10.21608/avmj.2025.368682.1632 Assiut University web-site: www.aun.edu.eg

RUTA GRAVEOLENS EXTRACT AMELIORATES TESTICULAR DAMAGE INDUCED BY ACRYLAMIDE IN RATS

ALI, SOHAIR [1]; FAROUK, MARWA [1]; AHMED, MAI A.M. [2] AND RADAD, KHALED [1]

[1] Department of Veterinary Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt
[2]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Received: 27 March 2025; **Accepted:** 19 June 2025

ABSTRACT

Due to the increasing reliance on processed food in our fast-paced world, exposure of people to acrylamide becomes inevitable, resulting in lifelong harm, most notably, to reproductive organs. The present study aimed to explore the ameliorative effects of *Ruta graveolens* hydroalcoholic extract against testicular damage and functional impairment induced by acrylamide in Sprague-Dawley rats. Thirty two rats were equally divided into 4 groups; the control group received normal saline (0.5 ml/rat). Acrylamide group treated with acrylamide (30 mg/kg b.w). Acrylamide and *Ruta graveolens* extract group co-administered with acrylamide (30 mg/kg b.w) and the extract (200 mg/kg b.w). *Ruta graveolens* group received only the extract (200 mg/kg b.w.). Normal saline, acrylamide and the extract were given orally for 3 weeks. Acrylamide significantly decreased the sperm concentration, initial sperm motility, and the percentage of live and normal sperms, and did not affect testosterone levels. Moreover, it insignificantly decreased GSH and increased MDA levels. Histopathologically, depletion in seminiferous epithelium and formation of multinucleate giant cells were constant lesions in most acrylamide-treated rats. There was also sloughing of seminiferous epithelial cells and stasis of seminiferous tubule fluid in the lumina of seminiferous tubules in some rats. On the other hand, concomitant treatment of rats with acrylamide and the extract significantly ameliorated the deleterious effect of acrylamide on sperm indices and markedly improved the histological architecture of testicular tissues. In conclusion, *Ruta graveolens* extract protects against acrylamide-induced testicular damage and improves rats' reproductive function. Elucidating Ruta graveolens underlying mechanisms is further needed.

Keywords: Acrylamide, *Ruta graveolens*, rats, testicle, extract

INTRODUCTION

Acrylamide (ACR) is a highly-water soluble synthetic monomer that is extensively utilized in polymer industries, treatment of wastewater, printing textiles, adhesives, cosmetics and laboratories (Riboldi *et al.*, 2014; Tepe and Çebi, 2019). Besides these exposure sources, smoking cigarettes is also a considerable source of exposure in humans (Friedman, 2015). In 2002, Stockholm University and Swedish National Food Agency discovered that ACR can be unintendedly formed when food rich in starch and protein, such as meat, potatoes crisps, biscuits, French fries, cereals and coffee are prepared at higher temperatures

Corresponding author: Prof. Dr. Khaled Radad
E-mail address: khaledradad@aun.edu.eg
Present address: Department of Veterinary Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

(Albalawi *et al.,* 2017). Formation of ACR occurs by the Maillard reaction, that catalyzes the interaction between sugars and asparagine (Sansano *et al.,* 2017). Since that time, great attention has been paid by scientists to ACR as a potential hazard to human health (Taeymans *et al.,* 2004).

Over nearly 2 decades, it has been shown that ACR is genotoxic (Abdel-Daim *et al.,* 2015), neurotoxic (Prasad *et al.,* 2014), hepatotoxic (Gelen *et al.,* 2022), nephrotoxic (Sengul *et al.,* 2021), carcinogenic (Shipp *et al.,* 2006) and toxic for the reproductive organs (Shahrzad *et al.,* 2020). Among them, toxicity to the male genital system is of great public health concern (Saleh *et al.,* 2024). In this context, ACR was reported to damage testicular tissues (Gül *et al.,* 2021; Ahmed *et al.,* 2022) and consequently disrupt male reproductive function, represented in reduction of sperm concentration and increasing sperm deformity index (Lebda *et al.,* 2014).

Recently, the shift to use natural medicinal materials instead of synthetic products is tremendously increased worldwide (Emerit *et al.,* 2012). Accordingly, plant natural products are in the focus of researchers to formulate new therapeutic agents that are effective against various illnesses (Dzobo, 2022). *Ruta graveolens* Linn (RG) that belongs to the Rutaceae family is a native plant growing in southern Europe and spread all over the world (Malik *et al.,* 2013).

RG has been used as a medicinal plant in ancient civilizations, already known by ancient Romans and Greeks, to treat ocular, ear, neurological, pharyngeal, respiratory, kidney, gynecological and gastrointestinal diseases as well as pain, spasm, inflammation and parasite infestation (Baharvand-Ahmadi *et al.,* 2015; Javadi and Emami, 2015).
Recently, RG has been receiving great attention by drug discovery groups, who discovered the presence of more than 100-120 compounds belonging to flavonoids, terpenoids, volatile compounds, alkaloids, coumarins and furoquinolines in its extracts (Kuzovkina *et al.,* 2004). This content of diverse compounds mediates the beneficial effects of RG extracts against many disease conditions. For example, Ratheesh *et al.* (2010) demonstrated the beneficial action of some RG fractions against inflammatory models in rats. Tarique *et al.* (2016) exhibited antiulcerogenic effects of RG extract in rats' stomachs. Campanile *et al.* (2022) reported that RG water extract ameliorated the ischemic insult and improved neurological functions in rats. Küçükler *et al.* (2024) showed that rutin, the main active ingredient RGE, mitigated deltamethrin-induced testicular toxicity in rats.

According to the aforementioned knowledge, our current study was built up to reveal the potential ameliorative effects of *Ruta graveolens* hydroalcoholic extract against testicular damage and functional impairment in Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Animals and chemicals

Thirty-two SD rats (7 weeks old and 180 - 200 g weight) were obtained from the Animal House, Department of Pathology, Faculty of Veterinary Medicine, Assiut University. Animals were kept at 4 rats per cage and housed under standard laboratory conditions (12 h light/dark cycle, 23±2 °C and 60% humidity). During the experiment, free access to food and water is allowed. ACR (CAS No. 79-06-1) was purchased from Sigma-Aldrich (Germany). *Ruta graveolens* hydroalcoholic extract was prepared at the Department of Pharmacognosy, Faculty of Pharmacy, Assuit University.

Experimental design

Animals were cared during the experiment in accordance to the European Union Council guidelines (86/60/EU). The design and all procedures during the experiment were reviewed and allowed by the animal ethics committee, Faculty of Veterinary

Medicine, Assiut University (No. IRP 06/2024/0226).

After one week of acclimatization, animals were randomly divided into 4 even groups (8 rats each). Control group received normal saline (0.5 ml/a rat). ACR group treated with ACR (30 mg/kg b.w.). ACR and RG group co-treated with ACR (30 mg/kg b.w.) and RG hydroalcoholic extract (200 mg/kg b.w.). RG group treated with RG hydroalcoholic extract (200 mg/kg b.w.). ACR and *Ruta graveolens* hydroalcoholic extract were dissolved in normal saline and given by gastric tube. All treatments were done daily for 3 weeks.

Methods

Preparation of *Ruta graveolens* hydroalcohol extract

Ruta graveolens L. (Family Rutaceae) plant (leaves and stems) were obtained from the Experimental Station of Medical Plants, Faculty of Pharmacy, Assiut University, Assiut, Egypt. The aerial parts of the plant were collected and air-dried in the shade to yield 125 g of plant material. Then, dried material was ground into fine powder and macerated in 0.5 L of hydro-alcoholic mixture (ethanol:distilled water, 7:3 v/v) over night. The obtained material was sonicated for 30 min and left to stand for 16 h. A total of 20.7g dry extract was obtained following decanting and evaporating the hydro-alcoholic layer by a vacuum rotary evaporator under reduced pressure at 45ºC (Täckholm, 1974).

Body weight gain

Body weight was measured for each rat at the beginning and the end of the experiment. The net weight gain was assessed based on the equation (start body weight - end body weight).

Blood samples and serum preparation

Blood samples were obtained from rats' orbital sinuses at the end of the experiment into blood tubes without anticoagulant. Then, blood samples were left for 1 h and centrifuged at 3500 rpm for 15 min to obtain the serum. Prepared sera were kept at -80 °C till use.

Sperm collection and examination

The testicles and epididymides were collected after humanely killing of animals at the end of each treatment. Epididymides were then separated, and the sperms were obtained from their tails. In brief, dissected cauda epididymis was put in a Petri dish containing warm PBS (pH 7.4). The spermatozoa were then released into the saline by gentle pressure on the tissues. Obtained sperm cell suspension was used for determination of sperms' concentration, motility, viability and morphology (Sanchez-Alvarez *et al.,* 2012).

Sperm concentration

Sperm concentration was calculated by dilution of 100 µl of sperm suspension to 2000 µl with 1.9 ml of normal saline. After then, 10 µl of the diluted sperm suspension was put on a hemocytometer. After 7 min to allow the sperms to stand, the number of sperms/25 squares was counted and expressed as 10^6/ml according to Nahid *et al.* (2016).

Sperm motility

Based on the method of Nahid *et al.* (2016), total sperm motility was measured by placing a drop of sperm suspension on a glass slide, which was then covered with a cover slip and examined under light microscopy at magnification 40×. The motility percentage of sperms was calculated as the percentage of mobile spermatozoa.

Sperm viability and morphology

Briefly, 40 µl of sperm suspension was carefully mixed with 10 µl of eosin-Nigrosin stain. Then, one drop of the mixture was put on a clean glass slide and examined under a light microscopy. Red stained sperms were considered dead, and unstained sperms were viable. Any abnormalities in the shape of sperms were recorded and the percentage calculated (Saleh *et al.,* 2024).

Biochemical analysis

GSH and MDA levels in testicular homogenates

For preparation of testicular homogenates, 100 mg of testicular tissue (n=16/group) was taken and homogenized in 1ml PBS using homogenizer type 302 (Poland). The homogenized material was then centrifuged at 20000 rpm for 30 min, and supernatants were obtained. Glutathione (GSH) and malondialdehyde (MDA) were measured in the supernatants using spectrophotometer (JENWAY, UK). GSH and MDA levels were measured based on the methods of Beutler *et al.* (1963) and Satoh *et al.* (1978), respectively.

Testosterone levels

Measurement of testosterone levels was done by using rat testosterone ELISA kit (Cat. No. 80550, Crystal Chem., USA) based on the method of Erdemli *et al.* (2019).

Histopathological examination

Obtained testicular specimens (n=16/group) were fixed in 10% neutral buffered formalin for 48-72 h and processed for histopathological examination. Briefly, specimens dehydrated in a graded series of alcohol, cleared in xylene and embedded in paraffin wax. Four micrometer-thickness tissue sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy, and photographed by a digital camera (Bancroft & Stevans, 1990).

Number of histologically damaged seminiferous tubules

Adopting light microscopy at a magnification 40×, seminiferous tubules that exhibited degenerated tubular epithelium, presence of multinucleate giant cells, and cell sloughing and obstruction of tubules' lumina were counted in 3 tissue sections for each testicle (n=48 sections/group) (Radad *et al.,* 2020).

Statistics

Obtained data were presented as means± standard error of means (SEM). Statistical analysis was done using ANOVA and differences between various groups were performed by the Tukey's post hoc test. Significance was considered when the p value is less than 0.05.

RESULTS

Clinical signs

No deaths were recorded during the whole experiment. ACR-treated rats showed low activity and uncoordinated movement after two weeks of treatment. The symptoms were progressed to a foot splay and hind limbs' dragging by the end of the experiment. Treatment with RG hydroalcoholic extract markedly relieved these clinical signs. Control and RG hydroalcoholic extract-treated rats did not show abnormal behavior.

Body and testicles weight

ACR significantly decreased body weight gain, the effect that could not be alleviated by concomitant treatment with ACR and RG hydroalcoholic extract. RG hydroalcoholic extract insignificantly decreased the body weight gain **(Figure 1A)**. ACR did not affect testicles' weight, and RG hydroalcoholic extract slightly decreased the weight of testis either with ACR or alone **(Figure 1B).**

Sperm examination

ACR was shown to significantly decrease sperm concentration, initial sperm motility, and numbers of live and normal sperms, and to increase the percentage of dead and abnormal sperms compared to controls. In contrast, co-administration of ACR and the extract resulted in a significant rise in sperm concentration, initial sperm motility, and the counts of live and normal sperms, and decreased the counts of dead and abnormal sperms. Treatment of rats with RG hydroalcoholic extract alone insignificantly decreased sperm motility and viability **(Figure 2)**.

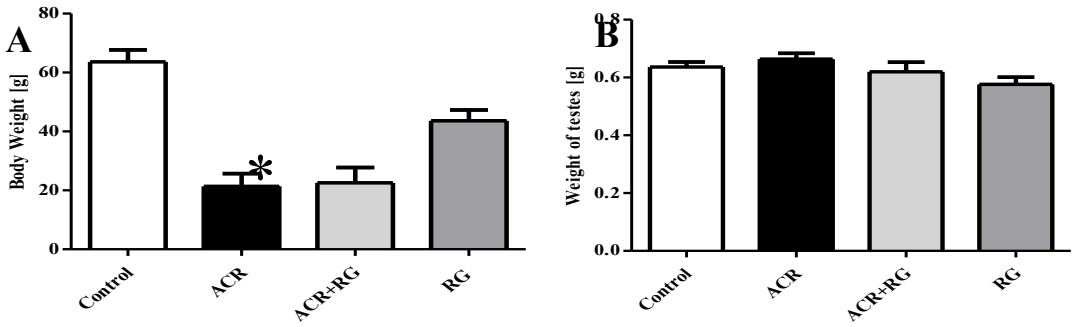

Figure 1: Body and testicles weight of rats. **A)** ACR exerts a significant decrease in the body weight gain (P=0.001), and co-treatment of rats with ACR and RG hydroalcoholic extract does not increase the gain in the body weight (P=0.941). **B)** No differences in the testicles weight between different treated groups are noticed.

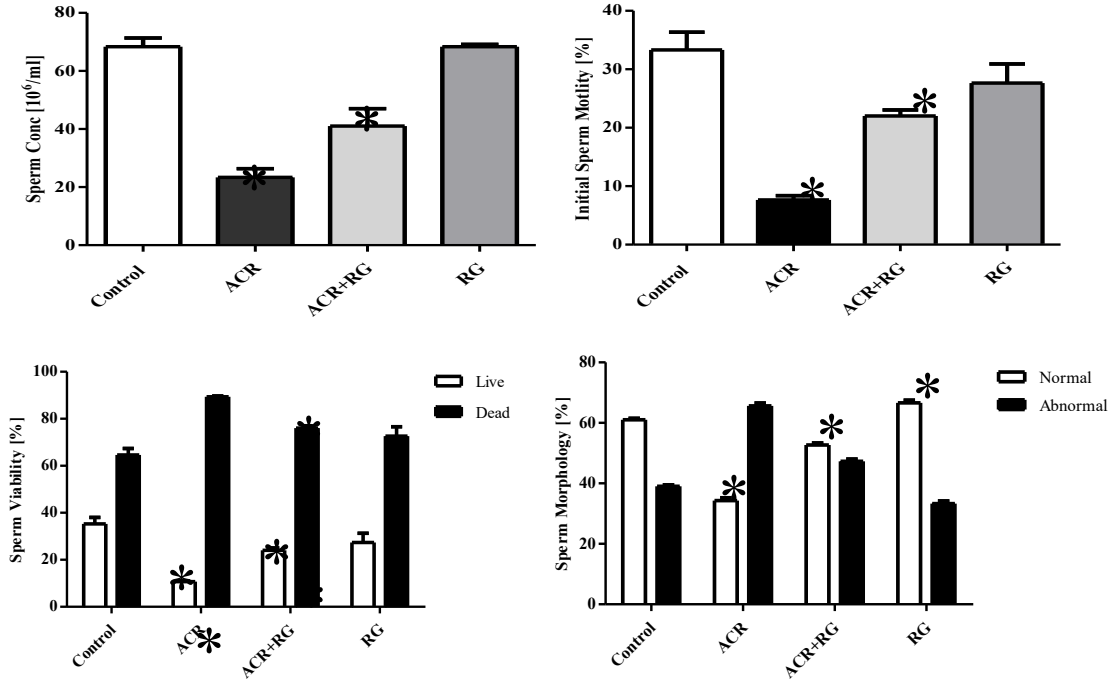

Figure 2: Concentration, initial motility, viability and morphology of sperms in different treated groups. Treatment of rats with ACR significantly decreases the sperm concentration (P=0.001), initial sperm motility (P=0.001), percentage of live (P=0.001) and normal sperms (P=0.001), and increases the percentage of dead (P=0.001) and abnormal sperms (P=0.001). Concomitant treatment of rats with ACR and RG hydroalcoholic extract significantly increases sperm concentration (P=0.01), initial motility (P=0.001), percentage of live (P=0.003) and normal sperms (P=0.001), and decreases the percentages of dead (P=0.003) and abnormal sperms (P=0.001) compared to the ACR-treated group.

Biochemical findings

Treatment of rats with ACR insignificantly lowered GSH levels **(Figure 3A)** and increased MDA levels **(Figure 3B)** in the homogenates, compared to the controls. Concomitant treatment of rats with ACR and RG hydroalcoholic extract slightly ameliorated the decrease in GSH levels **(Figure 3A)** and significantly decreased MDA levels **(Figure 3B)** compared to the ACR-treated group. RG hydroalcoholic extract alone exerted a slight increase and decrease in GSH and MDA levels, respectively, compared to the control group **(Figure 3A,B)**.

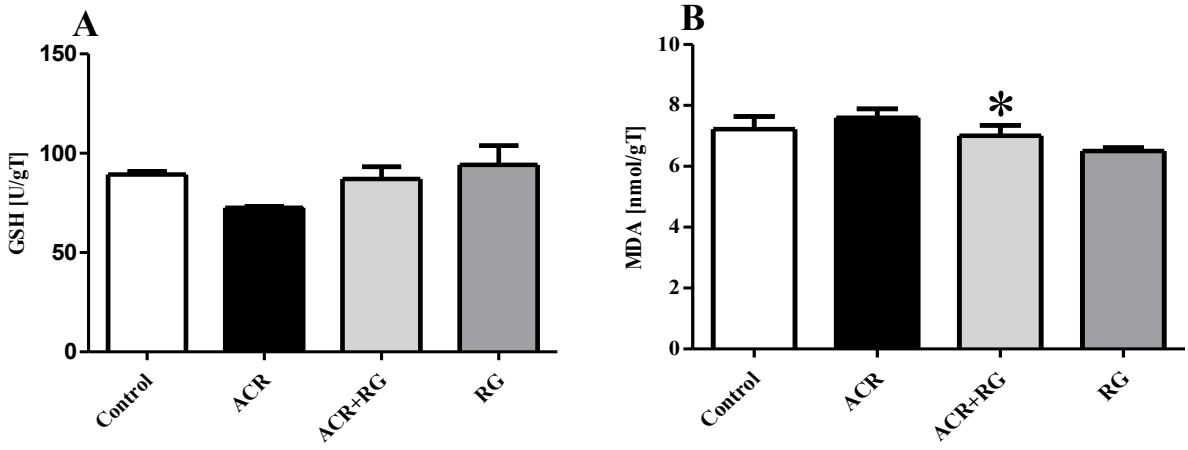

Figure 3: The levels of GSH and MDA in testicular homogenates. **A)** Treatment of rats with ACR insignificantly decreases GSH level (P=0.197), and co-treatment of rats with ACR and RG hydroalcoholic extract slightly ameliorates such decrease (P=0.306) compared to ACR group. **B)** Treatment of rats with ACR slightly increases MDA level (P=0.324), and co-treatment of rats with ACR and RG hydroalcoholic extract significantly decreased MDA level (P=0.05) compared to ACR group.

Serum testosterone

ACR treatment did not affect testosterone levels, compared to controls. Treatment of rats with RG hydroalcoholic extract alone or with ACR significantly decreased testosterone levels (Figure 4).

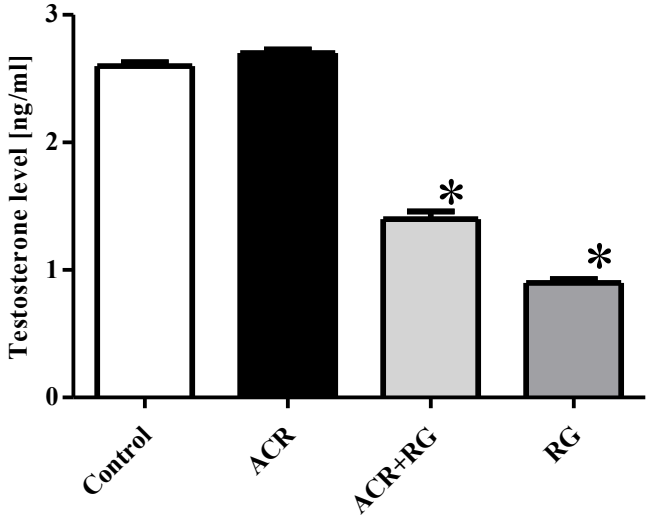

Figure 4. Effect of ACR and RG hydroalcoholic extract on serum testosterone level. ACR does not affect testosterone level (P=0.269), and RG hydroalcoholic extract both with ACR or alone significantly decreases testosterone levels (P=0.001).

Histopathological findings

Histopathological examination of testicular tissues from control rats showed normal seminiferous tubules and normal interstitial tissues containing Leydig cells **(Figure 5A)**. The seminiferous tubules appeared lined with normal layers of cells and had many sperms in the lumina **(Figure 5B)**. Most of rats in the ACR group showed depletion in seminiferous tubular cells and formation of multinucleate giant cells. Depletion affected all layers of seminiferous tubular epithelium,

including spermatogonia, spermatocytes, spermatids and spermatozoa **(Figure 5C,D)**. Moreover, there was also sloughing of tubular cells **(Figure 5E)** and stasis of seminiferous tubule fluid **(Figure 5F)** in the lumina of some tubules. Occasionally, some ACR-administered rats showed congestion and edema in the interstitial tissue **(Figure 6)**. In contrast, co-administration of rats with ACR and RG hydroalcoholic extract markedly alleviated most of ACR-induced histopathological lesions **(Figure 7A,B)**. RG hydroalcoholic extract alone did not disrupt the histological structure of seminiferous tubules **(Figure 7C,D)**.

Number of damaged seminiferous tubules

Damaged seminiferous tubules were significantly increased in number in ACR-treated rats (mean=66.00±2.35) compared to the control group (mean=17.00±0.36). Co-treatment of rats with ACR and RG hydroalcoholic extract significantly lowered the number of damaged tubules (mean=9.75±0.88) compared to ACR-treated rats. The number of damaged tubules in RG hydroalcoholic extract-treated rats (mean=10.50±0.19) and control group were not significantly different **(Figure 8)**.

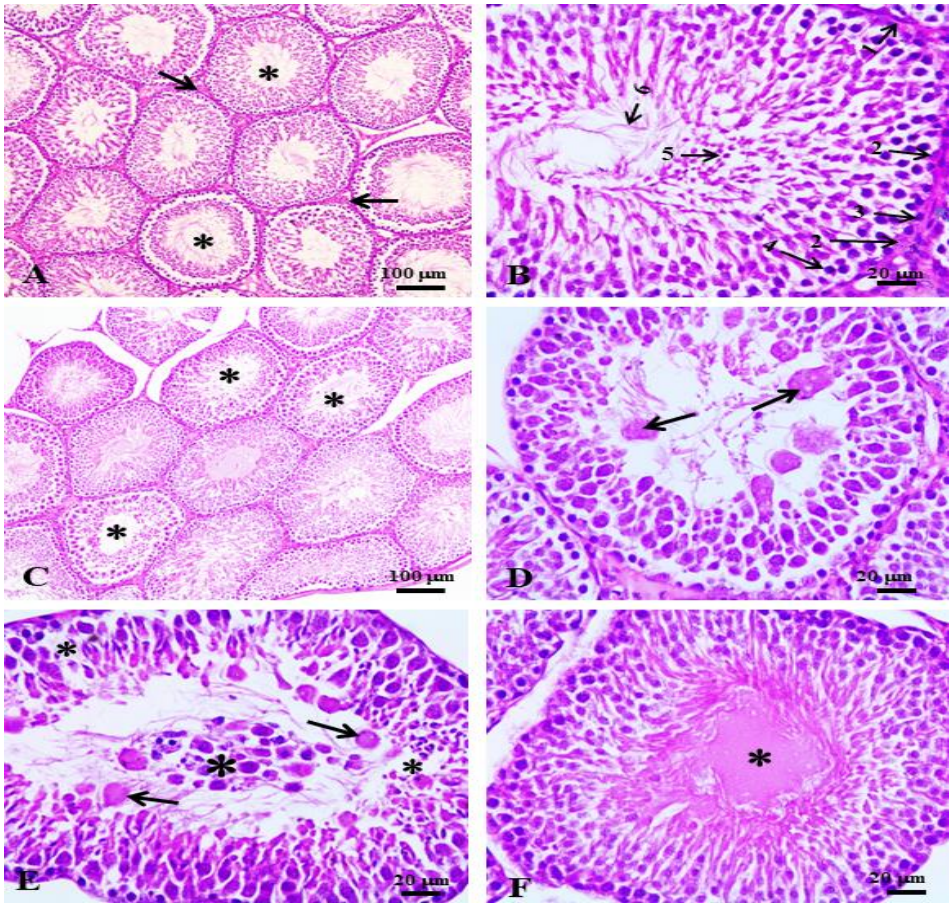

Figure 5: Representative photomicrographs of the testicles in various treated groups. **A)** A control rat shows normally arranged seminiferous tubules (asterisks) and normal interstitial tissues with Leydig cells (arrows). **B)** Higher magnification shows normal tubular lining including (1) myofibroblasts, (2) Sertoli cells, (3) spermatogonia, (4) spermatocytes, (5) spermatids and (6) spermatozoa. **C)** ACR-administered rat shows depletion of seminiferous tubular epithelium (asterisks). **D)** Seminiferous tubules from ACR-treated rats show formation of multinucleate giant cells (arrows) and depletion of the seminiferous tubular epithelium. **E)** A seminiferous tubule from ACR-treated rat shows formation of giant cells (arrows), and depletion (small asterisks) and sloughing of the seminiferous tubular epithelium in the lumina (large asterisk). **F)** A seminiferous tubule from ACR-treated rats rat shows stasis of seminiferous tubule fluid in the lumen (asterisk). H&E.

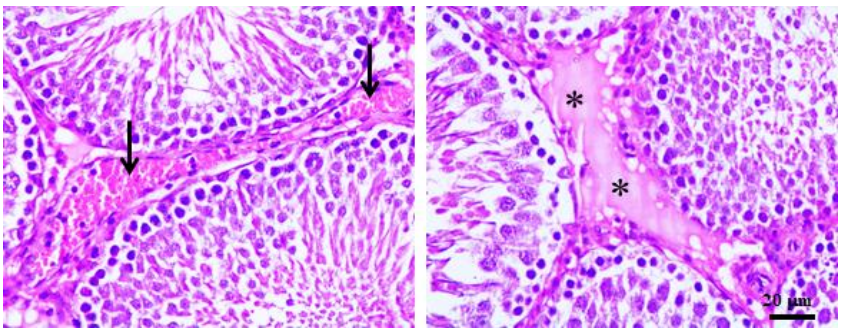

Figure 6: Representative photomicrographs of the testicles in ACR-treated group. Treatment with ACR causes congestion (arrows) and edema (asterisks) in interstitial tissue. H&E.

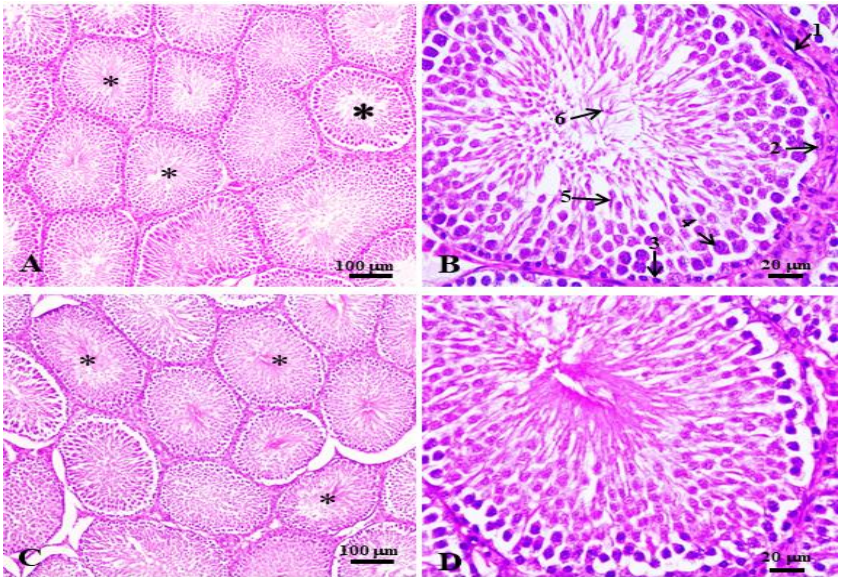

Figure 7: Representative photomicrographs of the testicles in different treated groups. **A)** A concomitantly treated rat with ACR and RG hydroalcoholic extract shows more or less normal seminiferous tubules (small asterisks) with cell depletion in few seminiferous tubules (large asterisk). **B)** A seminiferous tubule from a rat co-treated with ACR and RG hydroalcoholic extract appears lined with nearly normal seminiferous tubular epithelium (arrows 1 - 6). **C)** A rat testicle from RG hydroalcoholic extract-treated group shows normal seminiferous tubules (asterisks). **D)** A seminiferous tubule from a rat treated with RG hydroalcoholic extract appears lined with all layers of seminiferous tubular epithelium. H&E.

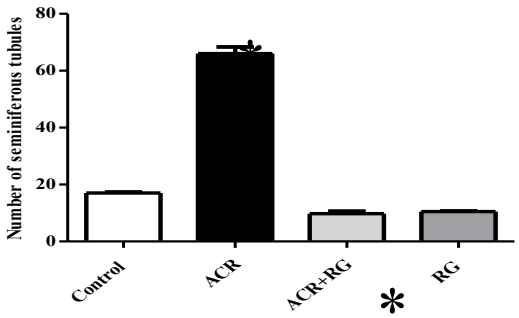

Figure 8: Number of damaged seminiferous tubules in different treated group**s.** ACR significantly increases the number of damaged seminiferous tubules (P=0.001), and co-treatment of rats with ACR and RG hydroalcoholic extract significantly decreases the number of damaged seminiferous tubules (P=0.001) compared to rats in the ACR group.

DISCUSSION

In the current study, ACR significantly decreased rats' weight gain and did not affect the relative weight of the testicles. Decreasing of body weight gain due to ACR has been reported in most experimental studies (see Hashem *et al.,* 2022; Zhang *et al.,* 2023). This may be due to decreasing the appetite and disrupting a variety of biological functions by reacting with the sulfhydryl group in enzymes, receptors and cytoskeletal proteins (Hashem *et al.,* 2022). ACR's effect on the testes weight is controversial in literature. Some studies reported that ACR decreased testicle weight (Gül *et al.,* 2021; Saleh *et al.,* 2024) and some denied (Nixon *et al.,* 2012; Zhang *et al.,* 2023). These controversial results may be attributed to the dose of ACR and the extent of testicular damage (Zhang *et al.,* 2023). RG hydroalcoholic extract did not affect ACR-lowering action on the body weight gain, and it alone slightly decreased body weight gain and testicles' weight compared to control rats. Decreasing gain in the body weight and testicles' weight by RG aqueous extract was reported by Adam *et al.* (2014) and Khouri and El-Akawi (2005).

Semen analysis revealed that ACR significantly decreased sperm concentration, initial sperm motility, and the percentage of live and normal sperms. Parallel to our findings, ACR was reported to adversely affect these sperm parameters (Rajeh and Al-Shehri, 2019; Ahmed *et al.,* 2022). These drastic effects on sperm parameters suggested spermatogenesis impairment by ACR (Assi and Bader, 2011). On the other hand, co-administration of ACR and RG hydroalcoholic extract significantly improved all these sperm parameters. However, treatment of rats with the extract alone slightly decreased sperm motility and viability. Consistently, Khouri and El-Akawi (2005) observed that oral treatment of rats with 500 mg/kg b.w. of RG aqueous extract decreased sperm density and motility after 60 days of treatment.

ELISA evaluation of serum testosterone revealed that ACR did not affect testosterone levels in our study. Very few studies reported increasing serum testosterone by ACR and attributed such effect to the hyperplasia of Leydig cells (Ahmed *et al.,* 2022). On the other hand, most previous studies showed that ACR negatively affected testosterone levels (see Erdemli *et al.,* 2009; Pourentezari *et al.,* 2014; Gül *et al.,* 2021; Zhang *et al.,* 2023; Yildrim *et al.,* 2024). Some authors returned ACR's lowering effect on testosterone to the reduction on Leydig cell viability and disruption of the steroidogenic pathway (Yang *et al.,* 2005). Testosterone is important for normal spermatogenesis, and decreasing its level can, in part, underline the adverse effects of ACR on sperm concentration, motility, viability and morphology (Pourentezari *et al.,* 2014; Saleh *et al.,* 2024: Shipa *et al.,* 2024). Treatment of rats with RG hydroalcoholic extract, either alone or with ACR, significantly decreased testosterone levels. Similarly, RG extracts were reported to decrease serum testosterone in rats. For example, Khouri and El-Akawi (2005) found that RG aqueous extract (500 mg/kg b.w.) for 60 days significantly decreased testosterone levels in rats.

Biochemical analysis using testicular homogenates revealed that ACR insignificantly decreased GSH levels and slightly increased MDA levels. Inconsistently, ACR was significantly reported to lower GSH and raised MDA levels in testicular tissues (Ahmed *et al.,* 2022; Gül *et al.,* 2021; Saleh *et al.,* 2024). Against ACR, RG hydroalcoholic extract exerted a low improving effect on GSH and MDA levels compared to ACR-treated rats. RG extracts were reported to alleviate oxidative stress, increase GSH levels and decreased MDA formation. In this context, Ratheesh *et al.* (2010) found that polyphenolic and alkaloid fractions from RG increased GSH levels in acute and chronic rats' arthritic models. Asgharian *et al.* (2020) reported the ability of RG extract to lower

serum MDA levels in rats suffering from spatial memory and passive avoidance memory. The effect of RG hydroalcoholic extract on testicular GSH and MDA in our study is believed to be minimal to underline some of RG beneficial effects against ACR-induced reproductive insults.

Histopatholgically, ACR was seen to induce clear histopathological alterations in testicles, most notably depletion in tubular cells, formation of multinucleate giant cells, and sloughing of some tubular cells and stasis of seminiferous tubule fluid in the lumina of some seminiferous tubules. Similar to our previous study (Radad *et al.,* 2020), seminiferous tubules as the result of ACR treatment appeared lined with only six layers of cells (myofibroblasts, Sertoli cells, spermatogonia, primary spermatocytes, secondary spermatocytes and round spermatids) instead of the eight normal layers (myofibroblasts, Sertoli cells, spermatogonia, primary spermatocytes, secondary spermatocytes, round spermatids, elongated spermatids, spermatozoa) in control groups. This was due to loss of some spermatogonia, spermatocytes, spermatids and spermatozoa within the testicular epithelium. Depletion was also similarly reported following ACR exposure (Wang *et al.,* 2010; Gül *et al.,* 2021; Saleh *et al.,* 2024; Shipa *et al.,* 2024). Formation of multinucleate giant cells due to ACR is a popular finding in most experimental studies and indicates a degenerative process (see Radad *et al.,* 2020; Zhang *et al.,* 2023; Yildrim *et al.,* 2024). As the result of such degenerative process, some sloughed cells appeared alongside multinucleate giant cells in some tubular lumina. In parallel, Lebda *et al.* (2014) reported that sloughing of seminiferous tubular epithelium could be seen in tubular lumina and epididymal ducts in ACR-treated rats. Sloughed epithelial cells were thought to occlude some seminiferous tubular lumina resulting in stasis. Interestingly, concomitant treatment of rats with ACR and RG hydroalcoholic extract markedly improved the testicular morphology and significantly decreased the incidence of the aforementioned histopathological findings. Correlating obtained data together suggests that the effect of RG hydroalcoholic extract seems to be mediated by local underlying mechanisms within the testicular tissues, which need to be further elucidated. In conclusion, RG hydroalcoholic extract markedly protects against ACR-induced testicular damage and improves rats' reproductive function in SD rats.

REFERENCES

Abdel-Daim, M.M.; Abd Eldaim, M.A. and Hassan, A.G. (2015): Trigonella foenum graecum ameliorates acrylamide-induced toxicity in rats: roles of oxidative stress, proinflammatory cytokines and DNA damage. Biochem. Cell Biol., 93, 192-198.

Adam, SH.I.Y.; Ahmed, N.N.A.; Eltayeb, N.A.M.; Saad, H.; and. Taha, K.A. (2014): Toxicity of Ruta graveolens Seeds' Extracts on Male Wistar Rats. International Journal of Animal and Veterinary Advances, 6, 92-96.

Ahmed, M.M.; Hammad, A.A.; Orabi, S.H.; Elbaz, H.T.; Elweza, A.E.; Tahoun, E.A.; Elseehy, M.M.; El-Shehawi, A.M. and Mousa, A.A. (2022): Reproductive Injury in Male Rats from Acrylamide Toxicity and Potential Protection by Earthwormn Methanolic Extract. Animals, 12, 1723.

Albalawi, A.; Alhasani, RHA.; Biswas, L.; Reilly, J. and Shu, X. (2017): Protective effect of carnosic acid against acrylamide-induced toxicity in RPE cells. Food Chem. Toxicol., 108, 543-553.

Asgharian, S.; Hojjati, M.R.; Ahrari, M.; Bijad, E.; Deris, F. and Lorigooini, Z. (2020): Ruta graveolens and rutin, as its major compound: investigating their effect on spatial memory and passive avoidance memory in rats. Pharm. Biol., 58, 447-453.

Assi, M.M. and Badr, M.R. (2011): Pathological effect of acrylamide on the reproductive performance of the male rabbit. Assiut Vet. Med. J., 57, 413-426.

Baharvand-Ahmadi, B.; Bahmani, M.; Zargaran, A.; Eftekhari, Z.; Saki, K.; Baharvand-Ahmadi, S.; Saki, K. and Rafieian-Kopaei, M. (2015): Ruta graveolens plant: a plant with a range of high therapeutic effect called cardiac plant. Der Pharmacia Lettre, 7,172-173.

Bancroft, J.D. and Stevans, A. (1990): Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh, 3rd ed. pp., 113-305.

Beutler, E.; Duron, O. and Kelly, B.M. (1963): Improved Method for the Determination of Blood Glutathione. Journal of Laboratory and Clinical Medicine, 61, 882-888.

Campanile, M.; Cuomo, O.; Brancaccio, P.; Vinciguerr, A.; Casamassa, A.; Pastorino, O.; Volpicelli, F.; Gentile, M.T.; Amoroso, S.; Annunziato, L.; D'Amato, L.C. and Pignataro, G. (2022): Ruta graveolens water extract (RGWE) ameliorates ischemic damage and improves neurological deficits in a rat model of transient focal brain ischemia. Biomedicine & Pharmacotherapy, 154, 113587.

Dzobo, K. (2022): The Role of Natural Products as Sources of Therapeutic Agents for Innovative Drug Discovery. Comprehensive Pharmacology, 9, 408-422.

Emerit, I.; Packer, L. and Auclair, C. (2012): Antioxidants in Therapy and Preventive Medicine; Springer Science & Business Media: Berlin/Heidelberg, Germany, p. 264.

Erdemli, Z.; Erdemli, M.E.; Turkoz, Y.; Gul, M.; Yigitcan, B. and Bag, H.G. (2019): The effects of acrylamide and Vitamin E administration during pregnancy on adult rats testis. Andrologia, 51, e13292.

Friedman, M. (2015): Acrylamide: Inhibition of formation in processed food and mitigation of toxicity in cells, animals and humans. Food Funct., 6, 1752-1772.

Gelen, V.; Serkan, Y.; Emin, S.; Ali, C.; Fikret, C.; Merve, K. and Melahat, G. (2022): Naringin attenuates oxidative stress, inflammation, apoptosis, and oxidative DNA damage in acrylamide-induced nephrotoxicity in rats. Asian Pac. J. Trop. Biomed., 12, 223-232.

Gül, M.; Kustepe, E.K.; Erdemli, M.E.; Altinöz, E.; Bag, H.G.G.; Gül, S. and Göktürk, N. (2021): Protective effects of crocin on acrylamide-induced testis damage. Andrologia, 53, e14176.

Hashem, M.M.; Abo-El-Sooud, Kh.; Abd El-Hakim, Y.M.; Badr, Y.A.; El-Metwally, A.E. and Bahy-El-Dien, A. (2022): The impact of long-term oral exposure to low doses of acrylamide on the hematological indicators, immune functions, and splenic tissue architecture in rats. Int. Immunopharmacol, 105, 108568.

Javadi, B. and Emami, SA. (2015): Avicenna's contribution to mechanisms of cardiovascular drugs. Iran J. Basic Med. Sci., 18,721-722.

Khouri, N.A. and El-Akawi, Z. (2005): Antiandrogenic activity of Ruta graveolens L in male Albino rats with emphasis on sexual and aggressive behavior. Neuro Endocrinol Lett., 26, 823-9.

Küçükler, S.; Çelik, O.; Özdemir, S.; Aydın, S.; Çomaklı, S. and Dalkılınç, E. (2024): Effects of rutin against deltamethrin-induced testicular toxicity in rats. Biochemical, molecular, and pathological studies. Food and Chemical Toxicology, 186, 114562.

Kuzovkina, I.; Al'terma, I. and Schneider, B. (2004): Specific accumulation and revised structures of acridone alkaloid glucosides in thetips of transformed roots of Ruta graveolens. Phytochemistry, 65, 1095-1100.

Lebda, M.; Gad, S. and Gaafar, H. (2014): Effects of lipoic acid on acrylamide induced testicular damage. Mater. Socio-Med., 26, 208-212.

Malik, AA.; Mir, SR. and Ahmad, J. (2013): Ruta graveolens L. essential oil composition under different nutritional treatments. Am. Eurasian J. Agric. Environ. Sci., 13, 1390e5.

Nahid, Y.; Sahabeh, E.; Ali, K.M.; Reza, T.A.; Akram, H. and Ali, N. (2016): Are any sperm cryopreservation methods superior over others? Comparison of motility, viability, and motile sperm organelle morphology examination (msome) of human spermatozoa. Int. Educ. Sci. Res., 2, 84-90.

Nixon, B.J.; Stanger, S.J.; Nixon, B. and Roman, S.D. (2012): Chronic exposure to acrylamide induces DNA damage in male germ cells of mice. Toxicol Sci., 129,135-45.

Pourentezari, M.; Talebi, A.; Abbasi, A.; Khalili, M.A.; Mangoli, E. and Anvari, M. (2014): Effects of acrylamide on sperm parameters, chromatin quality, and the level of blood testosterone in mice. Iran J. Reprod. Med., 12, 335-42.

Prasad, SN. (2014): Mitigation of acrylamide-induced behavioral deficits, oxi dative impairments and neurotoxicity by oral supplements of geraniol (a monoterpene) in a rat model. Chem. Biol. Interact., 223, 27-37.

Radad, KH.; El Amir, Y.; Al-Emam, A.; Al-Shraim, M.; Bin-Jaliah, I.; Krewenka, Ch. and Moldzio, R. (2020): Minocycline protects against acrylamide-induced neurotoxicity and testicular damage in Sprague-Dawley rats. J. Toxicol Pathol., 24, 33, 87-95.

Rajeh, N.A. and Al-Shehri, A.M. (2019): Antioxidant effect of Ferula hermonis Boiss on acrylamide induced testicular toxicity in male rats. Indian J. Exper. Biol., 57, 138-144.

Ratheesh, M.; Shyni, G.L.; Sindhu, G. and Helen, A. (2010): Protective effects of isolated polyphenolic and alkaloid fractions of Ruta graveolens L. on acute and chronic models of inflammation. Inflammation, 33, 18-24.

Riboldi, B.P.; Vinhas, A.M. and Moreira, J.D. (2104): Risks of dietary acrylamide exposure: a systematic review. Food Chem., 157, 310-322.

Saleh, D.O.; Baraka, S.M.; Abdel Jaleel, G.A.; Hassan, A. and Ahmed-Farid, O.A. (2024): Eugenol alleviates acrylamide-induced rat testicular toxicity by modulating AMPK/ p-AKT/mTOR signaling pathway and blood–testis barrier remodeling Scientific Reports, 14,1910.

Sanchez-Alvarez, J.; Cano-Corres, R.; and Fuentes-Arderiu, X. (2012): A complement for the WHO laboratory manual for the examination and processing of human semen. Ejifcc, 23, 103.

Sansano, M.; Heredia, A.; Peinado, I. and Andrés, A. (2017): Dietary acrylamide: What happens during digestion? Food Chem., 237, 58-64.

Satoh, K.E.I. (1978): Serum Lipid Peroxide in Cerebrovascular Disorders Determined by a New Colorimetric Method. Clinica Chimica Acta, 90, 37-43.

Sengul, E.; Gelen, V.; Yildirim, S.; Tekin, S. and Dag, Y. (2021): The effects of selenium in acrylamide-induced nephrotoxicity in rats: roles of oxidative stress, inflammation, apoptosis, and DNA damage. Biol. Trace Elem. Res., 199, 173-184.

Shahrzad, E.; Shariati, M.; Naimi, S. and Edalatmanesh, MA. (2020): Protective effect of N-acetylcysteine on changes in serum levels of pituitary–gonadal axis hormones and testicular tissue in acrylamide-treated adult rats. Adv. Hum. Biol., 10, 16-21.

Shipa, A.M.E.; Kahilo, K.A.; Elshazly, S.A.; Taher, E.S.; Nasr, N.E.; Alotaibi, B.S.; Almadaly, E.A.; Assas, M.; Abdo, W.; Abouzed, T.K.; Salem, E.A.; Kirci, D.; El-Seedi, H.R.; Refaey, M.S.; Rizk,

N.I.; Shukry, M. and Dorghamm, D.A. (2024): Protective effect of Petroselinum crispum methanolic extract against acrylamide-induced reproductive toxicity in male rats through NF-κB, kinesin, steroidogenesis pathways. Reproductive Toxicology, 126, 108586.

Shipp, A.; Lawrence, G.; Gentry, R.; McDonald, T.; Bartow, H.; Bounds, J.; Macdonald, N.; Clewell, H.; Allen, B.; and Van Landingham, C. (2006): Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit. Rev. Toxicol., 36, 481-608.

Täckholm, V. (1974): "Students' Flora of Egypt", 2nd ed., Cairo Univ. Press, Cairo, Egypt, pp. 23.

Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, RH.; Gondé, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Müller, D.; Tallmadge, D.; O'Brien, J.; Thomp son, S.; Silvani, D. and Whitmore, T. (2004): A review of acrylamide: an industry perspective on research, analysis, formation, and control. Crit. Rev. Food Sci. Nutr., 44, 323-347.

Tarique, M.; Siddiqui, H.H.; Khushtar, M. and Rahman Md.A. (2016): Protective effect of hydro-alcoholic extract of Ruta gra veolens Linn. leaves on indomethacin and pylorus ligation-induced gastric ulcer in rats. Journal of Ayurveda and Integrative Medicine, 7, 38e43.

Tepe, Y. and Çebi, A. (2019): Acrylamide in environmental water: a review on sources, exposure and public health. Health, 11, 3-12.

Wang, H.; Huang, P.; Lie, T.; Li, J.; Hutz, R.J.; Li, K. and Shi, F. (2010): Reproductive toxicity of acrylamide-treated male rats. Reprod. Toxicol., 29, 225-230.

Yang, HJ.; Lee, SH.; Jin, Y.; Choi, JH.; Han, CH. and Lee, MH. (2005): Genotoxicity and toxicological effects of acrylamide on reproductive system in male rats. J. Vet. Sci., 6, 103-109.

Yildirim, S.; Sengul, E.; Aksu, E.H.; Cinar, I.; Gelen, V.; Tekin, S. and Dag, Y. (2024): Selenium reduces acrylamide-induced testicular toxicity in rats by regulating HSD17B1, StAR, and CYP17A1 expression, oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. Environ Toxicol, 39, 1402-1414.

Zhang, J.; Zhu, X.; Xu, W.; Hu, J.; Shen, Q.; Zhu, D.; Xu, X.; Wei, Z.; Zhou, P. and Cao, Y. (2023): Exposure to acrylamide inhibits testosterone production in mice testes and Leydig cells by activating ERK1/2 phosphorylation. Food and Chemical Toxicology, 172, 113576.

يحمي مستخلص نبات السدب من تلف الخصية الناجم عن الأكريلاميد في جرذان سبراج داولي

سهير على ، مروة فاروق ، مى أحمد ، خالد رداد

Email: khaledradad@aun.edu.eg Assiut University web-site: www.aun.edu.eg

بسبب الأعتماد المتزايد على العديد من الأطعمة المصنعة في عالمنا سريع الخطى، أصبح تعرض الأشخاص للأكريلاميد أمرا لا مفر منه مما يؤدي إلى ضرر مدى الحياة وبالأخص على الأعضاء التناسلية. وبناءً على ذلك، هدفت دراستنا إلى تبيان الآثار الوقائية المحتملة لمستخلص نبات السدب ضد التلف والأختلال الوظيفي الناجم عن الأكريلاميد في خصي جرذان سبراغ داولي . تم توزيع أثنان وثلاثون جرذا على ٤ مجموعات ؛ تلقت المجموعة الضابطة محلول ملحي (٠,٥ مل/جرذ) . وعولجت مجموعة الأكريلاميد ب ٣٠ ملغ من الأكريلاميد/كغ من وزن الجسم . وتناولت مجموعة الأكريلاميد ومستخلص نبات السدب كل من ٣٠ ملغ من الأكريلاميد و٢٠٠ ملغ من مستخلص نبات السدب معا لكل كغ من وزن الجسم . وتلقت مجموعة نبات السدب ٢٠٠ ملغ/كغ من وزن الجسم من المستخلص فقط. تم إعطاء كل من المحلول الملحي والأكريلاميد والمستخلص عن طريق الفم لمدة ٣ أسابيع. نتج عن الأكريلاميد انخفاض ملحوظ في تركيز الحيوانات المنوية وحركتها الأولية ونسبة الحيوانات المنوية الحية والطبيعية ولم يؤثر على مستويات هرمون التستوستيرون. علاوة على ذلك، أثر الأكريلاميد بشكل طفيف علي مستويات كل من GSH وMDA. من الناحية النسيجية، كان استنزاف الظهارة الأنبوبية المنوية وتكوين الخلايا العملاقة المنوية من العلامات المرضية الشائعة في معظم الجرذان المعالجة بالأكريلاميد. وكان هناك أيضًا تساقط للخلايا الظهارية الأنبوبية المنوية وركود للسائل المنوي في تجويف الأنابيب المنوية في بعض الجرذان. من ناحية أخرى، أدى العلاج المشترك للجرذان بالأكريلاميد ومستخلص نبات السدب إلى انخفاض كبير في التأثير الضار للأكريلاميد على معايير الحيوانات المنوية والبنية النسيجية لأنسجة الخصية مقارنةً بالمجموعة المعالجة بالأكريلاميد فقط . وفي الختام ، يحمي مستخلص نبات السدب من تلف الخصية الناجم عن الأكريلاميد ويحسن من وظائفها في الجرذان .