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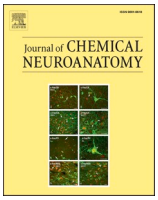

Effect of royal jelly on acrylamide-induced neurotoxicity in rats

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ABSTRACT

Royal Jelly (RJ) is a natural product made by nurse bees known for its multiple therapeutic properties. The research aims to discover the ability of RJ to improve the hematological alterations and neurotoxicity caused by acrylamide (AA). The study rats were separated equally into four groups (6 in each group), the control group, the AA (38.27 mg/kg bw) group, the RJ (150 mg/kg bw) + AA group, and the RJ (300 mg/kg bw) + AA group. Blood and brain samples were collected after 10 days to evaluate haematological and biochemical parameters and to examine histopathological and immunohistochemistry. The administration of AA increased the level of malondialdehyde (MDA), decreases levels of haematological parameters, superoxide dismutase (SOD), reduced glutathione (GSH), brain-derived neurotrophic factor (BDNF), neurotransmitters (serotonin, dopamine, and acetylcholine), and cleaved caspase-3, as well as increase the damage to the brain tissues. Meanwhile, RJ improved levels of haematological parameters, oxidative stress parameters (MDA, SOD, and GSH), BDNF, neurotransmitters, cleaved caspase-3, and brain tissue damage induced by AA. The study demonstrated the protective impact of RJ against the haematological alterations and neurotoxicity caused by AA.

1. Introduction

Acrylamide (AA) is a synthetic chemical used to make polyacrylamide, which is utilized in a variety of industries (Rivadeneyra-Domínguez et al., 2018). In addition, AA is formed naturally when foods rich in sugar are exposed to high temperatures (Rawi et al., 2012). AA has the potential to enter the bloodstream through inhalation, skin contact, or ingestion (Edres et al., 2021). Once it gets into the blood, AA causes haematological disorders, such as macrocytic anemia, leukopenia, eosinophilia, lymphocytopenia, and thrombocytopenia (Hashem et al., 2022). Furthermore, AA can penetrate the blood-brain barriers causing neurotoxicity (Zhao et al., 2022). In the brain, AA promotes oxidative stress, apoptosis, and inflammation (Gur et al., 2021) as well as, changes in brain functions and tissue (Edres et al., 2021). Moreover, AA can cause neuron necrosis, pyramidal and glial cell degeneration (Dasari et al., 2018), and vascular congestion in brain tissue (Farouk et al., 2021).

Royal jelly (RJ) is a natural product made by nurse bees used in health foods, medicines, and cosmetics (Paredes-Barquero et al., 2022). It contains a variety of active ingredients such as proteins, free amino acids, phenols, polysaccharides, lipids, flavonoids, vitamins, minerals, and other components (Guo et al., 2021). This multiplicity of bioactive compounds has endowed RJ with a wide range of therapeutic properties, including antibacterial, antioxidant, antihypercholesterolemic, anti-inflammatory, antiaging, anticancer, and antidiabetic properties (Ahmad et al., 2020).

It is known that RJ can improve haematologic changes induced by food additives (Helal, 2001), radiation (Azab et al., 2011), and azathioprine (Ahmed et al., 2014). Not only that but it also has a neuroprotective effect against the neurotoxicity of tartrazine (Mohamed et al., 2015), cadmium chloride (Almeer et al., 2019), and fluoride (Aslan et al., 2023). However, the role of RJ against AA toxicity has not been demonstrated yet. Therefore, our study aimed to reveal the role of RJ against haematotoxicity and neurotoxicity of AA.

2. Materials and methods

2.1. Chemical and drug

Acrylamide was bought as a powder from Alpha Chemika Company (India). While RJ was purchased as a dietary supplement from PHARCO Pharmaceuticals Company (Egypt). Each 340 mg of crude RJ contains 6% 10-hydroxy-2-decenoic acid (10-HDA).

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2.2. Experimental animals

The experiment was conducted on 24 male Wistar rats (180 ± 10 g), bought from the laboratory animal unit in Helwan Farm-VACSERA, Egypt. Rats were kept under constant conditions that included good ventilation, constant temperature at 22 °C, exposure to 12 h of light per day, and unrestricted access to food and drink. The protocol of our study was approved (ZD/FSc/BU-IACUC/2022–16b) by the IACUC at the Zoology Department, Faculty of Science, Benha University.

2.3. Rat groups

After 7 days of acclimatization, the study rats were separated equally into four groups (6 in each group) as follows:

Control group: Rats were given 0.5 ml of saline orally.

AA group: Rats were given 0.5 ml of AA (38.27 mg/kg bw) dissolved in saline orally (Gur et al., 2021).

RJ (150 mg/kg) + AA group: Rats were given an oral dose of RJ (150 mg/kg bw)1 h before the oral AA dose.

RJ (300 mg/kg) + AA group: Rats were given an oral dose of RJ (300 mg/kg bw)1 h before the oral AA dose.

After 10 days, blood and brain samples were taken from each rat to evaluate haematological and biochemical parameters and to examine histopathological and immunohistochemical changes in the brain tissue.

2.4. Preparations of samples

Blood samples collected from anesthetized rats were placed in two sets of tubes. Anticoagulants were added to the blood in the first set of tubes for use in the calculation of the haematological parameters. Whereas, the blood in the second set of tubes was left to coagulate for 2 h without anticoagulant to obtain serum that will be used to assess levels of neurotransmitters.

Rat brains were removed and washed in phosphate buffer saline (PBS). Brain tissue homogenate was prepared by taking 10 mg of brain tissue and homogenizing it in PBS. Then, the brain tissue homogenate was centrifuged to separate the supernatant that will be used in the assessment of BDNF levels and oxidative stress parameters. While brain tissue section was prepared by fixing the left cerebral cortex of each rat in 10% formalin.

2.5. Haematological parameters calculation

Haematological parameters [red blood cells (RBCs) number, haemoglobin (Hb) content, haematocrit (Hct) value, white blood cells (WBCs) number, and platelets (PLT) number] were calculated by using the MS4e automatic hematology analyzer (France). The results of the haematological parameters were compared with biological reference ranges reported by Rivadeneyra-Domínguez et al. (2018).

2.6. Biochemical analysis

Colorimetric assay kits (BioVision, USA) were used to evaluate the levels of malondialdehyde (MDA) and superoxide dismutase (SOD) in brain tissue homogenates. While the MyBioSource (USA) colorimetric assay kit was used to assess the level of reduced glutathione (GSH) in brain tissue homogenate.

Rat ELISA kits (MyBioSource, USA) were used to calculate the brain-derived neurotrophic factor (BDNF) level in brain tissue homogenate and the levels of neurotransmitters (serotonin, dopamine, and acetylcholine) in serum.

2.7. Histopathological and immunohistochemical examinations

Brain tissue sections were prepared by washing fixed samples in distilled water, dehydrating them in alcohol, embedding them in paraffin, and cutting them into section (5 μm thick). Then, the prepared sections were deparaffinized and rehydrated.

To examine histopathological changes in brain tissues, some prepared sections were stained with hematoxylin and eosin (H&E).

To study immunological caspase-3 expression, some prepared sections were incubated for 40 min with antigen retrieval solution and then cooled for 30 min at room temperature. Sections were treated with 3% hydrogen peroxide, followed by washing, to stop the activity of endogenous peroxidase. After that,10% bovine serum albumin was added to the sections to inhibit non-specific immunoreactivity. Next, the anti-cleaved caspase-3 primary antibodies were incubated with the sections overnight. Slides were washed and incubated with the secondary antibody for 1 h at 25 °C. Sections were washed three times with PBS, incubated with diaminobenzidine for 10 min, and washed three times with distilled water. Finally, the stained sections were dehydrated and coated with a glass slip for examination. Stained slides were examined by a Nikon Eclipse E800 microscope and the percentage area of immunoreactivity in brain tissue was calculated using Image Pro Plus (ver. 4.5.1.27) software.

2.8. Statistical analysis

The SPSS (version 20.00) program was used to analyz all the data in this study. The mean and standard deviation were calculated for each group using 6 values. Differences between group means were evaluated by performing a one-way ANOVA test followed by Duncan's multiple-range test ($P < 0.05$). The SigmaPlot (version 10.00) program was used to plot the graphs.

3. Results

3.1. Effect of RJ on haematological parameters

The values □□of hematological parameters (RBCs, Hb, Hct, WBCs, and PLT) in the control group were within the biological reference ranges except for the Hct value which was slightly elevated. The levels of haematological parameters were significantly ($P < 0.05$) lower in the AA group than in all other groups. Administration of two doses (150 or 300 mg/kg bw) of RJ increased levels of haematological parameters in the RJ plus AA-treated rats compared to those in the AA group. The high dose of RJ increased the levels of haematological parameters more than the low dose of RJ in the RJ plus AA-treated rats (Table 1).

3.2. Effect of RJ on oxidative stress parameters

Administration of AA resulted in a significant ($P < 0.05$) increase in the level of MDA and significant decreases in levels of SOD and GSH in the brain tissues of AA-treated rats compared to the other groups. Whereas administration of two doses (150 or 300 mg/kg bw) of RJ improved levels of oxidative stress parameters (MDA, SOD, and GSH) in brain tissues of the RJ plus AA-treated rats compared to those in the AA group. The improvement in levels of oxidative stress parameters was more clear in the RJ 300 + AA group compared to the RJ 150 + AA group (Table 2).

3.3. Effect of RJ on BDNF level

According to Fig. 1, the level of BDNF was significantly ($P < 0.05$) lower in the brain tissues of the AA group than in all other groups. While administration of two doses (150 or 300 mg/kg bw) of RJ significantly increased BDNF levels in the brain tissues of the RJ plus AA-treated rats compared to those in the AA group. The high dose of RJ increased the level of BDNF more than the low dose of RJ in the RJ plus AA-treated rats.

Table 1
Effect of royal jelly (RJ, 150 or 300 mg/kg bw) on haematological parameters in acrylamide (AA, 38.27 mg/kg bw)-treated male rats.

	Groups				Reference ranges*
	Control	AA	RJ 150 + AA	RJ 300 + AA	
RBCs (10^6 cells/ mm^3)	8.28 ± 0.25[a]	4.04 ± 0.08[d]	4.66 ± 0.11[c]	5.73 ± 0.16[b]	7.80–8.65
Hb (g/dl)	13.89 ± 0.11[a]	7.94 ± 0.07[d]	9.70 ± 0.13[c]	10.60 ± 0.12[b]	13.20–17.10
Hct (%)	45.42 ± 0.16[a]	27.47 ± 0.12[d]	32.83 ± 0.08[c]	35.60 ± 0.12[b]	35–45
WBCs (10^3 cells/ mm^3)	6.57 ± 0.13[a]	3.27 ± 0.11[d]	4.25 ± 0.09[c]	4.94 ± 0.05[b]	4.00–17.00
PLT (10^3 cells/ mm^3)	643.45 ± 0.53[a]	319.72 ± 0.31[d]	437.04 ± 0.28[c]	467.30 ± 0.22[b]	300.00–1500.00

All data were analyzed by the SPSS statistical software (Duncan's test) and are listed in the table as the mean± standard deviation (n = 6).
The letters from a to d refer to significant differences ($P < 0.05$) between the means of groups in the same row.
*Source: Rivadeneyra-Domínguez et al. (2018).

Table 2
Effect of royal jelly (RJ, 150 or 300 mg/kg bw) on oxidative stress parameters in the brain tissues of acrylamide (AA, 38.27 mg/kg bw)-treated rats.

	Groups			
	Control	AA	RJ 150 + AA	RJ 300 +AA
MDA (nmol/10 mg tissue)	1.56 ± 0.01[c]	5.42 ± 0.04[a]	4.32 ± 0.02[b]	4.26 ± 0.01[b]
SOD (U/10 mg tissue)	6.28 ± 0.12[a]	3.31 ± 0.06[d]	4.26 ± 0.11[c]	4.53 ± 0.15[b]
GSH (μmol/10 mg tissue)	64.42 ± 0.34[a]	42.54 ± 0.21[d]	51.33 ± 0.26[c]	52.09 ± 0.31[b]

All data were analyzed by the SPSS statistical software (Duncan's test) and are listed in the table as the mean± standard deviation (n = 6).
The letters from a to d refer to significant differences ($P < 0.05$) between the means of groups in the same row.

3.4. Effect of RJ on histopathological changes in brain tissues

The microscopic examination of rat brains in the control group revealed normal histoarchitecture of the cerebral cortex consisting of the molecular layer formed mainly from neuropil, granule cell layer formed from small neurons, and Purkinje cell layer which appeared as a single layer of large cell bodies located at the interface of the molecular and granule cell layers (Fig. 2A). The examined brain of rats intoxicated with acrylamide showed degenerated and necrotic Purkinje neurons with central chromatolysis. Multiple areas of cerebral edema evidenced by separation of the axonal fibers with clear spaces and vacuolation of neuropil were found (Fig. 2B). Focal areas of hemorrhages replaced the brain tissues were scattered through the examined brain (Fig. 2C). Moreover, leptomeningitis characterized by congestion of meningeal blood vessels with perivascular lymphocytic cuffing was prevalent (Fig. 2D). Rat brains in the RJ 150 + AA group showed congestion of meningeal blood capillaries with perivascular inflammatory cellular aggregation (Fig. 2E), brain edema and necrosis of some neurons with the microglial response (Fig. 2F). The neuroprotective effect of RJ was more distinct in the RJ 300 + AA group which was clarified by a great improvement in the microscopic picture of the examined brain. The foci of gliosis and congestion of blood capillaries with pericapillary hemorrhage were the only detectable microscopic changes in the examined rat brains in the RJ 300 + AA group (Fig. 2G).

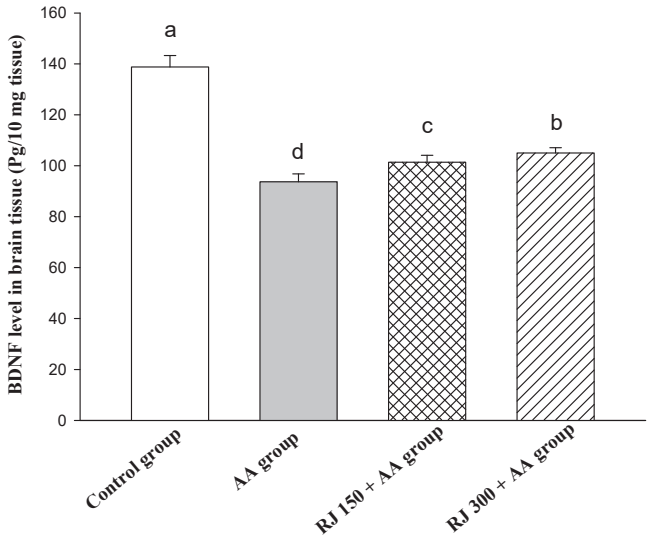

Fig. 1. Effect of royal jelly (RJ, 150 or 300 mg/kg bw) on BDNF levels in the brain tissues of acrylamide (AA, 38.27 mg/kg bw)-treated male rats. Bar graphs with error bars indicate the means and standard deviations of groups, respectively. The letters from a to d refer to significant differences ($P < 0.05$) between the means of groups.

3.5. Effect of RJ on caspase-3 immunoexpression

Immunohistochemistry results showed the presence of mild immunoreactivity for the cleaved caspase 3 in the brains of rats examined in the control group (Fig. 3A). Meanwhile, the AA group had greater numbers of cleaved caspase-3 positive cells than the control group. Active cleaved caspase-3-positive neurons, pyramidal cells, and epithelial cells lining the choroid plexus were recorded in the brain of rats intoxicated with acrylamide (Fig. 3B&D). The two oral royal jelly doses reduced the immunoexpression of cleaved caspase-3 in the tissue brains of the RJ plus AA-treated rats (Fig. 3E&F). Moreover, the high dose of RJ (300 mg/kg bw) significantly decreased the area percentage of cleaved caspase-3 immunoexpression compared to the low dose of RJ (150 mg/kg bw) in the tissue brains of the RJ plus AA-treated rats (Fig. 3G).

3.6. Effect of RJ on neurotransmitters

The administration of AA resulted in significant ($P < 0.05$) decreases in serum levels of neurotransmitters (serotonin, dopamine, and acetylcholine) in the AA group than in all other groups. Whereas administration of two doses (150 or 300 mg/kg bw) of RJ improved levels of serum neurotransmitters in the RJ plus AA-treated rats compared to those in the AA group. The high dose of RJ improved levels of serum neurotransmitters more than the low dose of RJ in the RJ plus AA-treated rats (Table 3).

4. Discussion

Anemia is a haematological disorder characterized by a low RBCs number, Hb content and Hct value (Buttarello, 2016). The decrease in RBCs count, Hb concentration and Hct value in the AA-treated rats in our study indicated the presence of anemia in the AA group. Rivadeneyra-Domínguez et al. (2018) observed that injection of AA (25 or 50 mg/kg bw) for 14 days in rats lowered levels of RBCs, Hct, Hb, and MCV, resulting in microcytic anemia. Alwan and Ghadhban (2021)

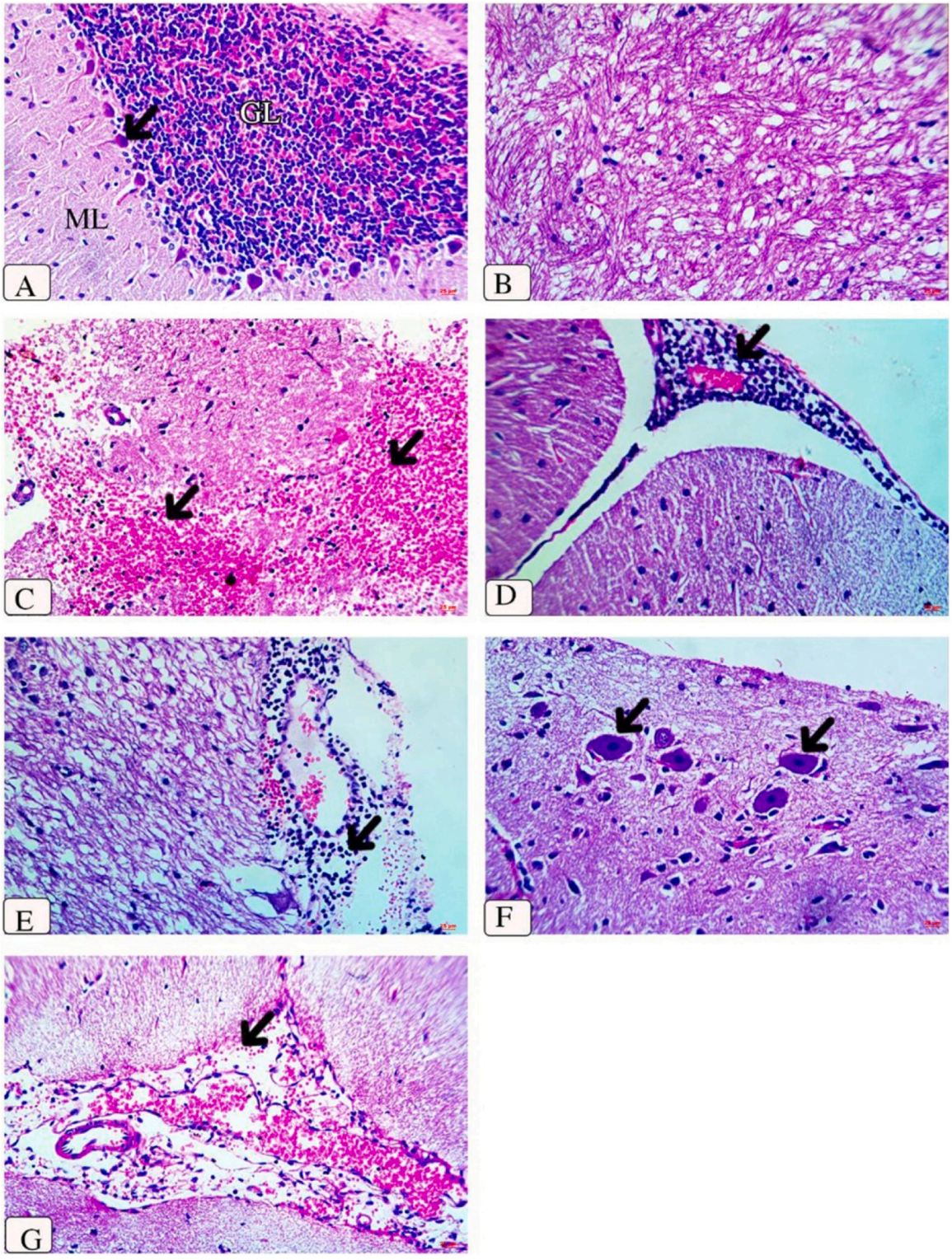

Fig. 2. Photomicrographs of brain sections of rats in the control group (A) showing normal cerebellar cortex formed from molecular layer (ml), Purkinje layer (arrow) and granular layer (GL), the intoxicated acrylamide group (B-D) showing vacuolation of neuropil (B), focal hemorrhage (arrows) replaced the brain tissue (C) and meningeal vascular congestion with perivascular lymphocytic cuffing (arrow) (D), RJ 150 + AA group (E-F) showing perivascular lymphocytic cuffing (arrow) with vacuolation of the neuropil (E) necrosis of some neurons (arrows) with aggregation of microglial cells around these neurons (F) and the RJ 300 + AA group showing congested meningeal blood capillaries with pericapillary hemorrhage (G). (H & E stain X200).

attributed the decrease in RBC count in male rats treated with AA to an increased rate of RBC breakdown within the hematopoietic organs. Additionally, oral administration of AA changed the viscosity properties of blood, caused erythrocyte membrane damage, and resulted in micronucleated erythrocytes (Arihan et al., 2011). Lower Hb concentration in AA-treated rats might be due to the ability of AA and glycidamide (AA metabolite) to form adducts with sulfhydryl groups on Hb and other proteins (Hashem et al., 2022). In addition, the present study

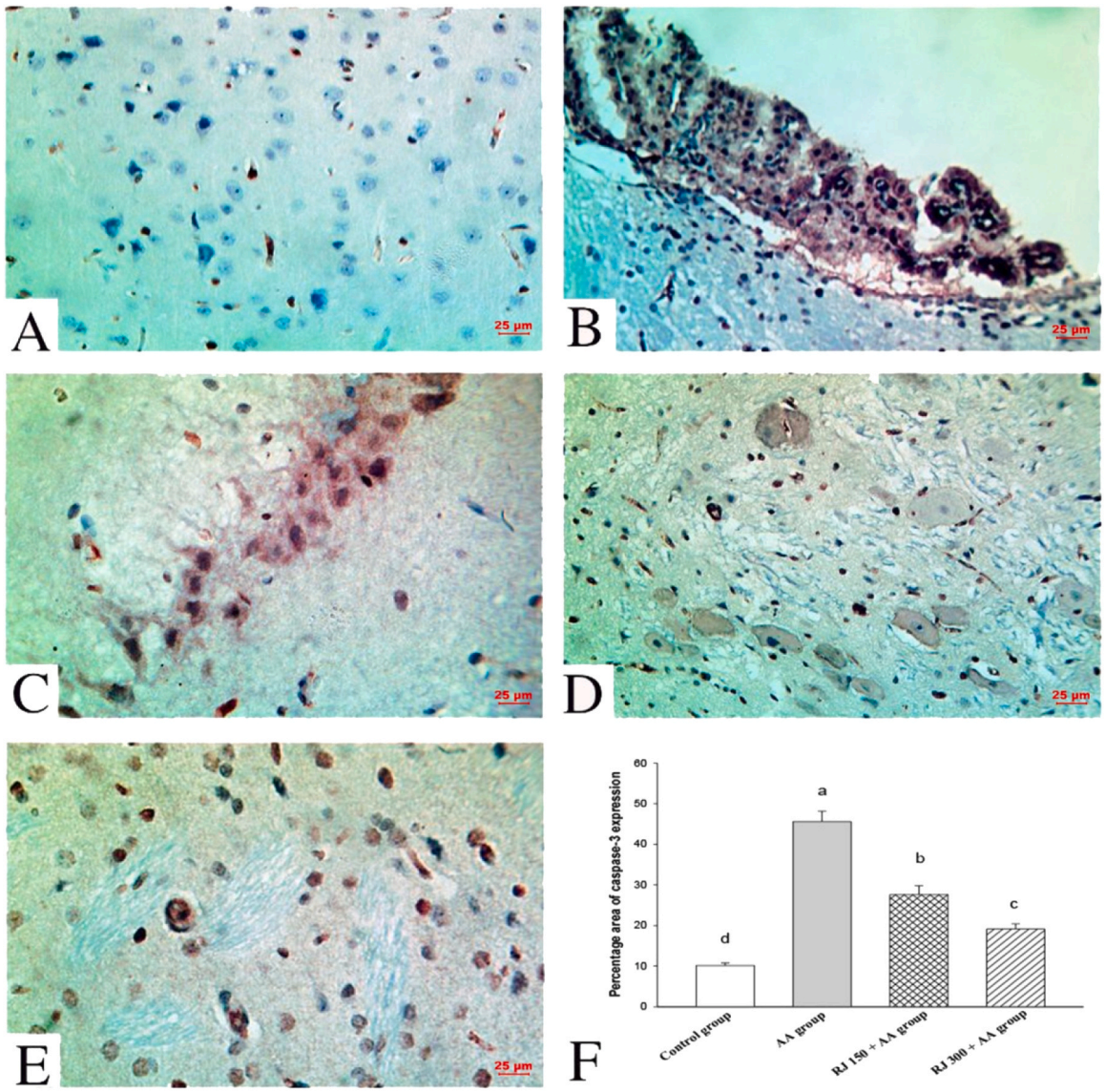

Fig. 3. Immunohistochemical Photomicrographs (A–E) of caspase-3 expression in the brain tissues of the control group (A), the acrylamide (AA) group (B&C), the RJ 150 + AA group (D), and the RJ 300 + AA group (E). Graph (F) shows the means and standard deviations of the percentage area of caspase-3 immunoreactivity for the groups. The letters from a to d refer to significant differences ($P < 0.05$) between the means of groups.

Table 3
Effect of royal jelly (RJ, 150 or 300 mg/kg bw) on serum levels of neurotransmitters in acrylamide (AA, 38.27 mg/kg bw)-treated rats.

	Groups			
	Control	AA	RJ 150 + AA	RJ 300 + AA
Serotonin (ng/ml)	127.34 ± 2.62[a]	94.02 ± 1.71[d]	102.91 ± 1.02[c]	105.49 ± 1.03[b]
Dopamine (ng/ml)	31.23 ± 0.14[a]	13.59 ± 0.09[d]	18.54 ± 0.11[c]	19.77 ± 0.17[b]
Acetylcholine (Pg/ml)	127.52 ± 1.16[a]	73.12 ± 0.58[d]	112.91 ± 1.09[b]	108.32 ± 1.06[c]

All data were analyzed by the SPSS statistical software (Duncan's test) and are listed in the table as the mean± standard deviation (n = 6).
The letters from a to d refer to significant differences ($P < 0.05$) between the means of groups in the same row.

demonstrated reductions in the number of total WBCs and PLT in AA-treated rats. The decrease in the total number of leukocytes in rats treated with AA might be due to the immunosuppressive effect (Rawi et al., 2012) or the immunotoxic effect of AA (Zamani et al.,2018). While Hashem et al. (2022) connected the reduction in the total number of leukocytes, lymphocytes, and neutrophil counts in AA-treated rats to the histological alterations caused by AA in the splenic tissue and bone marrow.

The ability of RJ to improve alterations in the haematological parameter induced by AA was demonstrated in this study. The presence of minerals such as iron, zinc, and copper and vitamins such as vitamin B6, folic acid, vitamin B12, vitamin C, vitamin E, and vitamin A in RJ (Botezan et al., 2023) may attributed to its potential to raise the number of erythrocytes and haemoglobin content. This suggestion was supported by Hayden et al. (2012) who reported that zinc, iron, folate, and vitamin B12 are essential factors for erythropoiesis. Chen et al. (2018) also confirmed that zinc supplementation stimulates the formation of erythrocytes in rats. Furthermore, RJ contains a variety of essential amino acids, such as histidine (Botezan et al., 2023), which is necessary for globin production and erythropoiesis (Vera-Aviles et al., 2018). RJ administration also increases the number of erythrocytes, leukocytes, and platelets by reducing bone marrow cell loss induced by cyclophosphamide in rats (Khazaei et al., 2020). Moreover, administration of

vitamin E increases the number of leukocytes in rats (Cay and Naziroğlu, 1999).

Acrylamide is known to be neurotoxic in both animals and humans (Li et al., 2006). The neurotoxicity of AA has been attributed to the oxidative stress it caused in rats (Ghasemzadeh Rahbardar et al., 2021). Our results confirmed the occurrence of oxidative stress in the brain tissues of AA-treated rats by increasing the level of MDA and decreasing the levels of SOD and GSH indicating the neurotoxic effect of AA. According to Gur et al. (2021), elevated levels of ROS caused oxidative stress in AA-treated rats. While Erdemli et al. (2019) attributed the oxidative stress of AA to the reduction in GSH, SOD, and CAT levels and the elevation in MDA levels. Additionally, the conjugation of AA and glycidamide to GSH might be the reason for the increased MDA content in AA-treated rats leading to oxidative stress (Mansour et al., 2017).

The results of the study confirmed the ability of RJ to improve oxidative damage induced by AA in the brain tissues by elevating the GSH and SOD levels as well as reducing MDA levels in the RJ plus AA-treated rats indicating its neuroprotective effect. Aslan et al. (2023) reported that the antioxidant activities of RJ were the reason for preventing brain tissue damage caused by fluoride in rats. The presence of polyphenols and proteins in the content of RJ components may be responsible for its antioxidant capabilities (Rafat et al., 2016). Additionally, the 10-HDA present in RJ decreased free radicals by scavenging hydroxyl radicals and raising GSH levels (Fan et al., 2022).

BDNF is an important neurotrophic factor for maintaining neuron growth, supporting the development of noradrenergic and serotonergic neurons, and protecting them from neurotoxic damage (Abou Zaid et al., 2017). Ingestion of 25 mg/kg bw of AA for 21 days caused a decrease in BDNF levels in the brains of rats (Mansour et al., 2017). The reduction in BDNF levels in AA-treated rats has been attributed to the oxidative stress generated by AA in rat brain tissues (Abou Zaid et al., 2017). The reduced levels of BDNF in the brain tissue of the AA-treated rats indicated in this study confirmed the neurodegenerative effect of AA.

The elevation in BDNF levels induced by RJ administration to AA-treated rats observed in this study reflected the ability of RJ to counteract the neurodegenerative effect of AA. According to Rahardjo and Santoso (2016), administration (100 mg/kg bw) of RJ for 7 days elevated the BDNF level in rats with a cerebral contusion. The presence of 10-HDA in RJ stimulated neurogenesis (Hattori et al., 2007).

In this study, brain tissues of AA-treated rats exhibited degeneration and necrosis in Purkinje neurons, the appearance of several regions of cerebral edema, hemorrhage, and congestion of meningeal blood vessels, as well as neuronal apoptosis. The histopathological changes and apoptosis indicated by the elevated cleaved caspase-3 level observed in the brain tissues of AA-treated rats may be due to oxidative stress and the decreased level of BDNF induced by AA. According to Abou Zaid et al. (2017), the oxidative stress produced by AA administration caused apoptosis, necrosis, increased degenerative plaques, and dilated blood capillaries in the brain striatum and hippocampus. High levels of free radicals produced by AA caused hemorrhage and congestion in the blood vessels in brain tissues of AA-treated rats (Farouk et al., 2021). Low levels of antioxidant enzymes in rats treated with AA caused pyramidal and glial cell degeneration as well as neuron necrosis (Dasari et al., 2018).

The ability of RJ to improve histopathological changes and reduce the level of cleaved caspase-3 in the brain tissues of the RJ plus AA-treated rats in this study reflected the neuroprotective effect of RJ against the neurotoxicity of AA. Administration (100 mg/kg bw) of RJ for 7 days reduced cerebral edema and apoptosis in the brain tissues in rats with cerebral contusion by decreasing IL-1β and increasing BDNF levels (Rahardjo and Santoso, 2016). Consumption of RJ reduced apoptosis in rabbits with spinal cord injury by increasing BDNF levels (Aslan et al., 2012).

In this study, lower levels of serum neurotransmitters (serotonin, dopamine, and acetylcholine) in AA-treated rats were markers of brain dysfunction. The decrease in dopamine levels in AA-treated rats has been attributed to the higher activity of monoamine oxidase, which results in elevated dopamine catabolism (Rawi et al., 2012). The interaction of AA with tryptophan and tyrosine (serotonin and dopamine precursors) may also reduce serotonin and dopamine levels (Husain et al., 1987). Edres et al. (2021) attributed the reduction in acetylcholine levels in AA-treated rats due to the higher activity of acetylcholinesterase, an enzyme that catalyzes acetylcholine degradation.

The improvement in serum levels of neurotransmitters in the RJ plus AA-treated rats induced by taking RJ in this study was another sign of RJ's ability to ameliorate the neurotoxic effect of AA. Our results supported the study of Almeer's (2019) demonstrating that RJ improves levels of norepinephrine, serotonin, and dopamine in rats poisoned with cadmium chloride. The presence of tryptophan, tyrosine, and acetylcholine among the RJ components (Ahmad et al., 2020; Guo et al., 2021) may be responsible for the elevated levels of neurotransmitters in the RJ plus AA-treated rats. The ability of RJ to reduce acetylcholinesterase and increase choline acetyltransferase (enzyme catalyzes acetylcholine synthesis) levels during neurological disorders (Pan et al., 2019) may be the reason for the higher level of acetylcholine. In addition, vitamin B5 in RJ stimulates the generation of coenzyme A, which is necessary for the production of acetylcholine (Chen et al., 2017).

5. Conclusion

RJ can ameliorate haematological alterations as well as the neuro-oxidative stress, neurodegenerative, neuronal histological changes, neuronal apoptosis, and alteration of neurotransmitter levels caused by AA.

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Present work did not receive any specific grant from funding agencies in the public, commercial, or not.

Ethical Statement

National Research Council's Guide for the Care and Use of Laboratory Animals has been followed. The protocol of our study was approved (ZD/FSc/BU-IACUC/2022–16b) by the IACUC at the Zoology Department, Faculty of Science, Benha University.

CRediT authorship contribution statement

Both authors designed and performed the experiment. Doaa S. Ibrahim: Writing and reviewing the paper. Eman M.S. Shahen: analysis and interpretation of data.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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