Trop J Nat Prod Res, November 2025; 9(11): 5427 - 5430
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Hepatoprotective Effect of *Hibiscus sabdariffa L.* in Severe Paracetamol-Induced Hepatotoxicity: High-Dose and Dose-Response Study

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ARTICLE INFO

Article history:
Received 28 December 2024
Revised 19 October 2025
Accepted 28 October 2025
Published online 01 December 2025

ABSTRACT

Liver disease remains a significant global health challenge, and despite progress in modern medicine, there is no pharmacological treatment that provides full protection or regeneration of liver tissue. Paracetamol, although widely used as an analgesic and antipyretic, is known to cause liver toxicity in overdose conditions. The objective of this research was to assess the hepatoprotective potential of *Hibiscus sabdariffa L*. extract in severe hepatotoxicity induced by high-dose paracetamol (2000 mg/kg BW), a dosage rarely explored in previous studies. The experimental rats were randomly divided into five distinct groups: a negative control, a positive control, and three treatment groups receiving *Hibiscus sabdariffa L* extract at 300, 550, and 1000 mg/kg BW, respectively. Histopathological analysis showed that the extract at 1000 mg/kg BW offered the highest level of protection, with minimal signs of hemorrhage, necrosis, or apoptosis, and improved hepatocellular morphology. The hepatoprotective effect of the extract may be attributed to the presence of antioxidant and anti-inflammatory properties, particularly anthocyanins and polyphenols, which have been shown to enhance antioxidant enzyme activity and reduce oxidative stress. The present results reinforce the possibility that *Hibiscus sabdariffa* L. may serve as a promising natural therapeutic agent that could offer hepatoprotective effect against drug-induced liver injury.

Keywords: *Hibiscus sabdariffa L*, Hepatoprotective, Paracetamol, Hepatotoxicity.

Introduction

Acetaminophen-induced hepatotoxicity has been widely studied, and the mechanisms underlying its pathophysiology have been well documented.[1] Paracetamol-induced hepatotoxicity results from the metabolic conversion into a reactive metabolite, N-acetyl-p-benzoquinonimine (NAPQI). Under physiological conditions, NAPQI is detoxified by binding with glutathione in hepatic tissue; however, when overdose occurs, glutathione reserves are depleted, allowing NAPQI to covalently interact with cellular proteins, causing hepatocyte necrosis and potentially liver failure.[2] Unlike most prior studies, this research explores the hepatoprotective effect of *Hibiscus sabdariffa L* against paracetamol-induced liver injury at a high dose of 2000 mg/kg BW. This approach enhances clinical relevance by simulating severe overdose scenarios, providing new insights into the therapeutic capacity of *Hibiscus sabdariffa L*. extract. Paracetamol overdose continues to be one of the primary causes of acute hepatic injury, particularly in Western countries.[3] The hepatotoxic mechanism involves oxidative stress, inflammation, and necrosis due to NAPQI.[4] *Hibiscus sabdariffa L* are rich in polyphenolic compounds, particularly anthocyanins, which exhibit strong antioxidant properties.[5] These compounds are believed to protect hepatocytes from oxidative damage induced by paracetamol metabolites.

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Citation: Abdulkadir WS, Cindana MA, Tungadi R, Akuba J, Ramadhani FN, Hutuba AH. Hepatoprotective Effect of *Hibiscus sabdariffa* L. in Severe Paracetamol-induced Hepatotoxicity: High-Dose and Dose-Response Study. Trop J Nat Prod Res. 2025; 9(11): 5427 – 5430 https://doi.org/10.26538/tjnpr/v9i11.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

However, the specific hepatoprotective mechanism of *Hibiscus sabdariffa L*. in high-dose paracetamol on hepatic toxicity has not been thoroughly investigated. Its anthocyanin content has been reported to upregulate antioxidant enzymes, including superoxide dismutase (SOD) and catalase, while lowering malondialdehyde (MDA) levels, an indicator of oxidative stress.[6]

Several studies have confirmed the liver-protective potential of *Hibiscus sabdariffa L*. extract. For instance, Nwozo *et al*. demonstrated its effectiveness against carbon tetrachloride (CCl_4)-induced liver damage in rats, marked by lower liver enzymes and improved tissue architecture.[7] Other reports also support its protective effects in liver injuries induced by alcohol and other chemical toxicants.[8] This study fills the research gap by employing a high-dose (2000 mg/kg BW) paracetamol-induced hepatotoxicity model to simulate severe overdose conditions and by establishing a dose–response relationship for Hibiscus sabdariffa L. extract under such conditions.

Materials and Methods

Plant collection and identification

The flowers (calyces) of *Hibiscus sabdariffa* Linn (Roselle) were collected from Gorontalo, Indonesia in June 2023. The plant material was taxonomically identified at the Laboratory of Pharmacy, Faculty of Sport and Health, Universitas Negeri Gorontalo, Indonesia. Voucher specimen with reference number 71/UN47.B7/LAB.FAR/XII/2023 was assigned.

Plant extraction

Hibiscus sabdariffa L. powder (500 g) was macerated in 2 L of 70% ethanol at laboratory conditions (approximately 27 ± 2°C) for 72 h. The extract was filtered, and the filtrate was concentrated using a rotary evaporator under vacuum at approximately −0.08 MPa and 60°C to obtain a thick extract.

Animals

Twenty (20) apparently healthy adult male Wistar rats (*Rattus norvegicus*), with body weights ranging from 150 - 250 g, were

obtained from the Animal Laboratory, Department of Pharmacy, Faculty of Sport and Health, Universitas Negeri Gorontalo, Indonesia. Rats were acclimatized for 7 days with free access to standard feed and water. There was no treatment during the adaptation period.

Ethical approval
The ethical approval with approval number 60/UN47.B7/KE/2023 (Protocol No. 00902275712111320230713000) was granted by the Health Research Ethics Committee, Universitas Negeri Gorontalo, Indonesia.

Experimental design
Twenty (20) male rats were randomly assigned to five experimental groups (I – V), each structured to assess the liver-protective effects of various doses of *Hibiscus sabdariffa L*. extract, with comparisons made to a negative control (untreated) and a positive control (Pro Liver medication).
Group I (negative control) was administered paracetamol tablets at a dose of 111 mg/kg BW. The dose of 111 mg/kg BW was selected based on previous studies that demonstrated its effectiveness in inducing hepatotoxicity in rat models. Group II (positive control) was administered Pro Liver drug (Good Life®, Indonesia) containing curcuminoids 20 mg, silymarin 70 mg, choline bitartrate 150 mg, and vitamin B6 2 mg per tablet. The administered dose corresponded to 20 mg/kg body weight, equivalent to approximately 0.34 mg per 189 g rat, adjusted from tablet stock solution (0.7520 g/tablet) to yield ~0.511 g per animal. Group III received *Hibiscus sabdariffa L*. extract at 300 mg/kg BW. Group IV was treated with *Hibiscus sabdariffa L*. extract at 550 mg/kg BW. Group V received *Hibiscus sabdariffa L*. extract at 1000 mg/kg BW. The extract and the Pro Liver drug was administered orally once daily for a duration of 7 th days, and on the 7th day paracetamol was administered as a single oral dose of 111 mg/kg BW. At the end of the treatments and induction period, the rats were sacrificed by cervical dislocation, the livers were harvested, and processed for histopathological analysis.

Histopathological examination of liver tissues
Histopathological preparations for all groups were carried out using the Hematoxylin-Eosin (H&E) staining method. Briedly, liver tissues were rinsed with 0.9% NaCl and fixed in 10% Buffered Neutral Formalin (BNF). Samples were processed for histopathological examination using Hematoxylin-Eosin (H&E) staining. The stained sections were observed under a trinocular light microscope (EIC 1153) at 40 - 100× magnification to assess hepatocyte abnormalities. Histological slides were prepared at the Veterinary Disease Investigation Center (Balai Besar Veteriner), Maros, South Sulawesi, Indonesia, and evaluated by a board-certified anatomical pathologist.

Results and Discussion

The histopathological findings of rats' liver under different treatment conditions are presented in Table 1. The results showed that *Hibiscus sabdariffa L.* extract exhibited showed marked protective effects on hepatic tissues subjected to paracetamol-induced injury. In groups treated with *Hibiscus sabdariffa L* extract at doses of 550 mg/kg BW and 1000 mg/kg BW, there were evidence of hepatocellular repair, with only minor damage remaining. At 1000 mg/kg BW, there were no indications of hemorrhage, fibrin, necrosis, or apoptosis. Edema and cell degeneration, while still present, were in a gradual repair process, as shown by normal hepatocellular morphology in the liver images. These results suggest that a 1000 mg/kg BW dose of *Hibiscus sabdariffa L* extract is effective in maintaining hepatocellular structure without signs of serious liver damage.
Histopathological observations as shown in Figure 1 indicate that the negative control group, treated with distilled water and high dose of paracetamol, showed severe liver damage with signs of edema, hemorrhage, fibrin, necrosis, and apoptosis. This finding aligns with previous studies suggesting that paracetamol, at high doses, can cause severe liver injury.[9] The positive control group, which received Pro Liver drug before paracetamol induction, exhibited no visible sign of histological damage, indicating normal liver structure with no degeneration or inflammatory cell infiltration. This outcome suggests that the administration of distilled water alone did not ameliorate paraceramol-induced liver damage. However, in the groups treated with *Hibiscus sabdariffa L* extract, histopathological findings revealed a decrease in liver damage compared to the negative control. At a dose of 300 mg/kg BW, cell degeneration and inflammatory infiltration were reduced. At 1000 mg/kg BW, liver damage diminished significantly, with minimal necrosis and better-organized sinusoidal structures.

Table 1: Histological findings of the liver of Wistar rats treated with *Hibiscus sabdariffa L* extract

Group	Treatment	Histopathological Observation	Description
I	Negative Control (Distilled Water)	Edema, Hemorrhage, Fibrin, Cell Degeneration, Cell Necrosis, Apoptosis	There was serious damage
II	Positive Control (Pro-Liver Supplement)	No Edema, No Hemorrhage, No Fibrin, No Cell Degeneration, No Apoptosis	There was no change in histoarchitecture
III	*Hibiscus sabdariffa* L. extract (300 mg/kg)	Edema, Hemorrhage, Cell Degeneration, Apoptosis	Moderate damage occurred
IV	*Hibiscus sabdariffa* L. extract (550 mg/kg)	Edema, Hemorrhage, Fibrin, Cell Degeneration	Minor damage occurred
V	*Hibiscus sabdariffa* L. extract (1000 mg/kg)	Edema, Cell Degeneration	Minor damage occurred

Note: Histopathological evaluation was conducted on hematoxylin–eosin (H&E) stained liver sections (400× magnification).

The hepatoprotective effect seen in this study may be attributed to the high antioxidant activity of *Hibiscus sabdariffa L*. Compounds like anthocyanins, flavonoids, and phenolic acids in *Hibiscus sabdariffa L* extract can neutralize free radicals generated from toxic paracetamol metabolites, thereby lowering oxidative stress on the hepatocytes.[10] Additionally, *Hibiscus sabdariffa L* extract has the potential to enhance the activity of intrinsic antioxidant enzymes, including superoxide dismutase (SOD) and catalase, which play vital roles in maintaining redox balance in liver cells. Enhanced enzyme activity may prevent free radical accumulation, protecting cells from oxidative damage.[11]
Previous studies have demonstrated that *Hibiscus sabdariffa L* possess anti-inflammatory activity by inhibiting pro-inflammatory cytokines and decreasing inflammatory cell infiltration in damaged tissue.[12] These findings are consistent with the reduction in inflammatory cell infiltration observed in the current study in rats treated with *Hibiscus sabdariffa* extract. *Hibiscus sabdariffa L* extract exhibits various biological activities *in vitro*, including antioxidant effects and inhibition of CYP enzymes, which suppresses pro-apoptotic protein expression in liver cells.[4] Free radicals, due to their unpaired electrons, are highly unstable and are capable of causing liver cell damage. Flavonoid compounds in *Hibiscus sabdariffa L* extract help stabilize free radicals, acting as antioxidants. The antioxidant mechanism of flavonoids can be direct, by donating hydrogen ions, or indirect, by inhibiting pathways such as NFκB leading to reduction in apoptosis.[13]
Pre-administration of *Hibiscus sabdariffa L* extract have been shown to prevent lipid peroxidation linked to paracetamol-induced cell damage, evidenced by decreased malondialdehyde (MDA) levels.[14] Antioxidants in *Hibiscus sabdariffa L* stabilize free radicals, protecting cell membranes from radical-induced damage.[15] Enzymes like SOD, catalase, and glutathione peroxidase are crucial cellular antioxidants

responsible for breaking down reactive oxygen species. These enzymes, which are typically reduced after exposure to toxins, showed increased activity following *Hibiscus sabdariffa L* extract administration, demonstrating the extract's antioxidant efficacy.[16] In addition to *Hibiscus sabdariffa*, the hepatoprotective properties of other medicinal plants such as *Buchholzia coriacea* and *Tithonia diversifolia* have also been demonstrated in models of chemically and paracetamol-induced liver injury, respectively, further reinforcing the therapeutic relevance of plant-based antioxidants in hepatoprotection.[17,18]

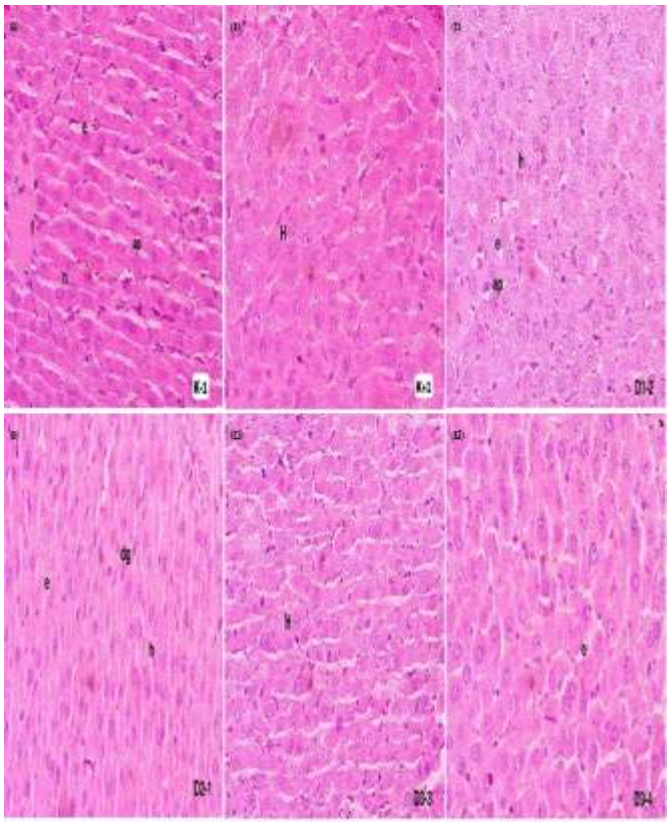

Figure 1: Photomicrographs of liver sections of rats under different treatments (H&E staining, 400×).
(A) Group I – Negative control (K–1): showing severe liver damage with edema (e), fibrin (f), nuclear degeneration (n), and apoptosis (ap). **(B)** Group II – Positive control (K+1): showing normal liver histoarchitecture with preserved hepatocytes (H) and no visible damage. **(C)** Group III – Extract 300 mg/kg BW (D1–2): showing moderate injury showing hemorrhage (h), edema (e), and apoptosis (ap). **(D)** Group IV – Extract 550 mg/kg BW (D2–1): showing minor injury with edema (e), hemorrhage (h), and cell degeneration (dg). **(E1)** Group V – Extract 1000 mg/kg BW (D3–3): showing mild cellular changes with preserved hepatocytes (H) and localized edema (e). **(E2)** Group V – Extract 1000 mg/kg BW (D3–4): showing tissue architecture well maintained; slight edema (e) observed.

The findings from this study have revealed that *Hibiscus sabdariffa L* extract holds promise as a natural hepatoprotective agent. Given the growing concerns over the side effects of synthetic drugs, medicinal plants like *Hibiscus sabdariffa L* offer a safer alternative to prevent liver damage, especially in populations at high risk of paracetamol overdose. Although these findings are promising, further research is necessary to validate the efficacy and safety of *Hibiscus sabdariffa L* in human subjects. Clinical trials with rigorous designs, such as randomized controlled trials, are crucial to determine the optimal dose, duration, and safety profile of this extract.

Conclusion

Histopathological evaluation of liver tissues from male rats administered with *Hibiscus sabdariffa* L. extract revealed marked hepatoprotective effects. Although mild edema and cellular degeneration were observed, the hepatic architecture remained largely intact, showing no evidence of hemorrhage, fibrin deposition, necrosis, or apoptosis. These observations indicate that *Hibiscus sabdariffa* L. extract provides strong protection against paracetamol-induced hepatic injury, particularly at higher dose levels**.** Further investigations are warranted to isolate and identify the specific bioactive constituents underlying this effect and to assess their therapeutic relevance using human hepatotoxicity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors appreciate Mr. I.I. Ogunlowo for his assistance during plant collection.

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ISSN 2616-0692 (Electronic)

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