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Protective Effect of Beetroot (*Beta vulgaris* L.) Extract Against 5-Fluorouracil-Induced Hepatotoxicity

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ABSTRACT

Background: The anticancer agent 5-fluorouracil (5-FU) exerts its cytotoxic effects partly through inhibition of thymidylate synthase and disruption of nucleic acid metabolism. However, treatment-related toxicities, including hepatotoxicity, can limit its clinical use. Beetroot (BR), *Beta vulgaris* L., is rich in bioactive compounds with antioxidant and anti-inflammatory properties that may protect against oxidative tissue injury. This study evaluated the protective effect of beetroot extract against 5-FU-induced hepatotoxicity in male albino rats.

Methods: Twenty-eight male albino rats were randomly allocated to four groups (n = 7 per group): control, BR, 5-FU, and 5-FU plus BR. BR extract (500 mg/kg) was administered orally once daily for 21 days, whereas 5-FU (50 mg/kg/day) was administered intraperitoneally on days 17 to 21. Blood samples were collected for assessment of serum liver biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein and albumin. Liver tissues were examined for hepatic oxidative stress markers, including malondialdehyde (MDA) and reduced glutathione (GSH) and superoxide dismutase (SOD) activity, as well as histopathological changes.

Results: Compared with the control group, 5-FU significantly increased serum ALT, AST, ALP, and total bilirubin, while decreasing serum total protein and albumin. In liver tissue, 5-FU significantly increased MDA and decreased GSH and SOD activity. Co-administration of BR extract significantly attenuated these biochemical and oxidative stress alterations and was associated with preservation of hepatic histological architecture compared with 5-FU treatment alone.

Conclusion: BR has a protective effect against 5-FU-induced hepatotoxicity, potentially attributed to its antioxidant properties. Further investigation of its potential hepatoprotective effects and underlying mechanisms in humans is recommended.

Keywords: Beetroot • 5-Fluorouracil • Hepatotoxicity • Oxidative stress • Rat

1. Introduction

Drug-induced liver injury (DILI) is one of the leading causes of acute liver failure globally.^(1,2) It may initially manifest as asymptomatic elevations in liver

enzymes or develop into more serious diseases such as cirrhosis, fulminant hepatic failure, and hepatitis.⁽³⁾ According to recent estimates, liver illnesses cause over 2 million deaths a year, or about 4% of all fatalities worldwide.⁽⁴⁾ Immune-mediated damage,



mitochondrial dysfunction, and oxidative stress are frequently involved in the pathophysiology of liver toxicity.^(5,6) Among chemotherapeutic agents, 5-fluorouracil (5-FU) has long been used to treat various malignancies, including colorectal, breast, gastric, and pancreatic cancers.^(7–10) However, a wide range of toxicities, such as those affecting the cardiovascular and gastrointestinal systems, kidneys, liver, and lungs, usually restricts its clinical value.^(11,12) Mechanistically, 5-FU's cytotoxic effects are mostly caused by thymidylate synthase inhibition through its active metabolite, which interferes with DNA synthesis.⁽¹³⁾ Additionally, its incorporation into RNA interferes with normal cellular processes, contributing to cytotoxicity.⁽¹⁴⁾

Commonly known as shamandar, beetroot (BR) is a plant cultivated in Europe, South America, Africa, and the Middle East, and has attracted interest due to its potential medicinal properties.⁽¹⁵⁾ Its high concentration of antioxidants, including polyphenols, flavonoids, saponins, betalains (such as betacyanins and betaxanthins), and inorganic nitrates, makes it especially noteworthy.⁽¹⁶⁾ Beetroot may provide protective effects against chemotherapy-induced liver toxicity, particularly in the setting of 5-FU toxicity, as these substances are known to combat oxidative stress, a major mediator of liver toxicity.

2. Methods

2.1. Beetroot collection and extraction

Fresh BR samples were obtained from a farm in Dhamar City, Yemen. The plant was identified and authenticated at the Department of Biology, Faculty of Science, Sana'a University; a voucher specimen was deposited under voucher No. 1177. BR samples were thoroughly washed with water to remove stems and surface impurities, peeled, and cut into uniformly small pieces. After being oven-dried at 40 °C, the dried pieces were ground into a fine powder and macerated in ethanol for 24 hours. The resulting

extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator.

2.2. Experimental animals and design

Twenty-eight male albino rats, weighing approximately 200 ± 20 g and aged 10–12 weeks, were obtained from the Animal House at the Biology Department of the Faculty of Science, Sana'a University. The rats were housed in groups of seven under controlled conditions. Throughout the study, they had free access to water and a standard pellet diet. Each rat was weighed and assigned a unique identification number to facilitate monitoring and data collection.

The animals were randomly divided into four experimental groups, each comprising seven rats. The control group received 2 mL of normal saline orally. The BR group received BR extract orally at 500 mg/kg/day for 21 days.⁽¹⁷⁾ The 5-FU group received 5-FU intraperitoneally at 50 mg/kg/day on days 17 to 21.⁽¹⁸⁾ The 5-FU plus BR group received BR extract orally at 500 mg/kg/day for 21 days and 5-FU intraperitoneally at 50 mg/kg/day on days 17 to 21.^(17,18)

2.3. Collection of blood and liver tissue specimens

On day 21 of the experiment, each rat was weighed individually. Twenty-four hours after the final injection, the rats were anesthetized with diethyl ether before blood and tissue collection. Blood was collected by cardiac puncture into serum-separator gel tubes and centrifuged at 3,000 rpm for 15 min. The separated serum was stored at –80°C until biochemical analysis. After blood collection, the liver was carefully excised and gently rinsed with normal saline. A portion of each liver sample was homogenized to measure oxidative stress parameters, while the remaining tissue was fixed in 10% formalin for histological analysis.

2.4. Biochemical analysis



Serum biochemical markers of liver injury and function were measured using commercially available assay kits (BioSystems S.A., Barcelona, Spain) according to the manufacturer's instructions. The measured parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin.

2.5. Oxidative stress analysis

Liver tissue homogenates were used to measure reduced glutathione (GSH) in mmol/L, malondialdehyde (MDA) in nmol/ml, and superoxide dismutase (SOD) in U/g protein using commercial colorimetric assay kits (Bio diagnostics Dokki, Giza, Egypt) with a Rayto RT-9200 semi-automatic chemistry analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China).

2.6. Histopathological examination

Liver tissue specimens were dehydrated by ascending concentrations of ethanol, cleared by xylene, embedded in paraffin, and sectioned at 4-μm thickness. Tissue sections were then stained with hematoxylin and eosin (H&E) and examined using an

Olympus BX53 digital microscope (Olympus Corporation, Hachioji, Tokyo, Japan).

2.7. Data analysis

Data were analyzed using GraphPad Prism, version 8.4 (GraphPad Software, San Diego, CA, USA). Data were compared as the mean ± standard deviation (SD), and differences among the groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of BR extract on liver biomarkers in 5-FU-treated rats

Compared with the control group, rats treated with 5-FU showed significantly lower serum total protein and albumin levels ($P < 0.001$) and significantly higher serum ALT, AST, and ALP activities and total bilirubin levels ($P < 0.001$). Compared with the 5-FU group, co-administration of BR extract significantly decreased serum ALT, AST, and ALP activities and total bilirubin levels ($P < 0.001$) and significantly increased serum total protein and albumin levels ($P < 0.001$) (Table 1).

Table 1: Effect of beetroot extract on serum liver biomarkers in 5-FU-treated albino rats

Biomarker	Groups (n = 7 per group)				P-value
	Control	5-FU	BR	5-FU plus BR	
	Mean ± SD				
ALT (U/L)	33.77 ± 8.30	69.75 ± 12.00	33.27 ± 7.90	44.60 ± 6.80	<0.001
AST (U/L)	35.10 ± 15.60	78.50 ± 11.50	33.80 ± 7.80	42.50 ± 11.00	<0.001
ALP (U/L)	198.80 ± 25.00	502.80 ± 27.00	183.00 ± 21.00	328.20 ± 47.00	<0.001
Total protein (mg/dL)	718.00 ± 57.00	571.00 ± 62.00	747.00 ± 40.00	668.00 ± 38.00	<0.001
Serum albumin (mg/dL)	364.40 ± 21.00	265.00 ± 43.00	381.00 ± 31.00	325.50 ± 20.00	<0.001
Total bilirubin (mg/dL)	0.33 ± 0.20	1.35 ± 0.30	0.15 ± 0.10	0.53 ± 0.14	<0.001

5-FU, 5-fluorouracil; BR, beetroot; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

3.2. Effect of BR extract on hepatic oxidative stress biomarkers in 5-FU-treated rats

Compared with the control group, rats receiving 5-FU showed significantly lower hepatic GSH levels and SOD activity ($P < 0.001$) and significantly higher

MDA levels ($P < 0.001$). In contrast, rats co-treated with BR extract had significantly lower hepatic MDA levels ($P < 0.001$) and significantly higher GSH levels and SOD activity ($P < 0.001$) compared with the 5-FU group (Figure 1).



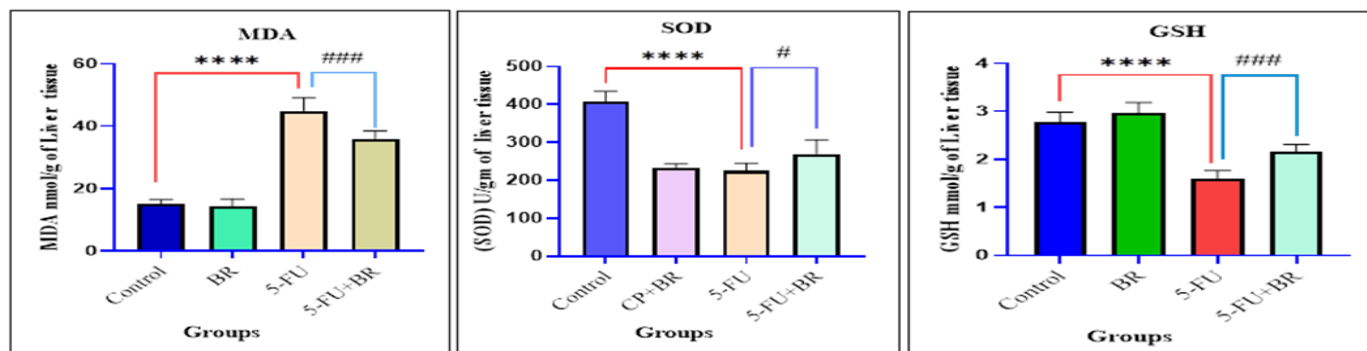


Figure 1: Effect of BR extract on hepatic oxidative stress markers in 5-FU-treated rats. Results are presented as mean $\pm$ SD; $n = 7$ rats per group. MDA, malondialdehyde; SOD, superoxide dismutase; GSH, reduced glutathione. **** $P < 0.001$ for the group treated with 5-FU compared with the control group; ### $P = 0.001$, # $P < 0.05$, and ### $P < 0.001$ for the group treated with 5-FU plus BR compared with the 5-FU-treated group

3.3. Effect of BR on histopathological findings in 5-FU-treated rats

H&E staining showed swollen, pale hepatocytes around the central vein, mild focal central venous congestion, and focal inflammatory cell infiltration in the 5-FU group. In contrast, the 5-FU plus BR group

exhibited preserved hepatic architecture, with well-organized hepatic cords and sinusoids radiating around the central vein and an intact portal tract comprising the portal vein, hepatic artery, and bile duct (Figure 2)

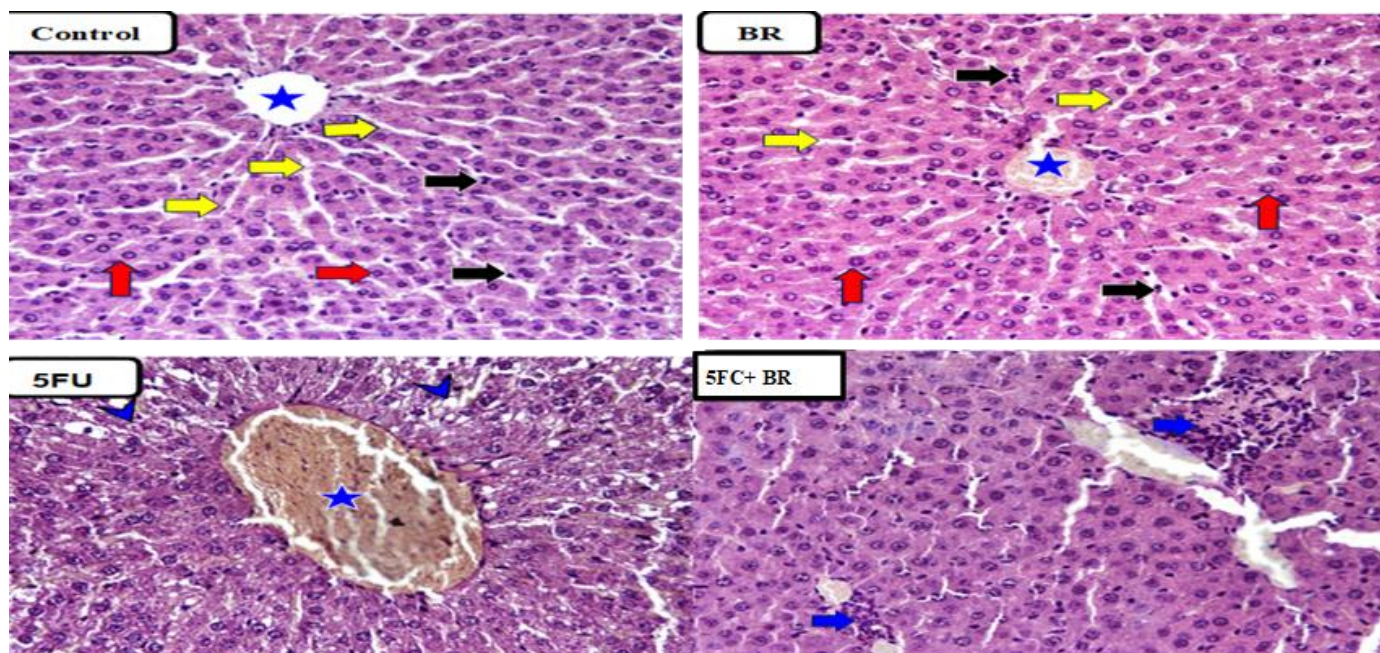


Figure 2: Photomicrographs of liver sections stained with H&E: Representative histological photomicrographs of liver sections from control rats, showing hepatocytes adjacent to the central vein that appear swollen and pale (arrowhead), with mild focal congestion of the central vein (★), and focal inflammatory cell infiltration (blue arrows). In contrast, the 5-FU plus BR group showed preserved hepatic architecture, with well-organized hepatic cords and sinusoids (yellow arrows) radiating around the central vein (★). The portal tract contained a portal vein (blue arrow), hepatic artery (red arrow), and bile duct (green arrow). Magnification: $\times 400$

4. Discussion

The present findings provide evidence that BR extract confers protective effects against 5-FU-induced hepatotoxicity, as demonstrated by

significant improvements in biochemical, oxidative, and histopathological parameters in the BR-treated group. These effects suggest that BR bioactive constituents may have hepatoprotective potential.



5-FU-induced hepatotoxicity involves several mechanisms, including inflammation, oxidative stress, and mitochondrial dysfunction.^(5,6) In the present study, the increased serum activities of ALT, AST, and ALP and the elevated total bilirubin level following 5-FU administration indicate hepatocellular injury and impaired hepatic function, whereas the reductions in total protein and albumin may reflect diminished hepatic synthetic capacity. These findings are consistent with previous reports of 5-FU-associated liver injury.^(12,14,23) Co-administration of BR attenuated these alterations, suggesting partial preservation of hepatic function. The observed hepatoprotective effects may be attributable to BR phytochemicals, particularly betalains, polyphenols, and flavonoids, which have demonstrated antioxidant and anti-inflammatory properties in various experimental models.^(15,16,19)

Oxidative stress is a key contributor to chemotherapy-induced organ toxicity.⁽²⁰⁾ The increased hepatic MDA level and decreased SOD activity and GSH level following 5-FU administration indicate enhanced lipid peroxidation and depletion of antioxidant defenses. These findings are consistent with those of Öztürk et al,⁽²¹⁾ who reported that 5-FU treatment induced oxidative damage. The attenuation of these changes by BR may be related to its antioxidant constituents; however, the underlying molecular pathways were not directly investigated in this study.⁽²²⁾

Histologically, rats treated with 5-FU exhibited hepatocyte swelling, central venous congestion, and inflammatory cell infiltration, supporting the biochemical evidence of hepatic injury. These findings are consistent with the 5-FU-induced tissue toxicity reported in a rat model of 5-FU toxicity.^(12,23) Co-treatment with BR largely preserved hepatic architecture, in agreement with previous experimental evidence of the tissue-protective effects of beetroot against toxic injury.^(24,25)

This study was limited by its small sample size, inclusion of only male rats, short duration, and use of single BR and 5-FU doses. Therefore, these pre-clinical findings require confirmation in further studies.

5. Conclusion

BR extract shows a protective effect against 5-FU-induced hepatotoxicity in this rat model, as reflected by improvements in biochemical and oxidative stress markers and preservation of hepatic architecture. Further well-designed studies, including clinical trials, are needed to confirm these findings and establish the safety and efficacy of BR extract before its clinical use as an adjunct to 5-FU therapy.

Ethical approval

All experimental procedures were conducted in accordance with the revised *Guide for the Care and Use of Laboratory Animals* (1996). The study protocol was approved by the Animal Research Ethics Committee of the Faculty of Pharmacy, USTY, Sana'a, Yemen (Approval No. UST/UREC/0010/1445).

Conflict of interest

The authors declare no conflicts of interest.

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