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ROLE OF ROYAL JELLY ON HEPATOTOXICITY INDUCED BY ACRYLAMIDE IN MALE RATS

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ABSTRACT

Acrylamide (AC) is an organic amide classified as a hepatotoxic compound. Its presence in cigarette smoke and some processed foods represents a hazard to humans. As a natural food with a high nutritional content, royal jelly (RJ) is used in the protection and therapy of several diseases that affect humans. This research examined the possibility of using RJ to improve hepatotoxicity caused by AC. The study included four groups of rats (each 6 rats): the first group was given oral saline (control group); the second group was given an oral dose of AC (38.27 mg/kg dissolved in saline); and the third and fourth groups received oral RJ an hour before the AC administration at 150 mg/kg (150 RJ + AC group) and 300 mg/kg (300 RJ + AC group), respectively. The trial period extended to 10 consecutive days. On the 11th day, blood and liver samples were obtained from rats in each group for biochemical, histological, and immunohistochemical examinations. The sera of AC-treated rats showed an elevated hepatic function panel, mild hyperglycemia, and dyslipidemia, while their hepatic tissues showed histological changes, oxidative stress, and inflammation. Administration of RJ at 150 and 300 mg/kg doses to AC-treated rats improved the hepatic function panel, blood glucose, lipid panel, histological changes, oxidative stress, and inflammation in the hepatic tissue. In conclusion, RJ was able to ameliorate hepatic dysfunction, mild hyperglycemia, and dyslipidemia, as well as improve histological changes, inflammation, and oxidation in the hepatic tissue induced by AC in rats.

Keywords: Acrylamide; Hepatotoxicity; Royal jelly; Hepatic function; Oxidative stress.

INTRODUCTION

Acrylamide (AC) is an organic amide classified as a toxic compound (Altunay *et al*., 2023). It is produced Industrially hydrolyzing acrylonitrile in many industries (Kahkeshani *et al*., 2015). In addition, it is naturally present in cigarette smoke and processed foods such as bread, biscuits, roasted coffee, cereals, cocoa, and roasted potatoes (Banc *et al*., 2022). The tiny size and hydrophilicity of AC allow it to spread quickly all over the body (Abdel-Moneim *et al*., 2019). Like any toxic substance, AC is carried to the liver to be metabolized (Ibrahim, 2024).

The liver is negatively affected by AC accumulation in several ways (Cerrah *et al*., 2023). Physiologically, AC causes liver dysfunction, represented by high levels of total bilirubin (TBil) and hepatic enzymes in the serum of rats (Rivadeneyra-Domínguez

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et al., 2018). Moreover, AC has been reported to cause disruptions in glucose and lipid metabolism in mice (Zhao *et al*., 2022). Histologically, AC induces hepatocyte necrosis, vascular congestion, and mononuclear cell infiltration in the hepatic tissue of rats (Mahmood *et al*., 2015). Cellularly, AC promotes oxidative stress, necrosis, apoptosis, and impaired autophagy in rat hepatocytes (Erfan *et al*., 2021).

Royal jelly (RJ) is a highly nutritious food prepared by worker bees to nourish their queen throughout her life (Bagameri *et al*., 2022). The high nutritional value of RJ is attributed to its content of amino acids, peptides, complex proteins, carbohydrates, fatty acids (mainly 10-hydroxy-2-decenoic acid "10-H2DA"), vitamins, polyphenols, and minerals (Botezan *et al*., 2023). For humans, RJ is used as a food source and production of some cosmetics and medicines (Ibrahim and Shahen, 2023). The pharmacological uses of RJ are numerous, as it is considered an anti-inflammatory, antitumor, antibacterial, antioxidant, hypolipidemic, hypoglycemic, and hepatoprotective agent (Almeer *et al*., 2018). Experimental studies on rats have demonstrated that RJ can ameliorate hepatotoxicity induced by azathioprine (Ahmed *et al*., 2014), diclofenac (Mostafa *et al*., 2020), hydroxyurea (Tohamy *et al*., 2022), and cadmium chloride (Hamza *et al*., 2022). RJ improves the hepatic function panel in mice with cadmium chloride hepatotoxicity by reducing the elevation in TBil and hepatic enzymes (Almeer *et al*., 2018). It also reduces hyperglycemia, hyperlipidemia, and hyperinsulinemia in metabolic disorders and obesity (Yoneshiro *et al*., 2018). RJ can protect hepatic tissue from histological abnormalities, inflammation, oxidation, and apoptosis (Tohamy *et al*., 2022). Therefore, RJ was selected to evaluate its capacity to protect the liver from AC toxicity.

MATERIALS AND METHODS

Drug and chemical:

RJ was obtained from PHARCO Pharmaceuticals Company (Alexandria, Egypt) in the form of dietary supplement capsules. 10-H2DA represents 6% of each RJ capsule (340 mg). Meanwhile, White AC powder (CAS No: 79-06-1) was obtained from Alpha Chemika Company (Mumbai, India).

Experimental animals:

24 male Wistar rats, each weighing 180±10g were obtained from the VACSERA animal farm in Helwan (Cairo, Egypt). Seven days before the start of the experiment, the rats were acclimatized in a chamber set at a consistent temperature of 23 degrees Celsius with adequate ventilation, a 12-hour light each day and unrestricted access to food and water. The experiment protocol was accepted by IACUC, Department of Zoology, Faculty of Science, Benha University, and registered under No. ZD/FSc/BU-IACUC/2022-16c.

Rat groups:

The rats used in the experiment were distributed into four groups (n = 6). Rats in the first group (control group) were given oral saline. An oral dose of AC (38.27 mg/kg) dissolved in saline was given to rats in the second group, according to Ibrahim (2024). Rats in the third and fourth groups received RJ orally an hour before the AC administration, at either 150 mg/kg (150 RJ + AC group) or 300 mg/kg (300 RJ + AC group), as described by Ibrahim and Shahen (2023). The trial period extended to 10 consecutive days. On the 11th day, blood and liver samples were obtained from rats in each group for biochemical, histological, and immunohistochemical examinations.

Biochemical examinations:

Blood samples from anesthetized rats were centrifuged to separate sera. These sera were then used to measure levels of hepatic enzymes (alanine aminotransferase "ALT", gamma-glutamyl transferase "GGT", alkaline phosphatase "ALP", and aspartate aminotransferase "AST"), total bilirubin

(TBil), glucose and lipid panel (triglycerides "TG", total cholesterol "TC", low-density lipoprotein-C "LDL-C" and high-density lipoprotein-C "HDL-C") spectrophotometrically using BioSystems (Spain) assay kits.

Each rat's liver was removed and cleaned in phosphate-buffered saline (PBS). Then, liver homogenates were obtained by mixing 10 mg of each liver in PBS and separating them by centrifugation. Liver homogenates were used to measure hepatic oxidative stress (catalase "CAT", superoxide dismutase "SOD", and malondialdehyde "MDA") levels spectrophotometrically using BioVision (USA) assay kits.

Histopathological and immunohistochemical examinations:
The hepatic tissue specimens were preserved in formalin (10%). Subsequently, they were embedded in paraffin and cut into 5 μm thick sections. These sections were then deparaffinized and rehydrated. Some sections were stained with hematoxylin and eosin for histological examination (Bancroft and Gamble, 2008).

The other liver sections were incubated with antigen retrieval solution, treated with hydrogen peroxide to halt endogenous peroxidase activity, and then blocked with bovine serum albumin to prevent non-specific immunoreactivity. Overnight incubation with anti–TNF-α primary antibodies was followed. After appropriate washing, sections were treated with a secondary antibody, incubated with diaminobenzidine, washed, dehydrated, and covered for examination. The hepatic tissue's percentage area of immunoreactivity was determined using Image Pro Plus software.

Statistical analysis:
All the statistical analyses in this study were conducted with SPSS statistical software (version 26.00), and the graphs were plotted using SigmaPlot software (version 12.00). The mean and standard deviation were computed by analyzing six data from each group. Group means were compared at significant differences $P < 0.05$ using a one-way ANOVA and then Duncan's multiple-range test was performed.

RESULTS

Hepatic function panel
The AC group recorded a significant elevation ($P < 0.05$) in the hepatic function panel (ALT, ALP, GGT, AST, and TBil) compared to the other groups. The hepatic function panel in the two RJ + AC groups was significantly lower than the AC group. The 300 RJ + AC group showed a greater reduction in the hepatic function panel than the 150 RJ + AC group (Table 1).

Blood glucose
In Figure 1, the blood glucose level in the AC group recorded a significant elevation ($P < 0.05$) compared to the other groups. While the blood glucose levels in the two RJ + AC groups were significantly lower than those in the AC group. The reduction in the level of blood glucose was more pronounced in the 300 RJ + AC group than in the 150 RJ + AC group.

Lipid panel
The serum of AC-treated rats had significantly ($P < 0.05$) higher levels of TG, TC, and LDL-C and a significantly lower level of HDL-C compared to the other groups. There was a significant improvement in the lipid panel of the two RJ + AC groups compared with those in the AC group. The improvement in lipid panel in the 300 RJ + AC group was more pronounced than in the 150 RJ + AC group (Table 2).

Oxidative stress biomarkers
The AC group had a significantly ($P < 0.05$) higher level of MDA and significantly lower activities of SOD and CAT compared with the other groups. Biomarkers of hepatic oxidative stress in the two RJ + AC groups were significantly improved than those in the AC group. The amelioration in biomarkers of hepatic oxidative stress in the

300 RJ + AC group was more pronounced than in the 150 RJ + AC group (Table 3).

Histopathological examination

The microscopic examination of the liver of rats in the control group showed normal hepatic lobules comprised of rows of hepatic cells that radiated out from central veins toward the periphery of the hepatic lobules (Fig. 2A). Meanwhile, the liver sections of the AC group revealed congestion of central and portal veins with hyperplasia of the bile ductal epithelium (Fig. 2B). In addition, mononuclear cellular infiltration of the portal areas particularly lymphocytes was also detected (Fig. 2C). Focal coagulative necrosis of hepatic cells which in some areas mixed with mononuclear inflammatory cells were scattered among the examined liver of rats in this group (Fig. 2D). The microscopic picture of the liver in the 150 RJ + AC group showed congestion of central veins with hydropic and vacuolar degeneration of the hepatic cells (Fig. 2E). The examined portal areas of these liver sections revealed bile ductal hyperplasia with aggregation of a few inflammatory cells (Fig. 2F). While the 300 RJ + AC group revealed a greater enhancement in the microscopic picture of the liver where with no evidence of hepatic necrosis and only foci of inflammatory cells with congestion of central veins and mild hydropic degeneration of some hepatic cells mainly around central veins were the only recorded microscopic lesions (Fig. 2G).

Immunohistochemical examination

The results of TNF-α immunohistochemical staining of the liver from the control group showed weak TNFα expression which was restricted to the walls of hepatic sinusoids (Fig. 3A). In contrast, remarked elevation of TNF-α expression with increases in the number of positive stained hepatocytes were recorded in the liver of the AC group (Fig. 3B). There were significant decreases in the area percentage of TNF-α expression in the AC groups pretreated with 150 and 300 mg/kg of RJ compared to that in the AC group (Fig. 3C&D). Moreover, the 300 RJ + AC group had fewer positively stained hepatocytes with a significant reduction in the area percentage of TNF-α expression compared to those in the 150 RJ + AC group (Fig. 3E).

Table 1: Royal jelly (RJ) effect on hepatic function panel in acrylamide (AC)-treated rats.

	Groups			
	Control	**AC**	**150 RJ + AC**	**300 RJ + AC**
Aspartate aminotransferase (AST) (U/L)	121.13 ± 0.76^{d}	192.27 ± 2.10^{a}	172.76 ± 1.12^{b}	157.24 ± 0.55^{c}
Alanine aminotransferase (ALT) (U/L)	34.27 ± 0.36^{d}	62.56 ± 0.73^{a}	53.33 ± 0.51^{b}	44.43 ± 0.42^{c}
Alkaline Phosphatase (ALP) (U/L)	211.78 ± 1.76^{d}	322.37 ± 2.04^{a}	253.30 ± 1.61^{b}	243.75 ± 1.02^{c}
Gamma-glutamyl transferase (GGT) (U/L)	4.01 ± 0.20^{d}	11.63 ± 0.41^{a}	8.44 ± 0.25^{b}	6.10 ± 0.20^{c}
Total bilirubin (TBil) (mg/dl)	0.36 ± 0.01^{d}	0.98 ± 0.02^{a}	0.72 ± 0.01^{b}	0.56 ± 0.01^{c}

All values were statistically analyzed using the SPSS program (Duncan's test) and are shown in the table as mean ± standard deviation (n=6). Statistically significant variations ($P < 0.05$) among the means were expressed using superscript letters (a to d).

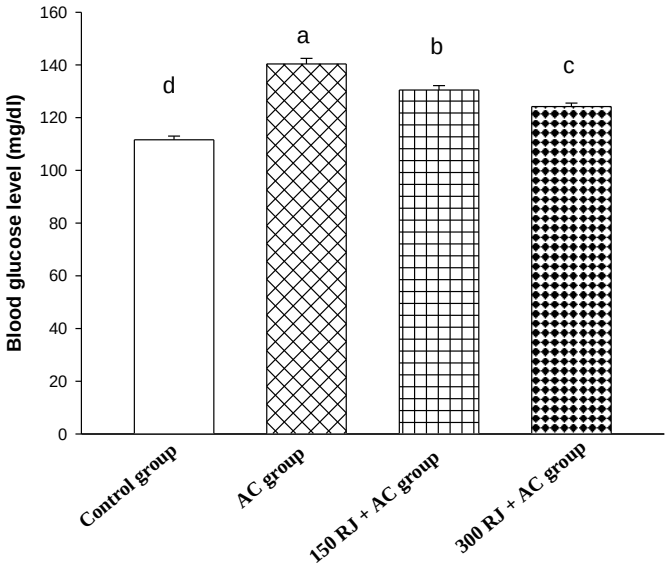

Fig. 1. Royal jelly (RJ) effect on blood glucose level in acrylamide (AC)-treated rats. All values were statistically analyzed using the SPSS program (Duncan's test) and represented as mean ± standard deviation (n=6). Statistically significant variations ($P < 0.05$) among the means were expressed using letters (a to d).

Table 2: Royal jelly (RJ) effect on lipid panel in acrylamide (AC)-treated rats.

	Groups			
	Control	**AC**	**150 RJ + AC**	**300 RJ + AC**
Triglycerides (TG) (mg/dl)	57.10 ± 0.82^{d}	133.90 ± 1.24^{a}	110.42 ± 1.04^{b}	88.21 ± 0.68^{c}
Total cholesterol (TC) (mg/dl)	73.23 ± 0.56^{d}	167.13 ± 1.39^{a}	129.20 ± 1.02^{b}	107.30 ± 0.87^{c}
Low-density lipoprotein-C (LDL-C) (mg/dl)	26.93 ± 0.41^{d}	48.43 ± 0.50^{a}	36.27 ± 0.24^{b}	30.83 ± 0.21^{c}
High-density lipoprotein-C (HDL-C) (mg/dl)	38.96 ± 0.35^{a}	27.51 ± 0.16^{d}	31.46 ± 0.40^{c}	33.90 ± 0.23^{b}

All values were statistically analyzed using the SPSS program (Duncan's test) and are shown in the table as mean ± standard deviation (n=6). Statistically significant variations ($P < 0.05$) among the means were expressed using superscript letters (a to d).

Table 3: Royal jelly (RJ) effect on biomarkers of hepatic oxidative stress in acrylamide (AC)-treated rats.

	Groups			
	Control	**AC**	**150 RJ + AC**	**300 RJ + AC**
Malondialdehyde (MDA) (nmol/10 mg tissue)	1.95 ± 0.03^{c}	6.01 ± 0.14^{a}	4.87 ± 0.01^{b}	4.82 ± 0.02^{b}
Superoxide dismutase (SOD) (U/10 mg tissue)	6.06 ± 0.08^{a}	2.80 ± 0.02^{d}	3.61 ± 0.02^{c}	3.74 ± 0.03^{b}
Catalase (CAT) (mU/10 mg tissue)	5.63 ± 0.06^{a}	2.13 ± 0.02^{d}	3.47 ± 0.04^{c}	3.54 ± 0.02^{b}

All values were statistically analyzed using the SPSS program (Duncan's test) and are shown in the table as mean ± standard deviation (n=6). Statistically significant variations ($P < 0.05$) among the means were expressed using superscript letters (a to d).

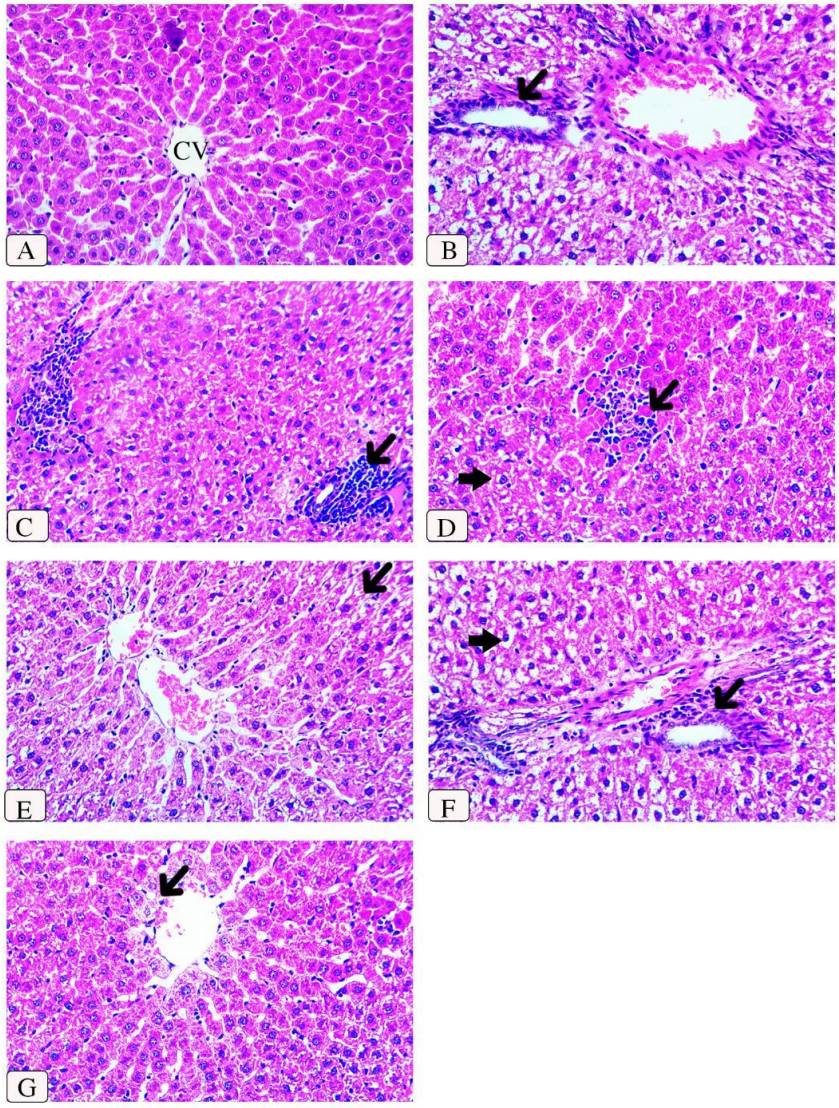

Fig. 2. Photomicrographs of liver sections (H&E, X200). The liver section of the control group (A) shows normal hepatic lobules comprised of rows of hepatic cells radiating out from the central vein (CV) towards the periphery. Liver sections from the AC group (B-D) show congestion of the portal vein with hyperplasia of the bile ductal epithelium (arrow) (B), mononuclear cellular infiltration of the portal areas particularly lymphocytes (arrow) (C), coagulative necrosis of some hepatic cells (thin arrow) mixed with inflammatory cells and degeneration of some hepatocytes (thick arrow) (D). Liver sections from the 150 RJ + AC group (E-F) show congestion with hydropic and vacuolar degeneration of the hepatic cells (arrow) (E) and bile ductal hyperplasia (thin arrow) with aggregation of few inflammatory cells and hydropic degeneration of hepatocytes (thick arrow) (F). The liver section of the 300 RJ + AC group (G) shows congestion of the central vein with mild hydropic degeneration of some hepatic cells (arrow).

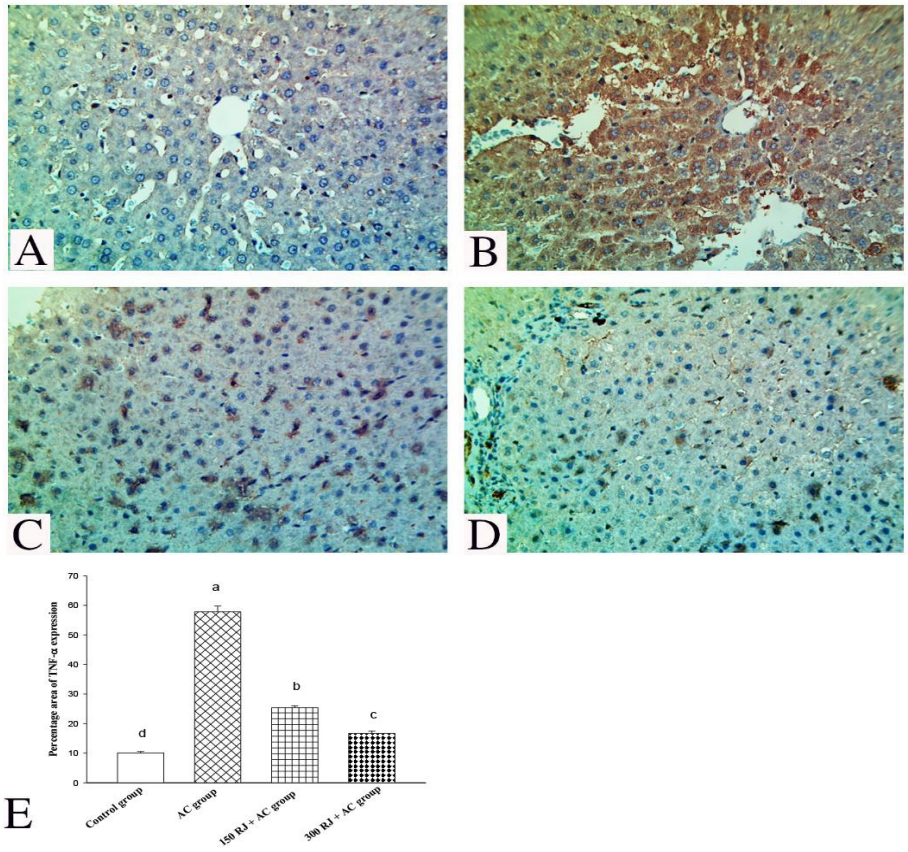

Fig. 3. Immunohistochemical photomicrographs of TNF-α expression in the hepatic tissues of the control group (A), the AC group (B), the 150 RJ + AC group (C), and the 300 RJ + AC group (D). Graph (E) represents the means and standard deviations of the percentage area of TNF-α immunoreactivity in the groups. Statistically significant variations ($P < 0.05$) among the means were expressed using letters (a to d).

DISCUSSION

Acrylamide is a hepatotoxic compound that causes hepatic dysfunction (Cerrah *et al*., 2023). Ozer *et al*. (2008) reported that elevation in serum indicators of hepatic functions (ALP, ALT, GGT, AST, TBil, and bile acids) indicates hepatotoxicity. In our study, the increase in the activity of hepatic enzymes (ALT, ALP, GGT, and AST) and the TBil concentration in the serum of AC-treated rats is evidence of hepatic dysfunction caused by AC hepatotoxicity. The damage, inflammation, and oxidation in the hepatic tissue of AC-treated rats observed in this study may be the reason for the elevation in serum indicators of hepatic functions in the AC group. Hamza *et al.* (2020) attributed the elevation in the activities of ALT and AST in AC-treated rats to hepatic tissue damage that led to hepatic enzyme leakage into the bloodstream.

In the current study, administering RJ at 150 and 300 mg/kg doses to AC-treated rats ameliorated the hepatic function panel, which may be related to the marked improvement in the hepatic tissue of the RJ + AC groups. Moreover, greater improvement in the hepatic function panel and hepatic tissue structure was observed in the 300 RJ + AC group than in the 150 RJ + AC group. Ahmed *et al*. (2014) reported that RJ could prevent azathioprine-induced hepatic dysfunction in rats by preventing leakage of hepatic enzymes from hepatocytes. Inhibiting lipid peroxidation in

hepatocyte membranes by RJ reduces the loss of hepatic enzymes in the bloodstream (Jalili *et al*., 2019).

This study further proved that AC intake caused excessive blood glucose and dyslipidemia in rats which may be due to the damage, inflammation, and oxidation in hepatic tissue induced by AC. The disturbance of glucose and lipid metabolism induced by oral administration of 50 mg/kg AC in mice for 1 week may be attributed to AC oxidation and inflammatory damage (Zhao *et al*., 2022). Marković Filipović *et al*. (2022) also reported that the oxidative damage that AC causes to beta cells is the reason for AC- hypoinsulinemia and hyperglycemia. Furthermore, Yue *et al*. (2020) reported that AC stimulates glycogenolysis and gluconeogenesis and inhibits glycolysis, leading to hyperglycemia in rats. The inhibition of glycolysis by AC is attributed to its ability to lower blood glucose intermediate levels (Song *et al*., 2021). In addition, high TG levels and low HDL-C levels may cause hyperglycemia (Parhofer, 2015). Ingestion of 15 mg/kg of AC for 8 weeks reduced thyroid hormone in rats (Hamdy *et al*., 2012), causing dyslipidemia (Abdel-Moneim *et al*., 2019). AC also raises acetyl-CoA levels, which can be used in the production of some fatty acids and TC (Hamza *et al*., 2020).

Administering RJ (150 and 300 mg/kg) to AC-treated rats in this study showed improvement in glucose and lipid levels in the RJ + AC groups. The improvement in glucose and lipid levels in the 300 RJ + AC group was more pronounced than in the 150 RJ + AC group. RJ decreases cholesterol-genesis by suppressing hydroxymethyl-glutaryl (HMG) CoA reductase (essential enzyme for cholesterogenesis) gene expression, leading to a reduction in its activity (Petelin *et al*., 2019). RJ proteins have a hypocholesterolemic capacity also resulting from their ability to minimize the absorption of TC from the intestine (Hamza *et al*., 2022). In addition, 10-H2DA in RJ lowers blood TC levels by inhibiting sterol production in the liver (Bahari *et al*., 2023). The in vitro study performed by Zhang *et al*. (2021) confirmed the ability of RJ proteins to reduce TG within HepG2 cells. Meanwhile, Maleki *et al*. (2019) related RJ's hypoglycemic ability to its antioxidant and inflammatory properties. Paredes-Barquero *et al*. (2022) also reported that 10-H2DA in RJ has a hypoglycemic capacity by stimulating glycolysis.

Mitochondria are considered the principal producers of ROS inside the majority of cells (Gao *et al*., 2022). Acrylamide suppresses the activities of mitochondrial complexes resulting in increased ROS formation (Song *et al*., 2021). Both excessive ROS formation and reduction in the activities of antioxidant enzymes in the hepatic tissue lead to oxidative stress (Rahbardar *et al*., 2021). Oxidative stress causes inflammation (Hussain *et al*., 2016) by activating NF-κB which stimulates the release of proinflammatory cytokines like TNF-α (Liu *et al*., 2017). Not only that but oxidative stress also causes hepatic tissue damage in rats (Erfan *et al*., 2021). In this study, the elevation in MDA and TNF-α immunoexpression levels and the reduction in CAT and SOD activities, as well as the histological changes in the hepatic tissue of AC-treated rats revealed that AC caused oxidation, inflammation, and histological changes in rats' hepatic tissues. The authors believe that AC-induced oxidative stress is responsible for the inflammation and histological changes in rats' hepatic tissues. Cerrah *et al*. (2023) confirmed that AC-induced oxidative stress leads to elevated NF-κB levels in the hepatic tissue of the AC-treated rats resulting in increased TNF-α levels. AC-induced oxidative stress also causes changes in rats' hepatic tissues (Hasanin *et al*., 2018).

The capacity of RJ to minimize histological abnormalities, inflammation, and oxidation in the hepatic tissue of the RJ + AC groups in this experiment can be attributed to its

antioxidant content. Meanwhile, pretreatment with 300 mg of RJ to AC-treated rats revealed a greater reduction in inflammation and oxidation in the hepatic tissue than pretreatment with 150 mg of RJ to AC-treated rats. Ibrahim and Shahen (2023) reported that antioxidant components such as polyphenols, 10-H2DA, and proteins present in RJ are the reason behind RJ's antioxidant capacity. Moreover, the ability of RJ proteins to ameliorate mitochondrial dysfunction (Zhang *et al*., 2021), could also explain RJ's antioxidant capacity. The anti-inflammatory compounds such as 10-hydroxydecanoic acid, sebacic acid, and 10-H2DA present in RJ are the reason behind RJ's anti-inflammatory capacity (Chen *et al*., 2016). RJ's anti-inflammatory capacity may be also attributed to its capacity to suppress the NF-kB pathway, leading to lower TNF-α formation (Botezan *et al*., 2023). Furthermore, 10-H2DA can reduce the synthesis of TNF-α and NF-κB (Yang *et al*., 2018). RJ's antioxidant capacity is responsible for improving the alteration in hepatic tissues (Tohamy *et al*., 2022). Hamza *et al*. (2022) also discovered that royalactin, one of RJ's proteins, regenerates the liver by promoting the growth of its hepatocytes.

CONCLUSION

This study verified that elevation of the hepatic function panel, excessive blood glucose, and dyslipidemia occurred with AC ingestion. Furthermore, it induces histological changes, inflammation, and oxidation in rats' hepatic tissues. The ability of RJ (150 and 300 mg/kg) to resist AC hepatotoxicity was also demonstrated in the study, as RJ was able to ameliorate hepatic dysfunction, excessive blood glucose, and dyslipidemia, as well as improve histological changes, inflammation, and oxidation in the hepatic tissue caused by AC.

Conflict of interest

The authors declare no conflict of interest.

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دور غذاء ملكات النحل في السمية الكبدية الناتجة عن مادة الأكريلاميد في ذكور الجرذان

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مادة الأكريلاميد هى عبارة عن أميد عضوي مُصنفه على أنها مركب سام للكبد. يشكل وجودها في دخان السجائر وبعض الأطعمة المصنعة خطراً على الإنسان. يستخدم غذاء ملكات النحل (RJ) كغذاء طبيعي ذو محتوى غذائي عالي في الوقاية والعلاج من العديد من الأمراض التي تصيب الإنسان. يهدف هذا البحث إلى دراسة إمكانية استخدام غذاء ملكات النحل لتحسين السمية الكبدية الناجمة عن غذاء ملكات النحل. وقد شملت الدراسة أربع مجموعات من الجرذان (كل منها ٦ جرذان)؛ أعطيت المجموعة الأولى محلول ملحي عن طريق الفم (المجموعة الضابطة)؛ أعطيت المجموعة الثانية جرعة عن طريق الفم من مادة الأكريلاميد (٣٨,٢٧ ملجم / كجم مذابة في محلول ملحي)؛ وتلقت المجموعتان الثالثة والرابعة مادة الأكريلاميد قبل ساعة من إعطاء غذاء ملكات النحل بجرعة ١٥٠ ملجم / كجم و٣٠٠ ملجم / كجم على التوالي. وامتدت فترة التجربة إلى ١٠ أيام متتالية. في اليوم الحادي عشر، تم أخذ عينات الدم والكبد من جرذان كل مجموعة لإجراء الفحوصات البيوكيميائية والنسيجية والمناعية النسيجية. وقد أظهرت النتائج أن مصل الجرذان المعالجة بمادة الأكريلاميد ارتفاعا فى لوحة وظائف كبد وفرط فى سكر الدم وخلل فى شحميات الدم، بينما أظهرت أنسجتها الكبدية تغيرات نسيجية وإجهادًا تأكسديًا والتهابًا. وقد أدى إعطاء غذاء ملكات النحل بجرعات ١٥٠ و ٣٠٠ ملجم / كجم للجرذان المعالجة بمادة الأكريلاميد إلى تحسين وظائف الكبد وسكر الدم والدهون والتغيرات النسيجية والإجهاد التأكسدي والالتهاب في أنسجة الكبد. في الختام، كان غذاء ملكات النحل قادرًا على تحسين الخلل الوظيفي الكبدي وفرط سكر الدم وخلل شحميات الدم، فضلاً عن تحسين التغيرات النسيجية والالتهاب والأكسدة في الأنسجة الكبدية الناجمة عن مادة الأكريلاميد في الجرذان.