

AMELIORATIVE ROLE OF *FOENICULUM VULGARE* SEED OIL ON PHYSIOLOGICAL DISORDERS ACCOMPANIED PHENYLHYDRAZINE-INDUCED TOXICITY IN MALE ALBINO RATS

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ABSTRACT

Phenylhydrazine (PhH) is an aromatic organic compound with broad applications in various fields, including chemistry, pharmaceuticals, and agriculture. Additionally, it serves as a model for studying toxicity in biological and medical research. This study aims to explore the potential of *Foeniculum vulgare* seed oil (FSO) as a natural remedy to mitigate the harmful effects of PhH toxicity. The experiment involved five groups of male rats: the control group was administered distilled water for 14 days; the FSO group received a daily oral dose of 0.5 ml/kg b.w. FSO for 14 days; the PhH group was intraperitoneally injected with 60 mg/kg b.w. PhH over three consecutive days; the FSO + PhH group was treated with FSO orally for 11 days before being injected with PhH; and the PhH + FSO group was given PhH injections for three days, followed by daily oral doses of FSO for 11 days. Blood samples were taken from the rats on day 15 to analyze haematological and biochemical levels. Rats treated with PhH exhibited elevated levels of malondialdehyde, leukocytes, liver panel, kidney panel, and erythropoietin, while catalase, superoxide dismutase, erythrocytes, haematocrit, haemoglobin, and thrombocytes levels were reduced relative to the control group. Administration of FSO to PhH-treated rats improved oxidative stress, haematological parameters, liver panel, kidney panel, and erythropoietin levels. In summary, FSO was effective in reducing the disturbances in oxidative stress, haematological parameters, liver and kidney panels, and erythropoietin levels induced by PhH treatment in rats.

Keywords: Phenylhydrazine; *Foeniculum vulgare*; Haematological parameters; Liver panel; Kidney panel; Oxidative stress.

INTRODUCTION

Phenylhydrazine (PhH) is an organic compound belonging to the class of hydrazines (Yadav *et al.*, 2024). It is widely used in organic chemistry for synthesizing hydrazones, pharmaceuticals, and dyes, as well as for the detection of sugars and aldehydes in analytical applications

(Berger, 2007). However, its use is limited due to its potential toxicity (Ousaaïd *et al.*, 2022). The toxicity of PhH has gained attention in the scientific community as a model compound in toxicological studies (Luangaram *et al.*, 2007). Hepatotoxicity induced by PhH is characterized by raised liver enzyme levels, higher total bilirubin, and the appearance of necrosis and inflammatory infiltrates in the hepatic tissues (Amin *et al.*, 2024). According to Amama *et al.* (2022), PhH nephrotoxicity is linked to increased serum levels of urea and creatinine, reduced excretion in urine, and the presence of oxidative stress, inflammation, and apoptosis in renal tissue.

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Meanwhile, PhH haematotoxicity was demonstrated in rats by a reduction in erythrocyte count, haemoglobin (Hb) level, haematocrit (Hct) value, and raised serum ferritin and erythropoietin levels (El-Shafey *et al.*, 2023).

Recent studies have focused on the potential of natural compounds to counteract the detrimental effects of toxins (Amin *et al.*, 2024). One such compound gaining attention is *Foeniculum vulgare*, commonly known as fennel (Ogbonna *et al.*, 2024). The seeds of this aromatic plant are rich in essential oils and bioactive compounds such as caffeic acid, estragole, quercetin, apigenin, limonene, rutin, and chlorogenic acid (Ahmed *et al.*, 2019). It also contains essential minerals like iron, calcium, sodium, potassium, and phosphorus, as well as vitamins such as thiamine, niacin, riboflavin, and vitamin C (Alghamdi, 2020). *Foeniculum vulgare* seed oil (FSO) has multiple beneficial properties, including antimicrobial, antithrombotic, antioxidant, antibacterial, antidiabetic, laxative, antitumor, anti-inflammatory, anti-cancer, antinociceptive, and antispasmodic properties (Barakat *et al.*, 2023). In addition, FSO was found to reduce hepatotoxicity induced by carbon tetrachloride (CCl₄) by lowering serum levels of liver enzymes and bilirubin in rats (Özbek *et al.*, 2003). FSO exhibits a renal protective effect against sodium valproate (SV) nephrotoxicity, which was confirmed by decreasing serum urea and creatinine levels, improving the renal tissue, and regenerating renal tubules (Al-Amoudi, 2017). FSO also mitigated carbendazim haematotoxicity by elevated erythrocyte, Hb, and Hct levels in mice (Alghamdi, 2020). The current study aims to investigate the potential of FSO in alleviating hepatotoxicity, nephrotoxicity, and haematotoxicity induced by PhH.

MATERIALS AND METHODS

Experimental materials:

Phenylhydrazine was purchased as a yellow powder from Alpha Chemika for Scientific

Chemicals Company (Egypt). While *Foeniculum vulgare* seed oil was purchased from Elcaptain Company (Egypt).

Experimental animals:

The current study was conducted on 30 male Albino rats (*Rattus norvegicus*, 175 ± 10 g) purchased from the Center of Laboratory Animals of VACSERA Animal Farm (Helwan, Egypt). Rats were randomly assigned to five groups and kept in separate cages under standard laboratory conditions [temperature (25 ± 2 °C) and photoperiod (12 h light and 12 h dark cycle)] throughout the study. Rats were handled humanely according to the ethical standards of the Faculty of Science, Benha University (Approval number: ZD/FSc/BU-IACUC/2022-15).

Experimental design:

The experiment rats were separated into the following five groups (n = 6):

- Rats in the first group (control group) were given distilled water orally for fourteen days.
- Rats in the second group (FSO group) were administered a daily oral dose of FSO (0.5 ml/kg b.w.) according to El-Sheikh and Galal (2015) for fourteen days.
- Rats in the third group (PhH group) were intraperitoneally injected with PhH (60 mg/kg b.w.) divided over three days as described by El-Shafey *et al.* (2023).
- Rats in the fourth group (FSO + PhH group) were given a daily oral dosage of FSO (0.5 ml/kg b.w.) for eleven days before being injected intraperitoneally with PhH (60 mg/kg b.w. divided over three days).
- Rats in the fifth group (PhH + FSO group) were intraperitoneally injected with PhH (60 mg/kg b.w.) divided over three days, followed by an oral dosage of FSO (0.5 ml/kg b.w.) every day for eleven days.

Sample preparations:

At the end of the 14-day experimental period, all animals were fasted overnight. Rats were weighed and anaesthetized using isoflurane inhalation (Li *et al.*, 2017).

Dissection was performed as described by Ibrahim and Abd El-maksoud (2025). Blood samples were collected from six rats per group into two sets of tubes: one containing EDTA as an anticoagulant for haematological examinations, and the other allowing the blood to clot. Separated sera were stored for biochemical examinations.

Biochemical examinations:

Serum samples were analyzed spectrophotometrically (Jasco V-530 UV/VIS spectrophotometer, Japan) to determine oxidative stress markers, including catalase (CAT, Cat. No. K773-100), superoxide dismutase (SOD, Cat. No. E4584-100), and malondialdehyde (MDA, Cat. No. K739-100), using assay kits from BioVision (USA).

Serum liver panel, including alanine aminotransferase (ALT, Cat. No. GPT113100), alkaline phosphatase (ALP, Cat. No. 217 001), aspartate aminotransferase (AST, Cat. No. GOT111060), and total bilirubin (TBil, Cat. No. 102001) levels, were analyzed spectrophotometrically using BioMed kits (Egypt) for ALT and AST, a Spectrum kit (Egypt) for ALP, and a Diamond kit (Egypt) for TBil.

The serum kidney panel, which includes creatinine, urea, and uric acid, was assessed spectrophotometrically using BioMed kits (Egypt) for creatinine (Cat. No. CRE105100) and uric acid (Cat. No. UA119090), while urea was measured with a Spectrum kit, Egypt (Cat. No. 320 001).

Erythropoietin levels in the serum were assessed using rat ELISA kits provided by CUSABIO, USA (Cat. No. CSB-E07323r).

Haematological examinations:

Haematological examination was conducted using an automated haematology cell counter (MS4e Haematology Analyser, France) to assess the levels of red blood cells (RBCs), haemoglobin (Hb), haematocrit (Hct), white blood cells (WBCs) and platelets (PLT).

Statistical analysis:

Statistical evaluations were performed using SPSS software (version 26.0), and graphs were generated with SigmaPlot software (version 12.0). The mean and standard deviation were calculated based on six data points from each group. A one-way ANOVA was used to compare group means, and significant differences were determined at $P < 0.05$. Duncan's multiple-range test was subsequently applied to identify specific group differences.

RESULTS

Oxidative stress markers

The PhH group exhibited significantly higher serum MDA levels and significantly reduced SOD and CAT activities compared to the other groups. In the FSO + PhH and PhH + FSO groups, serum oxidative stress markers showed significant improvement relative to the PhH group. The improvement in serum oxidative stress markers was more notable in the FSO + PhH group than in the PhH + FSO group (Fig. 1 & 2).

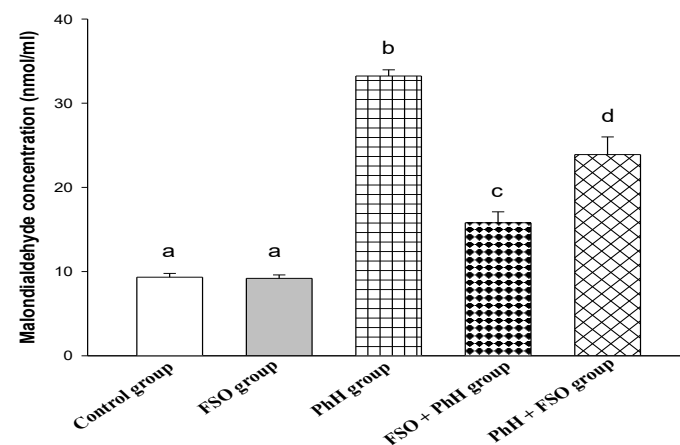


Figure 1: Serum malondialdehyde concentrations in control, *Foeniculum vulgare* seed oil (FSO, 0.5 ml/kg b.w.), phenylhydrazine (PhH, 60 mg/kg b.w.), FSO + PhH, and PhH + FSO groups. Data were analyzed using the SPSS software (Duncan's test) and provided as mean $\pm$ standard deviation. Letters (a-d) were used to indicate statistically significant differences ($P < 0.05$) between means.

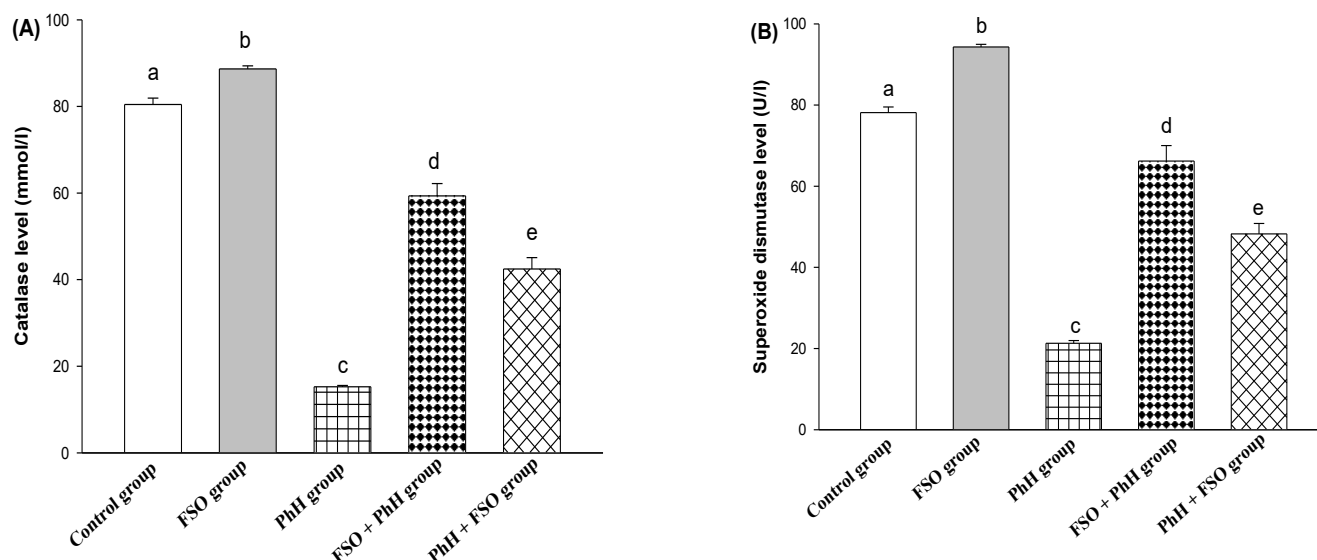


Figure 2: Serum catalase (A) and superoxide dismutase (B) activities in control, *Foeniculum vulgare* seed oil (FSO, 0.5 ml/kg b.w.), phenylhydrazine (PhH, 60 mg/kg b.w.), FSO + PhH, and PhH + FSO groups. Data were analyzed using the SPSS software (Duncan's test) and provided as mean $\pm$ standard deviation. Letters (a-e) were used to indicate statistically significant differences ($P < 0.05$) between means.

Liver panel

The PhH group demonstrated a significant rise in the liver panel (ALT, ALP, AST, and TBil) levels when compared to the other groups. In contrast, the liver panel levels in

the FSO + PhH and PhH + FSO groups were significantly lower than those in the PhH group. The FSO + PhH group showed a greater reduction in the liver panel levels than the PhH + FSO group (Table 1).

Table 1: Serum liver panel levels in control, *Foeniculum vulgare* seed oil (FSO, 0.5 ml/kg b.w.), phenylhydrazine (PhH, 60 mg/kg b.w.), FSO + PhH, and PhH + FSO groups.

	Groups				
	Control	FSO	PhH	FSO + PhH	PhH + FSO
ALT (U/L)	34.56 $\pm$ 0.65 ^a	34.50 $\pm$ 0.65 ^a	56.97 $\pm$ 1.36 ^b	39.30 $\pm$ 0.65 ^c	44.53 $\pm$ 0.65 ^d
AST (U/L)	109.23 $\pm$ 0.51 ^a	109.56 $\pm$ 0.62 ^a	144.53 $\pm$ 2.20 ^b	124.90 $\pm$ 1.05 ^c	131.93 $\pm$ 0.95 ^d
ALP (U/L)	114.47 $\pm$ 1.12 ^a	113.34 $\pm$ 0.06 ^a	225 $\pm$ 1.14 ^b	194.34 $\pm$ 0.83 ^c	205.43 $\pm$ 0.55 ^d
TBil (mg/dl)	0.65 $\pm$ 0.01 ^a	0.64 $\pm$ 0.01 ^a	3.87 $\pm$ 0.06 ^b	2.48 $\pm$ 0.05 ^c	2.91 $\pm$ 0.02 ^d

Data were analyzed using the SPSS software (Duncan's test) and provided as mean $\pm$ standard deviation. Letters (a-d) were used to indicate statistically significant differences ($P < 0.05$) between means. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TBil: total bilirubin.

Kidney panel

The PhH group exhibited a significant increase in kidney panel markers, including creatinine, urea, and uric acid, compared to the other groups. Meanwhile, the FSO + PhH and PhH + FSO groups had significantly lower kidney panel levels than the PhH group. The reduction in kidney panel markers was more pronounced in the FSO + PhH group than in the PhH + FSO group (Table 2).

Table 2: Serum kidney panel levels in control, *Foeniculum vulgare* seed oil (FSO, 0.5 ml/kg b.w.), phenylhydrazine (PhH, 60 mg/kg b.w.), FSO + PhH, and PhH + FSO groups.

	Groups				
	Control	FSO	PhH	FSO + PhH	PhH + FSO
Creatinine (mg/dl)	0.51 ± 0.01 ^a	0.55 ± 0.01 ^b	0.89 ± 0.04 ^c	0.70 ± 0.02 ^d	0.79 ± 0.03 ^e
Urea (mg/dl)	31.12 ± 1.13 ^a	32.00 ± 1.02 ^a	39.01 ± 2.25 ^b	35.06 ± 1.07 ^c	37.14 ± 1.11 ^d
Uric acid (mg/dl)	1.76 ± 0.96 ^a	1.71 ± 0.85 ^a	3.79 ± 0.05 ^b	2.11 ± 0.08 ^c	2.80 ± 0.85 ^d

Data were analyzed using the SPSS software (Duncan's test) and provided as mean ± standard deviation. Letters (a-e) were used to indicate statistically significant differences ($P < 0.05$) between means.

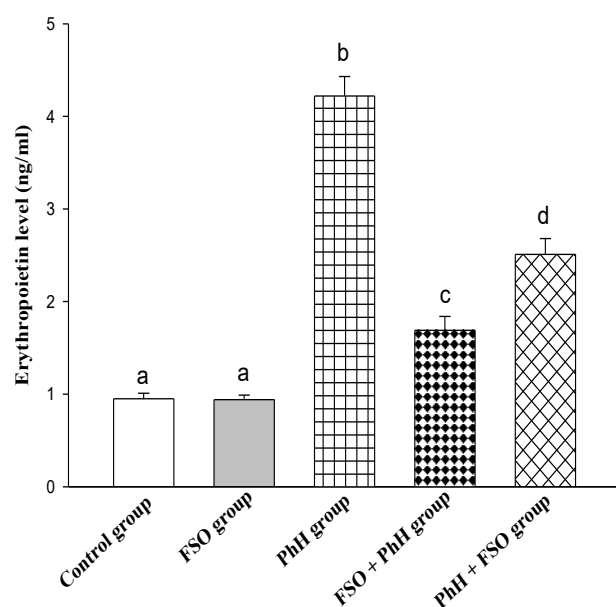


Figure 3: Serum erythropoietin level in control, *Foeniculum vulgare* seed oil (FSO, 0.5 ml/kg b.w.), phenylhydrazine (PhH, 60 mg/kg b.w.), FSO + PhH, and PhH + FSO groups. Data were analyzed using the SPSS software (Duncan's test) and provided as mean ± standard deviation. Letters (a-d) were used to indicate statistically significant differences ($P < 0.05$) between means.

Erythropoietin level

Figure 3 shows that serum erythropoietin levels in the PhH group were significantly higher compared to the other groups. In contrast, the FSO + PhH and PhH + FSO groups exhibited significantly lower serum erythropoietin levels than the PhH group. The decrease in serum erythropoietin levels was more evident in the FSO + PhH group compared to the PhH + FSO group.

Haematological examinations:

The PhH group showed significant decreases in RBC count, Hb content, Hct value, and PLT count, along with a significant increase in WBC count compared to all other groups. In contrast, the FSO + PhH and PhH + FSO groups exhibited higher RBC count, Hb content, Hct value, and PLT count, along with a lower WBC count, compared to the PhH group. The improvement in haematological parameters was more pronounced in the FSO + PhH group than in the PhH + FSO group (Table 3).

Table 3: Haematological parameters levels in control, *Foeniculum vulgare* seed oil (FSO, 0.5 ml/kg b.w.), phenylhydrazine (PhH, 60 mg/kg b.w.), FSO + PhH, and PhH + FSO groups.

	Groups				
	Control	FSO	PhH	FSO + PhH	PhH + FSO
RBCs (10 ⁶ cells/mm ³)	3.43 ± 0.37 ^a	3.89 ± 0.20 ^b	2.06 ± 0.20 ^c	2.81 ± 0.03 ^d	2.61 ± 0.13 ^d
Hb (g/dl)	10.86 ± 0.15 ^a	11.13 ± 0.3 ^a	7.36 ± 0.45 ^b	9.36 ± 0.15 ^c	8.76 ± 0.25 ^d
Hct (%)	30.80 ± 0.4 ^a	33.33 ± 0.95 ^b	23.53 ± 0.96 ^c	28.60 ± 0.20 ^d	26.48 ± 0.16 ^c
WBCs (10 ³ cells/mm ³)	6.43 ± 0.15 ^a	6.50 ± 0.36 ^a	8.63 ± 0.25 ^b	7.10 ± 0.10 ^c	7.26 ± 0.25 ^c
PLT (10 ³ cells/mm ³)	358.33 ± 7.63 ^a	375.66 ± 6.02 ^b	268.66 ± 7.88 ^c	336.33 ± 4.04 ^d	327.66 ± 2.08 ^d

Data were analyzed using the SPSS software (Duncan's test) and provided as mean ± standard deviation. Letters (a-e) were used to indicate statistically significant differences ($P < 0.05$) between means. RBCs: red blood cells; Hb: haemoglobin; Hct: haematocrit; WBCs: white blood cells; PLT: platelet.

DISCUSSION

Phenylhydrazine (PhH) is a potent oxidizing compound recognized for its ability to induce oxidative stress (Johri and Khan, 2021). The oxidative stress induced by PhH was confirmed in our study by a marked reduction in CAT and SOD activities, accompanied by a significant rise in MDA levels in the PhH group. Luangaram *et al.* (2007) reported that PhH has the ability to release reactive oxygen and nitrogen species (ROS & RNS) and elevate lipid peroxidation (LPO), which is represented by MDA. It also releases PhH-derived radicals, including benzene diazonium ions, phenyldiazene, and phenylhydrazyl radicals (El-Shehry *et al.*, 2023). It also declines the antioxidant enzymes such as CAT, glutathione peroxidase, and SOD levels in rats (El-Shafey *et al.*, 2023).

The ability of FSO to lower MDA and elevate antioxidant enzymes in PhH-treated rats in the present study confirmed the antioxidant activity of FSO. Imbabi *et al.* (2021) also attributed the antioxidant activity of FSO to its ability to increase the antioxidant enzymes. Additionally, high-performance liquid chromatography

(HPLC) analysis conducted by Barakat *et al.* (2023) identified nineteen phenolic compounds in FSO, including thirteen phenolic acids and six flavonoids, further establishing it as a potent antioxidant. According to Ahmed *et al.* (2019), the antioxidant properties of FSO are attributed to its high total phenolic content and effective radical scavenging power.

In this study, increased serum liver panel (ALT, TBil, AST, and ALP) observed in PhH-treated rats served as evidence of PhH-induced hepatotoxicity. The haemolysis, oxidative stress, and hyperbilirubinemia caused by PhH in this study may play a role in the development of hepatotoxicity. According to Zangeneh *et al.* (2019), PhH-triggered haemolysis can lead to hepatomegaly and chronic liver dysfunction. This process also elevates bilirubin levels, as noted by Nawaz *et al.* (2016). Oxidative stress caused by PhH in the liver contributes to liver dysfunction (Okafor and Atsu, 2022).

The administration of FSO to PhH-treated rats reduced serum liver panel levels, indicating the hepatoprotective effect of FSO. Similarly, the FSO hepatoprotective effect was confirmed by Özbek *et al.* (2003), who reported that FSO lowered

TBil and liver enzyme levels in CCl₄-treated rats. Limonene, a component of FSO (Ahmed *et al.*, 2019), also acts as a hepatoprotective agent with antioxidant, anti-inflammatory, and anti-apoptotic properties that protect the liver from damage (Amini *et al.*, 2020). Moreover, Al-Amoudi (2017) reported that FSO aids in regenerating the periportal zone in the livers of rats affected by sodium valproate.

Our findings revealed evidence of kidney dysfunction in the PhH group, as indicated by elevated kidney panel parameters. We hypothesize that this dysfunction is related to haemolysis and oxidative stress triggered by PhH exposure. Similarly, Amama *et al.* (2022) noted that oxidative stress, inflammation, and apoptosis in the kidneys were key contributors to kidney dysfunction caused by PhH. Soliman *et al.* (2023) also linked kidney dysfunction and tissue damage to the excessive production of ROS and LPO induced by PhH. Moreover, El-Shafey *et al.* (2023) suggested that severe haemolysis caused by PhH could further contribute to kidney injury.

The observed improvement in kidney panel levels by FSO in PhH-treated rats supports the nephroprotective action of FSO. Tesfa *et al.* (2025) revealed that FSO administration reduced renal panel levels while improving kidney tissue in cisplatin-treated mice, indicating that FSO has a nephroprotective effect. FSO administration reduces SV-induced kidney damage by restoring tubules and glomeruli to normal morphology (Al-Amoudi, 2017). According to Barakat *et al.* (2023), the phenolic compounds in FSO have antioxidative and free radical scavenging properties, which allow them to reduce nephrotoxicity. FSO also contains quercetin (Ahmed *et al.*, 2019), which has been found to attenuate nephrotoxicity via an increase in antioxidant enzymatic activity and a decrease in LPO in the kidney (Zaveri *et al.*, 2011).

Our results revealed significant declines in erythrocytes, Hct, Hb, and thrombocytes,

along with increases in leukocytes and erythropoietin levels in PhH-treated rats, indicating hemolytic anaemia. These findings align with the results reported by El-Shafey *et al.* (2023). PhH induces oxidative stress in erythrocytes by generating ROS (Luangaram *et al.*, 2007), which interact with haemoglobin to form methemoglobin, hemichromes, and Heinz bodies (El-Shehry *et al.*, 2023). These ROS also cause LPO and oxidative degradation of spectrin in the RBC membrane skeleton (Shukla *et al.*, 2012). Additionally, PhH disrupts the RBC membrane by translocating phosphatidylserine to the outer membrane surface, signalling apoptosis and macrophage uptake (Maines, 1997).

Administration of FSO improves haematological parameters in rats treated with PhH. This may be due to the ability of FSO to reduce LPO and elevate antioxidant enzymes. FSO is an abundant source of iron, thiamine, niacin, riboflavin, and vitamin C (Alghamdi, 2020). Vitamin C lowers the formation of Heins bodies and methemoglobin induced by PhH (Berger, 2007). Additionally, riboflavin plays a crucial role in safeguarding erythrocytes from damage caused by ROS (Fishman *et al.*, 2000). FSO also contains quercetin (Ahmed *et al.*, 2019), which has been reported to reduce oxidative damage and vascular dysfunction caused by PhH (Luangaram *et al.*, 2007).

CONCLUSION

This study demonstrated the hepatotoxicity, nephrotoxicity, and haematotoxicity of PhH in rats. Additionally, it confirmed the potential of FSO to mitigate the disturbances in oxidative stress, haematological values, and liver and kidney panels caused by PhH. This suggests that FSO could serve as a natural remedy for oxidative stress and the associated organ damage.

Conflict of interest: The authors declare no conflict of interest.

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الدور المحسن لزيت بذور نبات الشمر في علاج الاضطرابات الفسيولوجية المصاحبة للسمية الناجمة عن الفينيل هيدرازين في ذكور الجرذان البيضاء

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الفينيل هيدرازين (PhH) هو مركب عضوي عطري ذو تطبيقات واسعة في مجالات مختلفة، بما في ذلك الكيمياء والأدوية والزراعة. بالإضافة إلى ذلك، يُستخدم كنموذج لدراسة السمية في البحوث البيولوجية والطبية. تهدف هذه الدراسة إلى استكشاف إمكانية استخدام زيت بذور الشمر (FSO) كعلاج طبيعي للتخفيف من التأثيرات الضارة لسمية الفينيل هيدرازين. شملت التجربة خمس مجموعات من ذكور الجرذان: تلقت مجموعة التحكم ماءً مقطرًا لمدة ١٤ يومًا؛ بينما تناولت مجموعة FSO جرعة فموية يومية من زيت بذور الشمر بتركيز ٠,٥ مل / كجم لمدة ١٤ يومًا؛ أما مجموعة PhH فقد حُقنت داخل الصفاق بجرعة ٦٠ ملجم / كجم من الفينيل هيدرازين على ثلاثة أيام متتالية؛ في حين تلقت مجموعة FSO + PhH علاجًا بزيت بذور الشمر فمويًا لمدة ١١ يومًا قبل حقنها بالفينيل هيدرازين؛ وأخيرًا، تم حقن مجموعة PhH + FSO بالفينيل هيدرازين على ثلاثة أيام، تلاها إعطاء جرعات فموية يومية من زيت بذور الشمر لمدة ١١ يومًا. تم سحب عينات الدم من الجرذان في اليوم الخامس عشر لتحليل المستويات الدموية والكيميائية الحيوية. أظهرت الجرذان التي عولجت بالفينيل هيدرازين ارتفاعًا في المالونديالدهيد، وكريات الدم البيضاء، واختبارات وظائف الكبد، واختبارات وظائف الكلى، وإريثروبويتين، بينما انخفضت مستويات الكاليز، والسوبر أوكسيد ديسميوتاز، وكريات الدم الحمراء، والهيماتوكريت، والهيموجلوبين، والصفائح الدموية مقارنة بمجموعة التحكم. أدى إعطاء زيت بذور الشمر للجرذان المعالجة بالفينيل هيدرازين إلى تحسين الإجهاد التأكسدي، والمعايير الدموية، واختبارات وظائف الكبد، واختبارات وظائف الكلى، ومستويات الإريثروبويتين. باختصار، كان زيت بذور الشمر فعالًا في الحد من الاضطرابات في الإجهاد التأكسدي، والمعايير الدموية، واختبارات وظائف الكبد والكلى، ومستويات الإريثروبويتين الناجمة عن معالجة الجرذان بالفينيل هيدرازين.