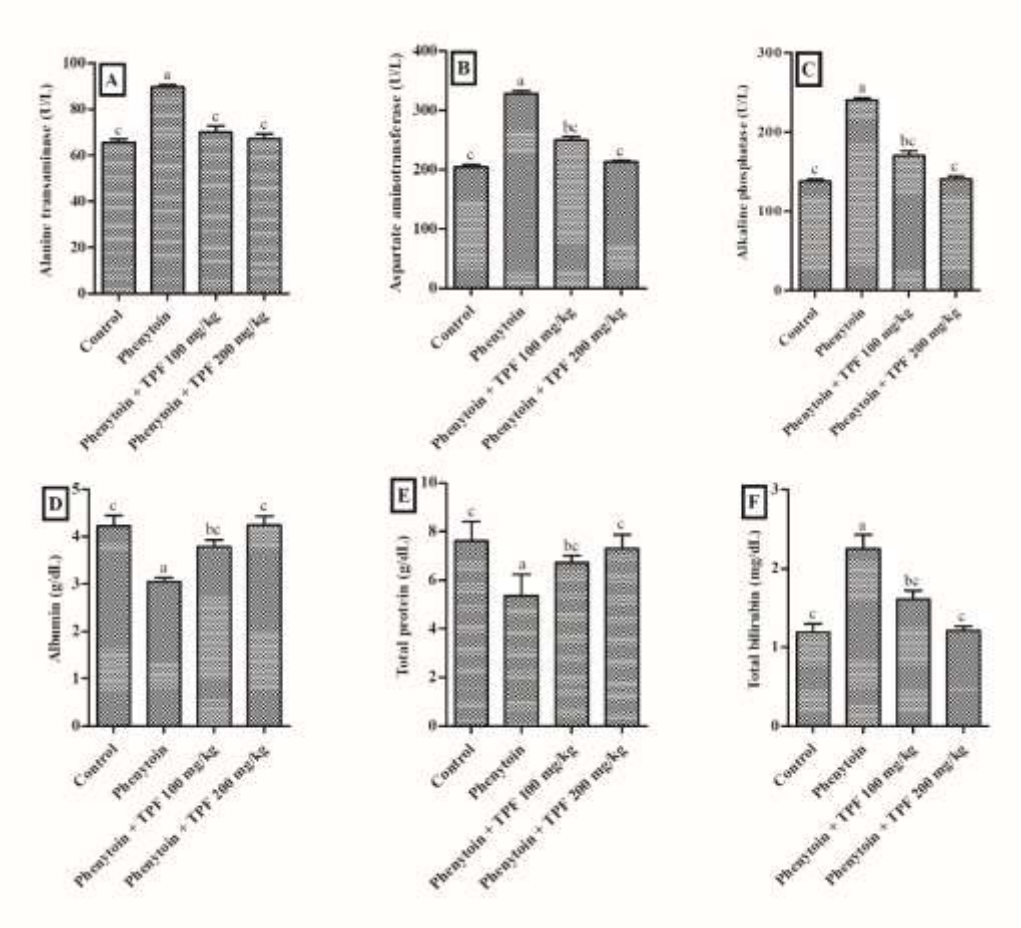
Administration of phenytoin at a dose of 20 mg/kg for a duration of 45 days led to significant increases in the levels of AST, ALT, ALP and total bilirubin, accompanied by a noteworthy decrease in the levels of total protein and albumin (Figure 1). TPF (100 mg/kg) demonstrated a significant ($p < 0.001$) reduction in the higher AST levels compared to phenytoin-treated rats, although these values did not fully return to the levels observed in the normal control group (Figure 1). Notably, TPF at its higher dose (200 mg/kg) effectively normalized the AST levels, bringing them close to those seen in the normal control animals (Figure 1).

Regarding ALT levels, TPF (100 mg/kg) significantly ($p < 0.001$) lowered them in comparison to phenytoin-treated animals, yet these values remained somewhat elevated compared to the normal control group (Figure 1). However, TPF at a dose of 200 mg/kg effectively reduced the ALT levels, restoring them to near-normal values (Figure 1). At a dose of 100 mg/kg, TPF significantly ($p < 0.001$) lowered total bilirubin levels, and at 200 mg/kg, these values approached those of the normal control group (Figure 1).

In terms of ALP levels, TPF at 100 mg/kg significantly ($p < 0.001$) lowered them, although they remained somewhat elevated compared to normal values (Figure 1). Nevertheless, the antioxidant, when administered at its higher dose of 200 mg/kg, effectively reduced ALP levels, bringing them near those of the normal control group (Figure 1).

At 100 and 200 mg/kg, it significantly enhanced albumin levels in a dose-dependent manner (Figure 1). Notably, at the higher dose of 200 mg/kg, a significant ($p < 0.001$) raise in albumin levels, approaching those of the normal control, was observed (Figure 1). Regarding total protein levels, TPF at 100 and 200 mg/kg, it significantly ($p < 0.001$) enhanced total protein levels (Figure 1).

Figure 1. Impact of TPF on liver parameter changes induced by phenytoin. Values are expressed as mean ± SEM of 6 animals, where $^{a}p < 0.001$ vs. control group, $^{b}p < 0.05$ vs. control group, and $^{c}p < 0.001$ vs. phenytoin group.



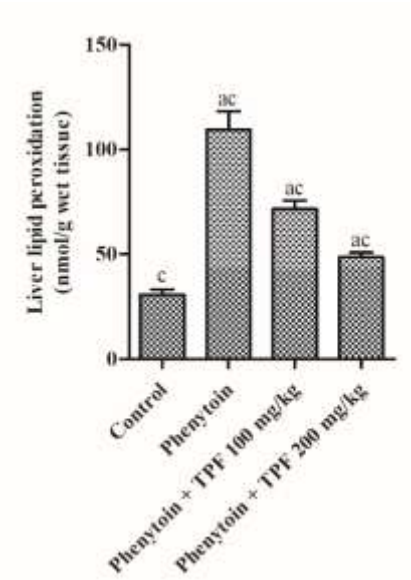


Figure 2. Influence of TPF on phenytoin-induced enhancement of liver lipid peroxidation. Values are expressed as mean ± SEM of 6 animals, where $^{a}p < 0.001$ vs. control group, $^{b}p < 0.05$ vs. control group, and $^{c}p < 0.001$ vs. phenytoin group.

Influence of TPF on phenytoin-induced enhancement of liver lipid peroxidation

The administration of phenytoin at a dose of 20 mg/kg over a 45-day period led to a significant increase in liver lipid peroxide content. TPF, administered at two doses (100 and 200 mg/kg), exhibited a significant ($p < 0.001$) reduction in liver lipid peroxidation in a dose-dependent manner. However, it's important to note that the values did not fully return to the normal levels (Figure 2).

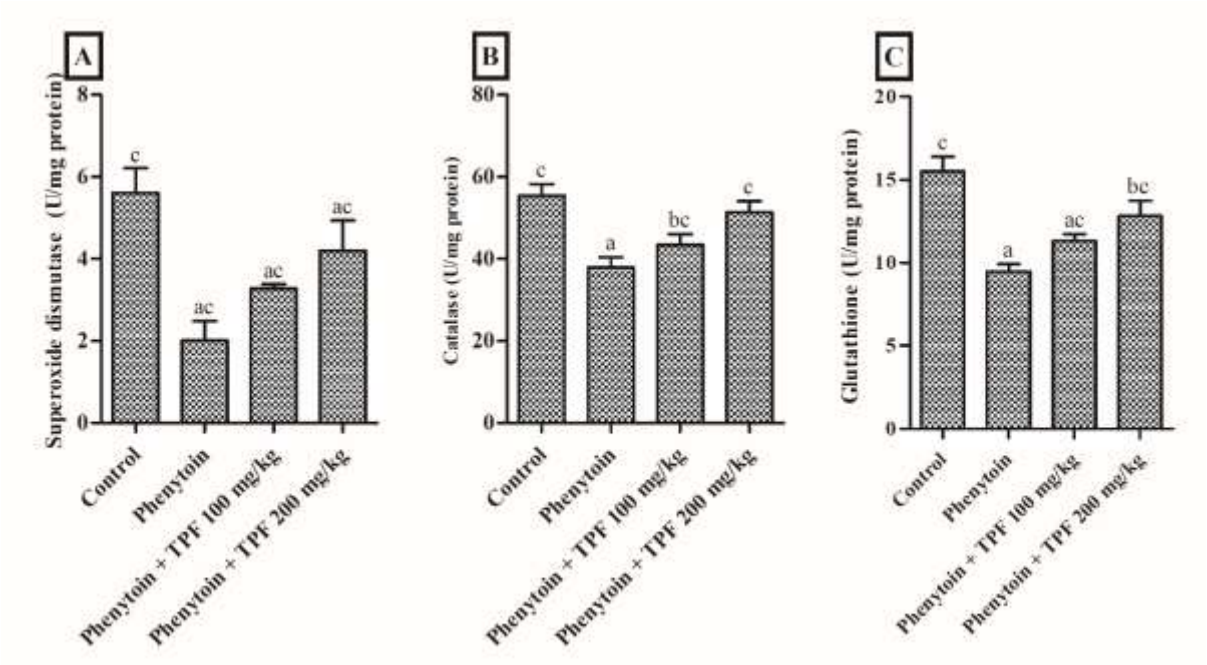


Figure 3. Impact of TPF on endogenous enzymatic and non-enzymatic antioxidants depleted by phenytoin. Values are expressed as mean ± SEM of 6 animals, where $^{a}p < 0.001$ vs. control group, $^{b}p < 0.05$ vs. control group, and $^{c}p < 0.001$ vs. phenytoin group.

Impact of TPF on Endogenous Enzymatic and Non-Enzymatic Antioxidants Depleted By Phenytoin

The administration of phenytoin at a dose of 20 mg/kg for a 45-day duration resulted in a significant decrease in endogenous enzymatic antioxidants, including SOD and catalase, as well as the non-enzymatic antioxidant GSH levels in the liver. TPF, when administered at two doses (100 and 200 mg/kg), demonstrated a significant raise in the levels

of endogenous antioxidants that had been reduced by phenytoin, following a dose-dependent pattern. However, it's noteworthy that these values did not fully return to normal levels (Figure 3).

Influence of TPF On Phenytoin-Induced Changes In Body Weight, Absolute Liver Weight, And Relative Liver Weight

After the 45-day treatment period with phenytoin, a statistically significant decrease in body weight and an increase in both absolute and relative liver weights were observed when compared to the control group. However, when TPF was administered at a dose of 100 and 200 mg/kg, it effectively reversed the phenytoin-induced weight loss and significantly reduced both the absolute and relative liver weights when compared to the phenytoin-treated group (Table 1). This suggests that TPF may play a role in mitigating the adverse effects of phenytoin on body weight and liver weight, potentially contributing to overall better health outcomes.

Table 1. Influence of TPF on phenytoin-induced changes in body weight, absolute liver weight, and relative liver weight

Groups	**Body weight (g)**			**Absolute liver weight (g)**	**Relative liver weight (g)**
	Initial (g)	**Final (g)**	**% Change**		
Control	212.5±10.2	240.2±12.1^{c}	13.04±0.99^{c}	11.7±0.81^{c}	4.4±0.45^{c}
Phenytoin	218.7±12.5	196.5±8.5^{a}	-10.15±0.61^{a}	13.6±0.56^{a}	6.9±0.70^{a}
Phenytoin + TPF 100 mg/kg	215.9±13.3	216.4±10.5^{bc}	0.23±0.01^{ac}	12.1±0.90^{bc}	5.6±0.21^{ac}
Phenytoin + TPF 200 mg/kg	216.8±15.5	235.4±12.9^{c}	8.58±0.17^{ac}	11.8±0.11^{c}	5.2±0.15^{ac}

Values are expressed as mean ± SEM of 6 animals, where $^{a}p < 0.001$ vs. control group, $^{b}p < 0.05$ vs. control group, and $^{c}p < 0.001$ vs. phenytoin group.

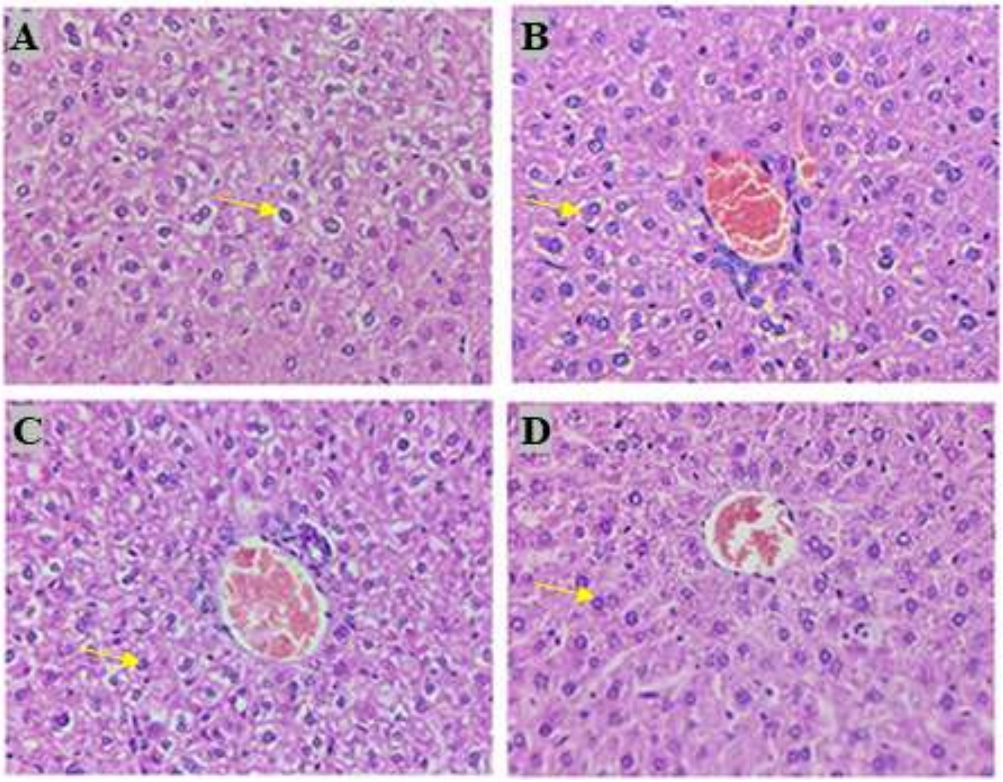


Figure 4. Visual representation of liver histopathology changes induced by phenytoin and TPF. (A) Control group: Normal hepatocytes, (B) Phenytoin-treated group: Periportal inflammation, (C) Phenytoin + TPF (100 mg/Kg) group: Fatty degeneration with mild hepatic necrosis, and (D) Phenytoin + TPF (200 mg/Kg) group: Fatty degeneration with restored hepatic parenchyma

Impact of TPF on Liver Histopathological Changes Induced By Phenytoin

Histopathological examination of the liver tissues revealed distinct findings among the experimental groups. The livers of rats in the control group exhibited a normal and healthy hepatic architecture, indicating no signs of damage or inflammation (Figure 4A). Liver sections from the phenytoin-

treated group displayed severe congestion, periportal inflammation characterized by centrilobular congestion, fatty degeneration, and hepatocellular necrosis. These findings reflected the significant hepatic damage induced by phenytoin (Figure 4B). Rats treated with both phenytoin and TPF at a dose of 100 mg/kg exhibited milder hepatic necrosis compared to the phenytoin group. This suggests that TPF at this dose mitigated the extent of hepatic damage induced by phenytoin (Figure 4C). The group treated with phenytoin and TPF at a dose of 200 mg/kg displayed a liver histopathology consistent with normal hepatic parenchyma. This suggests that TPF at this higher dose effectively protected the liver tissue from phenytoin-induced damage, resulting in a histological appearance similar to that of the control group (Figure 4D). These findings underscore the potential hepatoprotective effects of TPF, particularly at higher doses, in mitigating the hepatic damage caused by phenytoin, as evidenced by improved liver histopathology.

4. Discussion

Phenytoin, a commonly prescribed antiepileptic drug, is known to induce hepatotoxicity through various mechanisms [22]. Accumulation of arene oxide metabolites of phenytoin has been linked to hepatotoxicity, and oxidative stress is considered one of the primary mechanisms responsible for phenytoin-associated liver damage [7]. This oxidative stress arises from the metabolites formed during the biotransformation of phenytoin, both in humans and rats [10]. Oxidative stress can lead to the depletion of mitochondrial antioxidant defenses in the liver, contributing to the hepatotoxicity associated with phenytoin therapy [10,23,24]. These studies collectively underscore the role of oxidative stress mediated by reactive oxygen species as a significant contributor to phenytoin-induced liver damage [25]. In this study, phenytoin administration resulted in increased lipid peroxidation and decreased levels of endogenous antioxidants such as SOD, catalase, and GSH, indicating the presence of oxidative stress in the liver. However, TPF supplementation counteracts the alterations induced by phenytoin.

Serum markers such as AST, ALT, ALP, bilirubin, albumin, and total protein are commonly used to assess hepatic damage. Elevated levels of AST, ALT, ALP, and bilirubin, along with decreased levels of albumin and total protein, are indicative of liver damage [26]. Additionally, low serum albumin levels are associated with poor liver function [6]. In this study, phenytoin-treated rats exhibited significant alterations in these markers, confirming the hepatotoxic nature of phenytoin. Phenytoin also had a detrimental impact on body weight, resulting in weight loss, and increased the relative liver weight, further indicating liver damage [12]. In the current study, TPF supplementation demonstrated a significant reduction in markers of hepatotoxicity, including AST, ALT, and bilirubin, which had been higher by phenytoin. TPF also rebuilt the levels of total protein and albumin, which had been reduced by phenytoin treatment. Notably, TPF improved the total body weight of the rats and reduced the relative liver weight, countering the alterations induced by phenytoin.

Histopathological examination of liver tissues from phenytoin-treated rats revealed severe damage, including periportal inflammation, hemorrhage, sinusoidal congestion, and hepatocellular necrosis. These histological changes aligned with the alterations in biochemical parameters and provided evidence of liver damage caused by phenytoin, likely mediated by oxidative stress [5,6,11]. Histopathologically, TPF supplementation ameliorated the hepatic damage induced by phenytoin. TPF, particularly at a dose of 200 mg/kg, significantly protected against phenytoin-induced toxicity. This protective effect can be attributed to TPF's ability to mitigate oxidative stress and lipid peroxidation through its free radical scavenging activity, ultimately enhancing the levels of the antioxidant defense system.

Overall, this study highlights the potential hepatoprotective role of TPF against phenytoin-induced hepatotoxicity. TPF's ability to counter oxidative stress, restore antioxidant levels, and improve liver function markers underscores its therapeutic potential in safeguarding the liver against the adverse effects of phenytoin therapy. The antioxidant properties of TPF and its role in glutathione recycling have spurred its utilization in liver damage management. Further investigations into the precise mechanisms and clinical applications of TPF in hepatoprotection are warranted to advance our understanding and therapeutic options in the management of drug-induced liver damage.

5. Conclusion

The outcomes of the current study unveiled the hepatoprotective potential of TPF against phenytoin-induced oxidative stress and hepatotoxicity. TPF also effectively mitigated the histopathological alterations induced by phenytoin in the liver. Particularly, TPF at doses of 200 mg/kg demonstrated significant efficacy in ameliorating oxidative stress and hepatic damage induced by phenytoin. It is noteworthy that the relatively lower efficacy observed with TPF at 100 mg/kg may be attributed to the enzyme-inducing properties of phenytoin. This investigation underscores the favorable impact of TPF in countering phenytoin-

induced hepatotoxicity, primarily mediated through the mitigation of oxidative stress.

6. References

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