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Original article

Protective roles of vitamin C and 5-aminosalicylic acid on reproduction in acrylamide intoxicated male mice

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ABSTRACT

Context: Serious health risks have been connected to ongoing, escalating exposure to environmental toxins and one of them is acrylamide (ACR), an organic compound. Although there are many published reports on ACR toxicity, limited information is available regarding the use of two potential antioxidants against ACR-instigated reproductive toxicity.
Aims: The study focused on investigating the protective effects of vitamin C and 5-ASA against ACR-incited reproductive toxicity.
Methods: A total of 50 male mice aged 4 weeks old were treated for 90 days with different concentrations either of ACR or ACR and vitamin C or ACR and 5- ASA or ACR, vitamin C, and 5- ASA.
Key results: ACR significantly reduced serum testosterone level (p = 0.0037), sperm concentration (p = 0.0004), and percentage of sperm motility (p = 0.003), as well as increased sperm abnormality; head (p = 0.0058), tail (p = 0.001), and midpiece (p = 0.0339). Besides, the weight (p = 0.0006) and length (p = 0.0105) of testes, as well as weight (p = 0.0001) and length (p = 0.0021) of epididymis were decreased along with atrophy of seminiferous tubules of the testis, and disintegration of the tubular epithelium of epididymis on ACR exposed mice which were improved by vitamin C and 5-ASA administration.
Conclusions: Vitamin C and 5-ASA can potentially mitigate the negative effects of ACR on male reproduction; however, combined application is recommended for better performance.
Implications: In Bangladesh, this work is anticipated to address the health benefits of vitamin C and 5-ASA, particularly in improving the reproductive health of males against ACR toxicity.

1. Introduction

Exposure to acrylamide (ACR), which is generated in various foods due to high-temperature processing; has triggered a revived interest in animal and public health. ACR, a toxic substance is generated at high-temperature (above 120 °C) during cooking or processing, especially from carbohydrate-rich foods (Visvanathan R, 2014). It is primarily found in plant sources such as potatoes, cereals, grains, and even coffee; and in their manufactured products such as rolls, treats, snacks, bread, chips, and so other bakery products (Visvanathan R, 2014) (Zamani et al., 2018). Moreover, ACR is an industrial chemical utilized in the synthesis of water-soluble polymers (Tyl & Friedman, 2003) that are employed as rarefaction and emulsification assists in packaging, papermaking, mining, textile, cosmetic, plastics, and processing of water (Matoso et al., 2019) (Elbashir et al., 2014) (Rifai & Saleh, 2020). Intact skin, mucous membranes, lungs, and gastrointestinal tract are all possible routes of ACR exposure (Ibrahim et al., 2019). Due to its potentially toxic nature, ACR has negative impacts on skin, alimentary tract, heart and blood vessels, respiratory organs, brain and nerves,

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endocrine and reproductive organs, thus alters digestion, circulation, reproduction, neurological activity, and so on (Rajeh & Al-Dhaheri, 2017). ACR can easily pass through most cell membranes and be converted into a more active epoxide derivative, glycidamide by metabolism with CYP2E1 (Cytochrome P4502E1) oxidase that stimulates the toxic activity of ACR (Farag et al., 2021). Free radicals (ROS) are created during the metabolism process of ACR within the cell (Farag et al., 2021). ACR exerts its effect by generating oxidative stress bringing out reactive oxygen species (ROS) (Omidi et al. 2020). Additionally, the mechanism of ACR toxicity is related to the attraction for sulfhydryl groups on proteins that might render inactivate such proteins or enzymes required in repairing of DNA (Fiedan et al., 2015). ACR toxicity is recognized to down regulate testosterone levels (Farag et al., 2021) along with a reduction of sperm viability, motility, and membrane integrity (Kermani-Alghoraishi et al., 2010). Moreover, reproductive organs like testes undergoes necrosis of epithelial lining, vacuolation of the seminiferous tubules, and some degenerative changes in Leydig cells due to ACR exposure (Fiedan et al., 2015) (Zhang et al., 2009). In addition, ACR induces epithelial damage, cell degeneration, and connective tissue hyperplasia of epididymis (Yuxin Ma et al., 2011).

As ACR exerts its effect generating oxidative stress, substances having antioxidative efficiency may be used as efficient preventive agents against ACR toxicity (Rajeh & Khayyat, 2017). A renowned antioxidant vitamin C can scavenge superoxide and peroxyl radicals, hydrogen peroxide, hypochlorous acid, and oxidants (Farjana et al., 2020). It has protective action against inflammation, oxidative stress, autophagy chaos, and immune dysfunction (Farjana et al., 2020). By advancing reproductive hormones and structures, the antioxidant activity of vitamin C upholds reproductive soundness (Sadeghzadeh et al., 2019). Moreover, a non-steroid anti-inflammatory medicine (NSAID) called 5-ASA (an amino derivative of salicylic acid) has the potential to lessen the toxicity of ACR (Rajeh & Al-Dhaheri, 2017) (Yan et al., 2019). The antioxidant 5-ASA serves to remove free radicals that can damage metabolic by-products (Yan et al., 2019). Consumption of acetylsalicylic acid in drinking water may have the potentiality to improve reproductive parameters (Hassan et al., 2003). There is very little data regarding vitamin C plus 5-ASA against ACR toxicity, even though numerous investigations have already established that ACR toxicity causes reproductive damage. Whereas, vitamin C can protect germinal cells from oxidative stress throughout spermatogenesis (Angulo et al., 2011), and 5 ASA have the potential to protect reproductive organs from oxidative damage (Rajeh & Khayyat, 2017). Consequently, the purpose of the current research is to sum up the protective actions of vitamin C and 5-ASA on testosterone level, sperm physiology (sperm concentration, motility percentage, and morphology), gross (weight and length) as well as microscopic (histopathological) changes of testes and epididymis in ACR treated male mice.

2. Materials and methods

2.1. Drugs

ACR, vitamin C, and 5-ASA all were procured from Sigma-Aldrich Company, Spruce Street, St. Louis, USA (Lot # BCCD 0221).

2.2. Ethic al statement

All the techniques used in the current experiment with animals were accredited by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University (AWEEC/BAU/2022 (64)).

2.3. Animals and treatments

For this study, a total of fifty (50) Swiss Albino male mice (aged 4 weeks) were acquired from International Center for Diarrheal Disease Research, Bangladesh (icddr,b). They were assigned randomly into 5 groups (n = 10); Group A was considered as untreated vehicle control, whereas mice of group B were treated with ACR @45 mg/kgbwt daily (P.O.), mice of group C were treated with ACR @45 mg/kgbwt daily and vitamin C @200 mg/kgbwt daily (P. O.), mice of group D were treated with ACR @45 mg/kgbwt daily and 5-ASA @25 mg/kgbwt daily (P.O.), and mice of group E were treated with ACR @45 mg/kgbwt daily, vitamin C @200 mg/kgbwt daily and 5-ASA @25 mg/kg bwt/day (P.O.). The treatment was carried out for 90 days. The dose of ACR (45 mg/kg bw/day P.O.) through drinking water which was selected as effective dose for inducing ACR toxicity after a comprehensive literature review (Zhao et al., 2022) (Rajeh, 2020) (DaSari et al., 2018) (Rajeh & Khayyat, 2017) (Fang et al., 2014). The dose of vitamin C @200 mg/kgbwt daily (P.O.) (Alorabi et al., 2022), and 5-ASA @25 mg/kgbwt daily (P.O.) (Rajeh & Khayyat, 2017) through drinking water was selected following previous literature where it is established that they can counter oxidative stress in these concentrations.

2.4. Collection and processing of sample

At the end of experiment, mice were sacrificed to collect the samples (Day 90). Under the anesthesia of diethyl ether (Research-Lab Fine Chem Industries, India), approximately 1500 μL of blood were directly drawn from mice's heart using a sterile syringe. For the preparation of serum, one-half of the blood was kept in a sterile Eppendorf tube containing the anticoagulant tri-sodium citrate (Research-Lab Fine Chem Industries, India). The other half of the blood was taken in another Eppendorf tube without anticoagulant and undergone centrifugation at 1,500 rpm for 30 min to collect serum (Akter et al., 2021). For biochemical analyses, serum was preserved at −20 °C. Testes as well as epididymis were removed following a full blood draw and perfused with phosphate-buffered saline to evaluate the sperm and investigate the gross architecture. The isolated organs were then preserved in fixative (10% neutral buffered formalin) for 24 h to study histological changes (Akter et al., 2021).

2.5. Hormonal analysis

125I-testosterone RIA test kit (Cat#RK-61CT) (Berthold, Bad Wildbad, Germany) indirect quantitative radioimmunoassay was used to measure the level of serum testosterone hormone (Rima et al., 2018). 6 standards bottle (S0-S5) had T concentrations of 0, 0.1, 0.5, 2, 8, and 20 ng/ml, respectively. The rivalry between the 125I-labeled hormone and hormone in the sample or the standard for sites on hormone-specific antibodies determined the testing methods. The fraction of hormone that was 125I-labeled and binds to the antibody is inversely proportional to the hormone concentration in the unidentified sample. Then the precipitating solutions were added after incubation for a predetermined amount of time to separate the bound from the free. By operating a gamma counter (PC-RIA-MAS, Stratec Biomedical Systems, Germany), the radioactivity was counted (60 s per tube) (Talukder et al., 2014). The final step was to calculate the results. The minimum detectable range was 0.70 ± 0.21 ng/ml. The intra and inter-assay coefficients of variation were < 10% and < 15% respectively. Whereas, the sensitivity of testosterone assay was 75%, and the specificity was 95% (Talukder et al., 2014).

2.6. Preparation and observation of sperm

Epididymal sperm collection from cauda epididymis and the number of sperms were counted according to standard protocol (Christina & Daniel, 2017). Briefly, the cauda epididymis was first gathered in a petridish and finely minced with scissors. The torn epididymis was then put into a test tube with 5 mL physiological saline which was then incubated at room temperature for 2 min. After 2 min, the supernatant was collected and diluted (1:100) with 5 g sodium bicarbonate along with 1 mL of 35% formalin solution. About10 μL suspension of sperm was shifted to Neubauer's counting chamber (hemocytometer). The sperms were endorsed to disperse for 5 min and then counted under a microscope at higher magnification (40X objectives), The number of sperms was counted in five squares (16-celled). Total number of sperm was multiplied by 5 and reported as [X] $\times$ 10^6 /mL, where [X] denotes the number of sperm in a 16-celled square (Saalu et al., 2010). The motility of epididymal sperm was observed and reported as per standard protocol (Saalu et al., 2010). By placing a drop of diluted sperm sample on a prewarmed slide and covering it with a coverslip, the motility percentage of sperm was assessed. To determine the percentage of motile sperms, at least ten widely dispersed fields were viewed at 10X objectives (Sujan et al., 2021). The sperms were categorized into two categories of motility: Motile and non-motile sperms. The sperms exhibiting unidirectional or multidirectional movement, those slightly motile without any progression were considered as motile sperm. Sperms that displayed no movement at all were judged as non-motile (Kalaivani et al., 2018). Then the motility percentage (%) of sperm was reported. As per standard protocol, William's stain was used to assess sperm morphology (Mansur et al., 2018). First, a marked test tube received 1 mL of sperm solution. Absolute alcohol and chloramine were made and used to treat a thin semen stain. Finally, the smear was stained with carbolfuschsin for 8 min. At higher magnification (100X objectives), approximately hundred sperms/mice were observed from each group of mice. Morphology (size, shape and structure) of the head, midpiece, and tail of sperm was studied and number of abnormalities found in those structures were counted (Lüke et al., 2014).

2.7. Measurement of testes and epididymis

After collection, the weight and length of both testes and epididymis were measured by using a digital weight balance and slide calipers respectively; and recorded accordingly. Both the right and left parts of the organs were measured and the average value of an animal was calculated (Sadeghzadeh et al., 2019).

2.8. Detection of histological changes

The testes and epididymis from each group of mice were collected after complete removal of blood by perfusion with phosphate-buffered saline and kept in 10% neutral buffered formalin for 24 h for. The well-fixed tissues were processed, sectioned, and stained as per standard procedure (Banchroft et al., 1996). The epididymal body sections were used to study the histology of epididymis and similarly, same sections were used to study the histology of the testes. The tissues were trimmed and washed overnight in running tape water to remove formalin. The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per the following schedules. Then, the tissues were dehydrated in 50%, 70%, 80%, 90%, 100%, 100% and 100% ethanol, one hour in each. The tissues were cleaned in chloroform for 3 h to remove ethanol (1 and half hour in each, two changes). Impregnation was done in melted paraffin (56–60 °C) for 3 h. Then the tissues were sectioned with a microtome and small amount of gelatin was added to the water bath for better adhesion of the section of the slide. The sections were allowed to spread in a warm water bath at 40–42 °C. The sections were taken on grease-free clear slides. Finally, the slides containing the section were air-dried and kept in a cool place. The stained slides were studied under the OLYMPUS CX41 microscope at both 10X and 40X objectives.

2.9. Analysis of statistical data

All the experimental data were analyzed statistically by using GraphPad Prism (9.3.1). Data were expressed as mean ± SD; whereas, differences between two groups of mice were compared by using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test; setting statistical significance at $p < 0.05$.

3. Results

3.1. Serum testosterone level

To know the effects of ACR, vitamin C and 5-ASA on testosterone concentration in different treated groups of male mice were analyzed at the end of experiments. ACR-treated mice revealed a significant ($p = 0.0037$) decrease of serum testosterone level compared to the untreated mice of the control group (Fig. 1). Similarly, ACR and vitamin C treated mice also showed a significant ($p = 0.0311$) decrease of serum testosterone levels compared to the untreated mice of the control group (Fig. 1). However, vitamin C and 5-ASA combined treated mice revealed a significant ($p = 0.0231$) restoration of the testosterone level compared to ACR treated mice (Fig. 1).

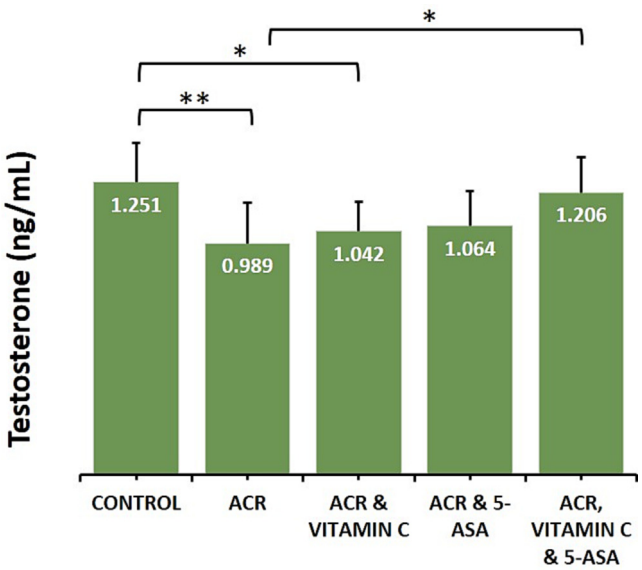

Fig. 1. Effect of vitamin C and 5-ASA on testosterone concentration against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR, or ACR and vitamin C or ACR and 5-ASA or ACR, vitamin C and 5-ASA. Control means the mice were kept as untreated with any drugs. Statistical analysis was performed using Graph Pad Prism 9.3.1. All data were expressed as mean ± SD and differences between two groups of animals were compared using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test. Here, ** indicates significant decrease ($p = 0.0037$) in ACR treated mice compared to untreated control; * indicates significant decrease ($p = 0.0311$) in ACR plus vitamin C treated mice compared to untreated control and significant increase ($p = 0.0231$) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice.

3.2. Sperm physiological parameters

To assess the effects of ACR, vitamin C and 5-ASA on sperm physiology (sperm concentration, motility percentage, and sperm morphology) in different treated groups of male mice were analyzed at the end of experiments. This study found a significant (p = 0.0004) reduction in sperm concentration in the case of ACR treated mice compared to the untreated of the control mice (Fig. 2). On the other hand, vitamin C and 5-ASA combined treated mice revealed a significant increase (p = 0.0047) in sperm concentration compared to the ACR-treated mice (Fig. 2). Exposure to ACR induced a significant (p = 0.003) decline in sperm motility percentage compared to the untreated control mice (Fig. 2). Whereas, mice combinedly treated with vitamin C and 5-ASA revealed a significant increase (p = 0.0146) in sperm motility percentage compared to the ACR-treated mice (Fig. 2). Sperm abnormality percentages increased significantly; head (p = 0.0058), tail (p = 0.001), and midpiece (p = 0.0339) in the ACR-treated mice; similarly, in ACR and vitamin C treated mice (p = 0.0155) in the case of tail abnormality compared to the untreated control mice (Fig. 3) (Table 1). On the other contrary, abnormality percentages were significantly declined; head (p = 0.0053), tail (p = 0.0407) in ACR, vitamin C and 5-ASA combined treated mice compared to the ACR-treated mice (Fig. 3) (Table 1). In the morphological structures, the sperm of untreated control mice showed a normal head and tail morphology with fewer abnormalities (Fig. 4). However, mice treated with ACR alone showed increased numbers of sperms with amorphous heads, bent necks, folded and coiled tails (Fig. 4). On the other hand, in mice treated with vitamin C and 5-ASA along with ACR, fewer differences in sperm like bent neck and folded tails were observed compared to the untreated control mice (Fig. 4).

3.3. Gross morphology of the testes and epididymis

To know the effects of ACR, vitamin C and 5-ASA on weight and length of the testes and epididymis in different treated groups of male mice were analyzed at the end of experiments. ACR treatment significantly decreased the weight (p = 0.0006) and length (p = 0.0105) of testes compared to the untreated control mice (Fig. 5). ACR and vitamin C treated mice in the case of testes weight also showed a significant (p = 0.0322) decrease compared to the untreated control mice (Fig. 5). Similarly, in the case of ACR and 5-ASA treated mice, testes weight and length were decreased significantly (p = 0.0103 & p = 0.0497 respectively) compared to the untreated control mice (Fig. 5). Whereas, vitamin C and 5-ASA combined treated mice showed a significant rise in the weight (p = 0.0075) and length (p = 0.0344) of testes compared to the ACR-treated mice (Fig. 5). Exposure to ACR significantly decreased the weight (p = 0.0001) and length (p = 0.0021) of epididymis compared to the untreated control mice (Fig. 6). ACR and vitamin C as well as ACR and 5-ASA treated mice also showed a significant (p = 0.0388 & p = 0.0269 respectively) decrease in the weight of epididymis compared to the untreated control mice (Fig. 6). Whereas, vitamin C and 5-ASA combined treated mice showed a significant rise in the weight (p = 0.0036) and length (p = 0.0122) of epididymis compared to the ACR-treated mice (Fig. 6).

3.4. Histopathological changes of the testes and epididymis

To know the effects of ACR, vitamin C and 5-ASA on histology of the testes and epididymis in different treated groups of male mice were analyzed at the end of experiments. Testes of untreated control mice showed healthy tissue structures with regularly organized seminiferous tubules representing regular cell structures such as spermatogenic cells as well as Sertoli, and Leydig cells (Fig. 7). But in the case of ACR-treated mice, there was atrophy of seminiferous tubules and increased inter-tubular spaces associated with loss of spermatozoa and spermatogenic cells, probably due to the arrest of spermatogenesis (Fig. 7). In the case of the ACR and vitamin C treated group, as well as ACR and 5-ASA treated groups, lack of sperm was observed in a few numbers of seminiferous tubules compared to ACR treated group (Fig. 7). However, vitamin C and 5-ASA combined treated mice showed no noticeable changes in the seminiferous tubules and inter-tubular spaces (Fig. 7). The epididymis of the untreated control mice showed normal tubules containing sperm with the normal lining of columnar epithelial cells and normal inter-tubular spaces (Fig. 8). However, in ACR-treated mice, there was a disarrangement of lining epithelial structure and the epididymal tubules lacked sperm and spermatogenic cells (Fig. 8). On the other hand, in ACR and vitamin C, as well as

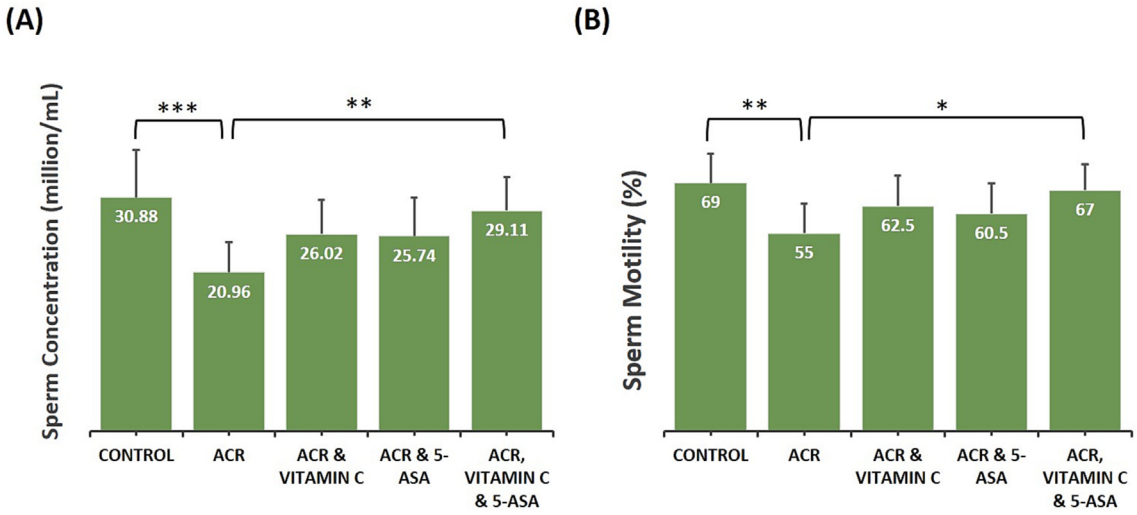

Fig. 2. Effect of vitamin C and 5-ASA on A) Sperm concentration, and B) Sperm motility percentage against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR, or ACR and vitamin C or ACR and 5-ASA or ACR, vitamin C and 5-ASA. Control means the mice were kept as untreated with any drugs. Statistical analysis was performed using Graph Pad Prism 9.3.1. All data were expressed as mean ± SD and differences between two groups of animals were compared using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test. Here, in case of sperm concentration, *** indicates significant decrease (p = 0.0004) in ACR treated mice compared to untreated control; ** indicates significant increase (p = 0.0047) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice. In case of sperm motility, ** indicates significant decrease (p = 0.003) in ACR treated mice compared to untreated control; * indicates significant increase (p = 0.0146) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice.

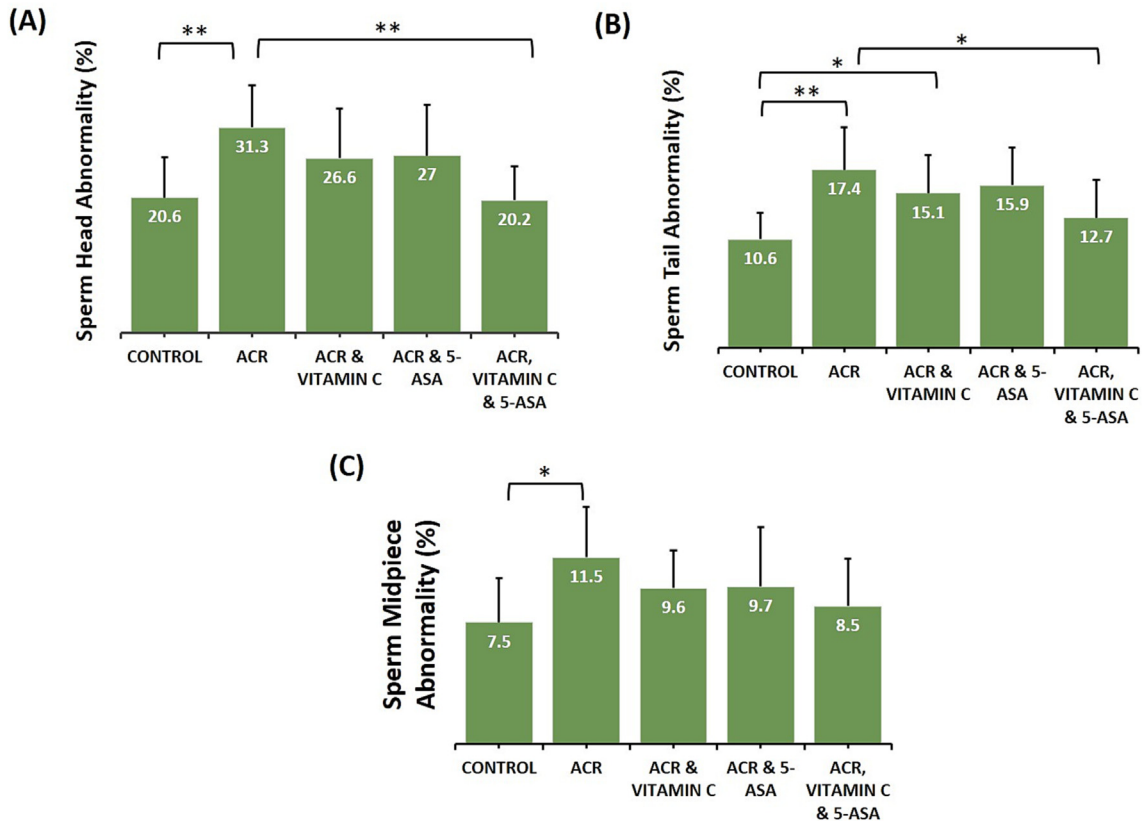

Fig. 3. Effect of vitamin C and 5-ASA on sperm abnormality percentages; A) Head abnormality, B) Tail abnormality and C) Midpiece abnormality against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR, or ACR and vitamin C or ACR and 5-ASA or ACR, vitamin C and 5-ASA. Control means the mice were kept as untreated with any drugs. Statistical analysis was performed using Graph Pad Prism 9.3.1. All data were expressed as mean ± SD and differences between two groups of animals were compared using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test. Here, in case of sperm head abnormality, ** indicates significant increase (p = 0.0058) in ACR treated mice compared to untreated control; whereas significant decrease (p = 0.0053) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice. In case of sperm tail abnormality, ** indicates significant increase (p = 0.001) in ACR treated mice compared to untreated control; * indicates significant increase (p = 0.0155) in ACR plus vitamin C treated mice compared to untreated control and significant decrease (p = 0.0407) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice. In case of sperm midpiece abnormality, * indicates significant increase (p = 0.0339) in ACR treated mice compared to untreated control.

Table 1
Effect of vitamin C and 5-ASA on sperm abnormality percentages; A) Head abnormality, B) Tail abnormality and C) Midpiece abnormality against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR, or ACR and vitamin C or ACR and 5-ASA or ACR, vitamin C and 5-ASA. Control means the mice were kept as untreated with any drugs. Statistical analysis was performed using Graph Pad Prism 9.3.1. All data were expressed as mean ± SD and differences between two groups of animals were compared using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test.

Sperm Abnormality (%)				
Groups	**Treatment**	**Head Abnormality (%) (Mean ± SD)**	**Mid-piece Abnormality (%) (Mean ± SD)**	**Tail Abnormality (%) (Mean ± SD)**
A	Control	20.6 ± 6.09	7.5 ± 2.71	10.6 ± 2.59
B	ACR	31.3 ± 6.44**	11.5 ± 3.14*	17.4 ± 4.11**
C	ACR + Vitamin-C	26.6 ± 7.57	9.6 ± 2.31	15.1 ± 3.72*
D	ACR + 5-ASA	27 ± 7.77	9.7 ± 3.65	15.9 ± 0.3.63
E	ACR + Vitamin-C + 5-ASA	20.2 ± 5.11##	8.5 ± 2.91	12.7 ± 3.71#
		**=Control vs Group B (p < 0.01) ##=Group B vs Group E (p < 0.01)	*=Control vs Group B (p < 0.05)	**=Control vs Group B (p < 0.05) *=Control vs Group C (p < 0.05) #=Group B vs Group E (p < 0.05)

ACR and 5-ASA treated mice, there were some minor changes like lack of sperms within some tubules (Fig. 8). Whereas, vitamin C and 5-ASA combined treated mice showed almost similar structures compared to untreated control (Fig. 8).

4. Discussion

Acrylamide toxicity may create oxidative stress accelerating the production of reactive oxygen species (ROS) (Farag et al., 2021). Oxidative stress strives a vital role in its toxicity to affect the body's biological mechanisms and physiological activities. At physiological concentrations, ROS function as molecular mediators of signal transduction pathways that control the spermatogenesis, steroidogenesis, and hypothalamic-pituitary–gonadal axis (Baskaran et al., 2021). Antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation (Obstr & Dis, 2018). However, there are many reports about ACR intoxication, but the information is limited regarding the combined administration of two potential antioxidants like vitamin C and 5-ASA against

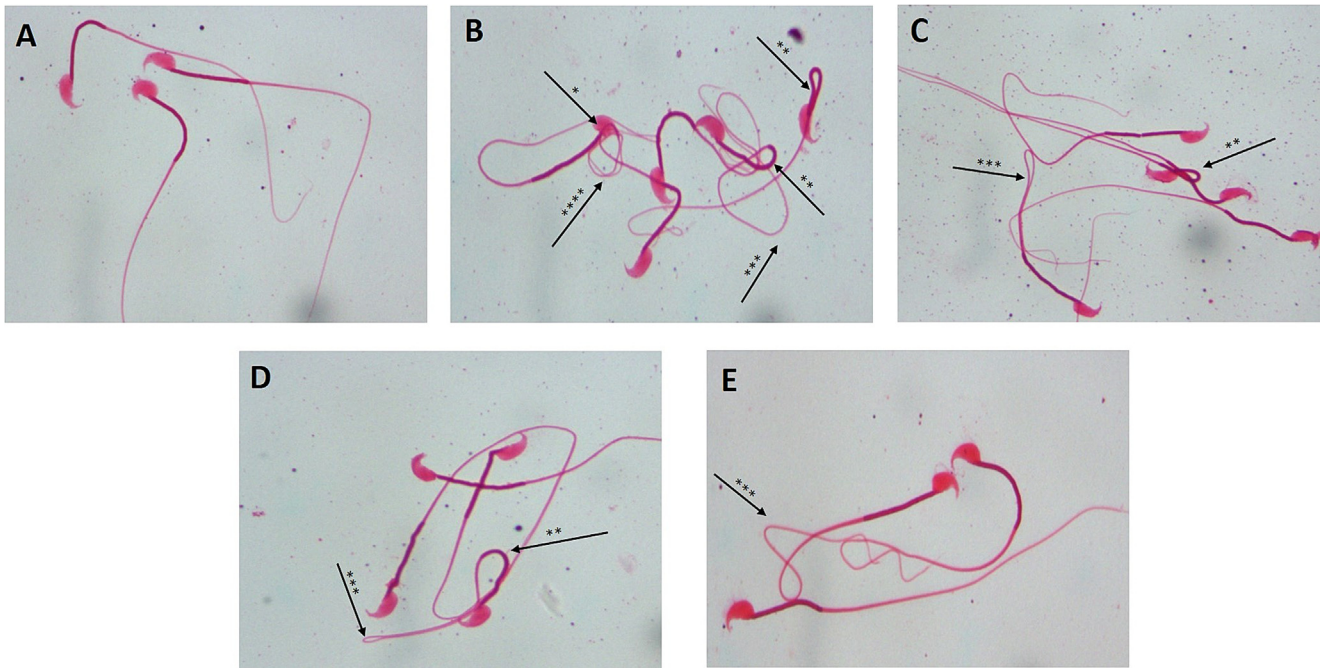

Fig. 4. Effect of vitamin C and 5-ASA on sperm morphology against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR (Group B), or ACR and vitamin C (Group C) or ACR and 5-ASA (Group D) or ACR, vitamin C and 5-ASA (Group E). Control (Group A) means the mice were kept as untreated with any drugs. Photomicrograph of sperm morphology of different groups of treated mice at the end of the treatment (William's stained, observed at 100X). Here, an arrow with * indicates "Amorphous head," an arrow with ** indicates "Bent neck," an arrow with *** indicates "Folded tail," an arrow with **** indicates "Coiled tail.".

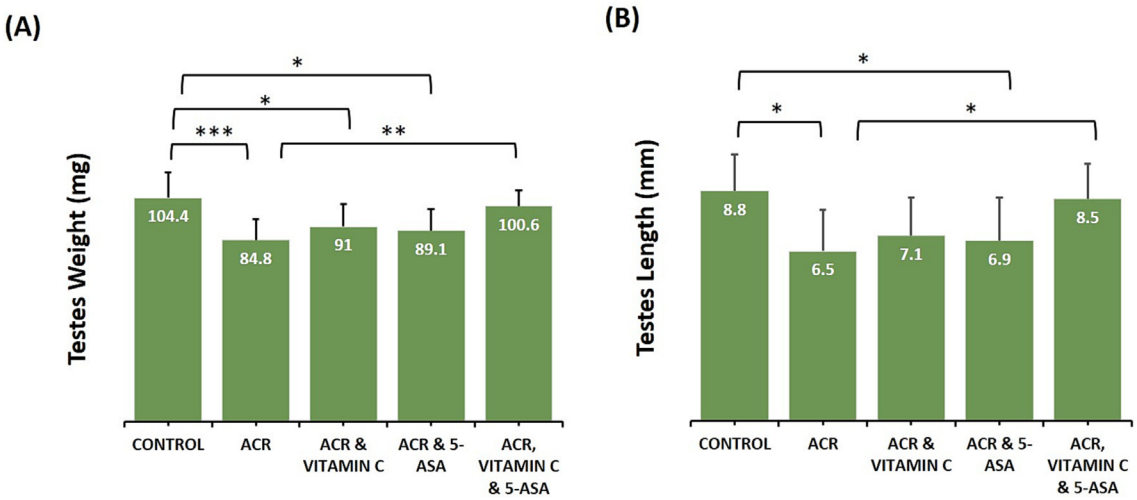

Fig. 5. Effect of vitamin C and 5-ASA on A) Weight and B) Length of the testes against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR, or ACR and vitamin C or ACR and 5-ASA or ACR, vitamin C and 5-ASA. Control means the mice were kept as untreated with any drugs. Statistical analysis was performed using Graph Pad Prism 9.3.1. All data were expressed as mean ± SD and differences between two groups of animals were compared using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test. Here, in case of testes weight, *** indicates significant decrease (p = 0.0006) in ACR treated mice compared to untreated control; * indicates significant decrease in ACR plus vitamin C (p = 0.0322) as well as ACR plus 5-ASA (p = 0.0103) treated mice compared to untreated control; ** indicates significant increase (p = 0.0075) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice. In case of testes length, * indicates significant decrease in ACR (p = 0.0105) as well as ACR plus 5-ASA (p = 0.0497) treated mice compared to untreated control; and significant increase (p = 0.0344) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice.

ACR induced reproductive toxicity. In our current study, the suppression of circulating testosterone, an important reproductive hormone level following exposure to ACR may be due to its activating role on the hypothalamic-pituitary–gonadal axis indicating an increase in luteinizing hormone (LH) (Camacho et al., 2012). Moreover, by increasing ROS concentration, ACR can induce damage to Leydig cells assisted by LH, which play a role in decreasing testosterone hormone concentration (Hamdy et al., 2012). However, the restoration of testosterone levels with the combined administration of vitamin C and 5-ASA is in accordance with a previous study which observed a significant recovery in the testosterone hormone level in antioxidant treated mice (Dirican & Kalender, 2012).

Alterations in sperm concentration, motility, and abnormalities in sperm morphology are useful indicators of an individual's fertility prospective (Dcunha et al., 2022). The reduction in sperm concentration in our study is in tune with previous studies according

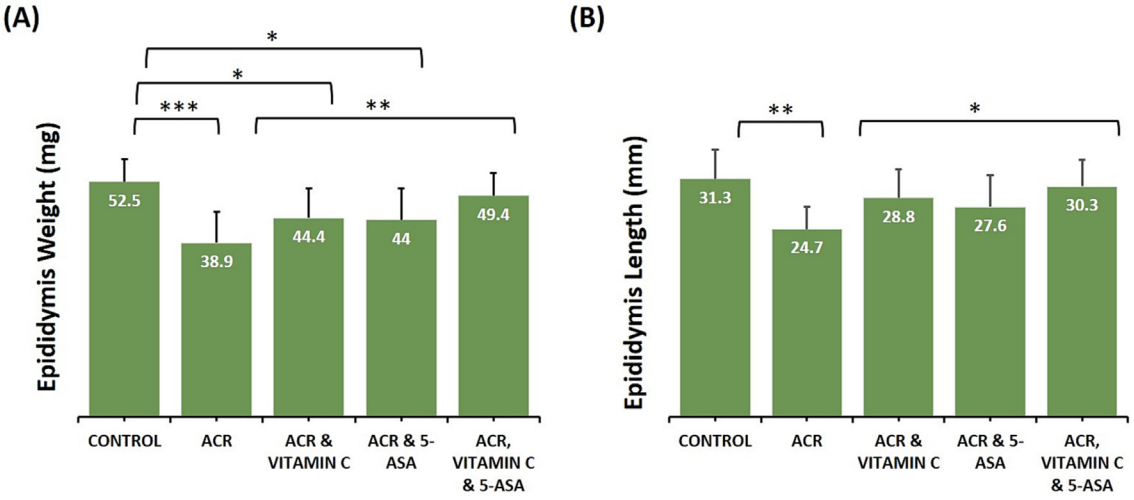

Fig. 6. Effect of vitamin C and 5-ASA on A) Weight and B) Length of the epididymis against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR, or ACR and vitamin C or ACR and 5-ASA or ACR, vitamin C and 5-ASA. Control means the mice were kept as untreated with any drugs. Statistical analysis was performed using Graph Pad Prism 9.3.1. All data were expressed as mean ± SD and differences between two groups of animals were compared using one-way repeated measure ANOVA followed by Tukey's multiple comparisons test. Here, in case of epididymis weight, *** indicates significant decrease (p = 0.0001) in ACR treated mice compared to untreated control; * indicates significant decrease in ACR plus vitamin C (p = 0.0388) as well as ACR plus 5-ASA (p = 0.0269) treated mice compared to untreated control; ** indicates significant increase (p = 0.0036) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice. In case of testes length, ** indicates significant decrease (p = 0.0021) in ACR treated mice compared to untreated control; and * indicates significant increase (p = 0.0122) in ACR, vitamin C and 5-ASA treated mice compared to ACR treated mice.

to whom it may be linked with the reduction of testosterone concentration which indicates Leydig cell damage leading to suppression of the rate of spermatogenesis (Pourentezari et al., 2014). On the other hand, vitamin C was reported to have positive effect on increasing sperm concentration (Rahayu et al., 2019). Moreover, 5-ASA possess the ability to improve all fertility parameters of a male including sperm concentration (Park & Kim, 2020). These were reflected in the sperm analysis from the cauda epididymis by the supplement of vitamin C and 5-ASA. In the case of sperm motility percentage, drop-off value due to ACR exposure supports those of prior researches who found that ACR reduces sperm cell progressive motility and mean percentages of sperm concentration (Kermani-Alghoraishi et al., 2010). One important reason for the declination of sperm motility incited by ACR could be that it may interfere with sperm energy metabolism (Yuxin Ma et al., 2011). ACR binding to motor proteins (e.g. kinesin and dynein) may be considered as another reason which affects sperm motility (Tyl & Friedman, 2003). On the contrary, a prior study conducted observed that vitamin C significantly improved sperm motility in rats (Vijayprasad et al., 2014), and Park & Kim delineated that 5-ASA exerts the ability to improve all male fertility parameters including sperm motility percentages (Park & Kim, 2020). Moreover, a similar report, strengthening the current finding with combined action of vitamin C and 5-ASA was observed in a study a highly significant elevation in sperm progressive motility after treating with an antioxidant and an anti-inflammatory substance (Mehni et al., 2014). ACR has the potentiality to become toxic to sperm, particularly the sperm head, and to disrupt the spermatogenesis and sperm maturation process (Tyl & Friedman, 2003). It is established that the head of the sperm accumulates genetic material (Kermani-Alghoraishi et al., 2010). As a result, trauma to the head might directly affect sperm DNA and result in genetic abnormality (Yuxin Ma et al., 2011). However, administration of vitamin C has a significant influence on elevating the sperm quality of rats (Rahayu et al., 2019) and 5-ASA exerts the ability to decrease abnormal sperm morphology (Park & Kim, 2020). Moreover, the findings of a previous report about the combined effort of two antioxidants to reduce the morphological abnormalities of sperm can be relatable to the current study (Rajeh & Khayyat, 2017).

Like body weight gross changes of testes and epididymis may be related to stress conditions (Kalaivani et al., 2018). Moreover, the changes are in accordance with a prior research that also reported that these measurements of testes and epididymis can be reduced due to ACR intoxication (ALKarim et al., 2015) (Kalaivani et al., 2018). The reduction in the measurements of reproductive organs may be linked with the impairment of spermatogenesis (Kalaivani et al., 2018). On the contrary, the combined action of two antioxidants was found to be effective in the restoration of weight and length of testes and epididymis supporting the ameliorating effects of vitamin C and 5-ASA in our research study (Hasan et al., 2021) (Ommurugan et al., 2018).

The histological alterations of the testes of only ACR exposed mice in the current study might be the result of testicular degeneration, which manifests as a reduction in spermatogenic cells and a notably increased number of multinucleated spermatid giant cells (Farag et al., 2021). After treating rats with ACR, similar kinds of lesions was also found such as the absence of spermatid and other stages of spermatozoa in seminiferous tubules with edema of intracellular tissue and a decrease of Leydig cells (ALKarim et al., 2015). A study revealed that ACR-treated rats exposed to 5-ASA protected from ACR-mediated toxicity, with nearly normal histological appearance of testes and normal spermatogenesis were observed, with no tubular atrophy (Dirican & Kalender, 2012). Additionally, combining vitamin C and vitamin E therapy rather than utilizing each vitamin separately provides a significantly protective impact against hazardous chemical-induced changes in testicular histology (El Kotb et al., 2020). In the case of epididymis, the histological findings of the epididymis are consistent with other researchers who stated that the epididymal ducts are affected by ACR, which causes significant vacuolation and degradation of the lining epithelium that contains few sperm (Farag et al., 2021). ACR on the epididymis results in fewer columnar cells with cytoplasmic vacuoles and a reduction in luminal capacitance (Kalaivani et al., 2018). Additionally, another investigation noted epithelial damage in the epididymis of mice given ACR, including disruption of the epithelial structure, cellular degeneration, and a marked decrease in sperm production (Yuxin Ma et al., 2011). Whereas, our findings regarding the protective effect of vitamin

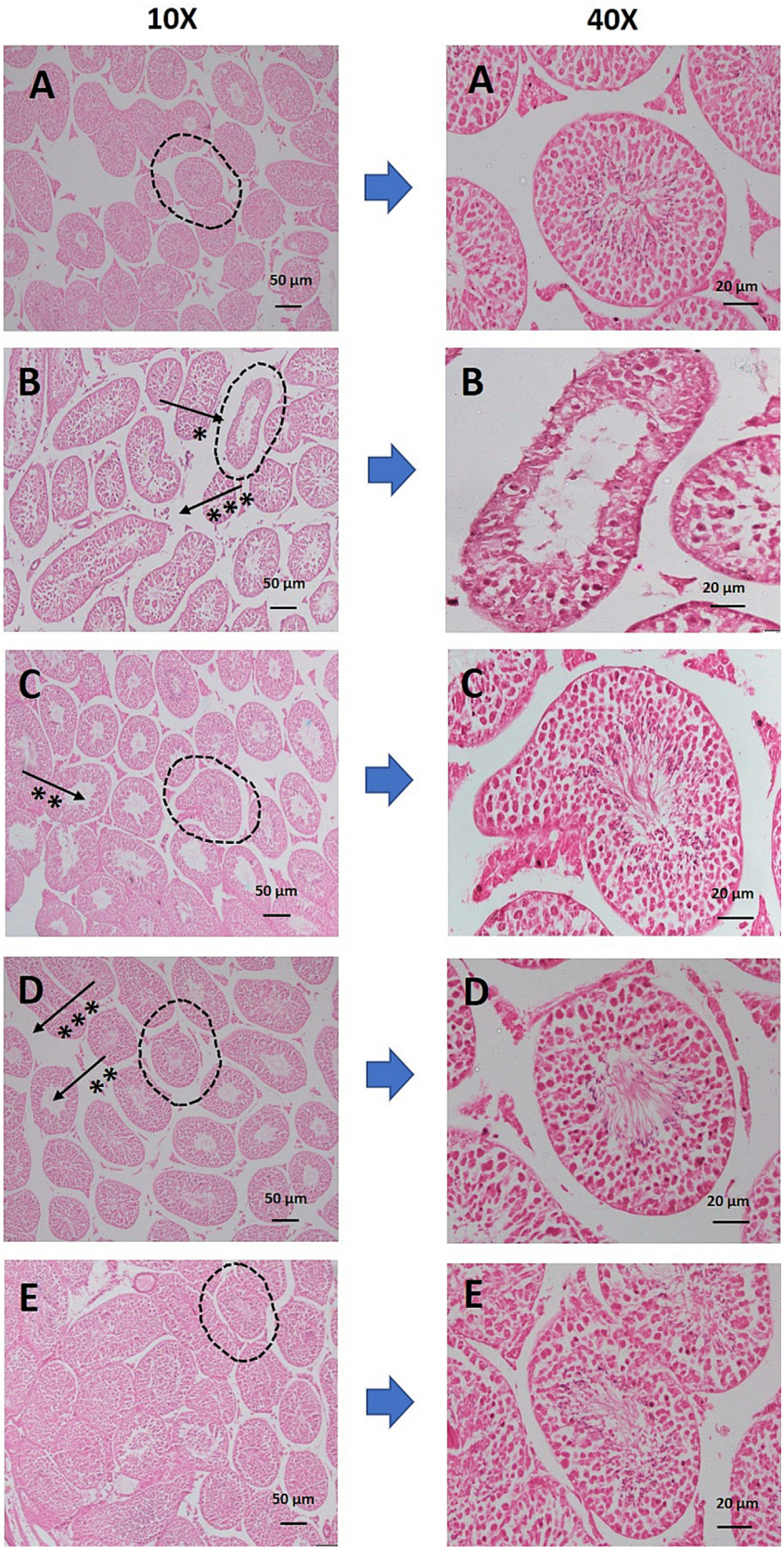

Fig. 7. Effect of vitamin C and 5-ASA on histostructure of testes against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR (Group B), or ACR and vitamin C (Group C) or ACR and 5-ASA (Group D) or ACR, vitamin C and 5-ASA (Group E). Control (Group A) means the mice were kept as untreated with any drugs. The slides were stained with Hematoxylin and Eosin, and the structures were studied at both 10X (Left column) and 40X (Right column) objectives. Here, arrow with * indicates "tubular atrophy due to arrest of spermatogenesis; arrow with ** indicates lack of sperm within the seminiferous tubules; arrow with *** indicates dilated inter-tubular spaces.".

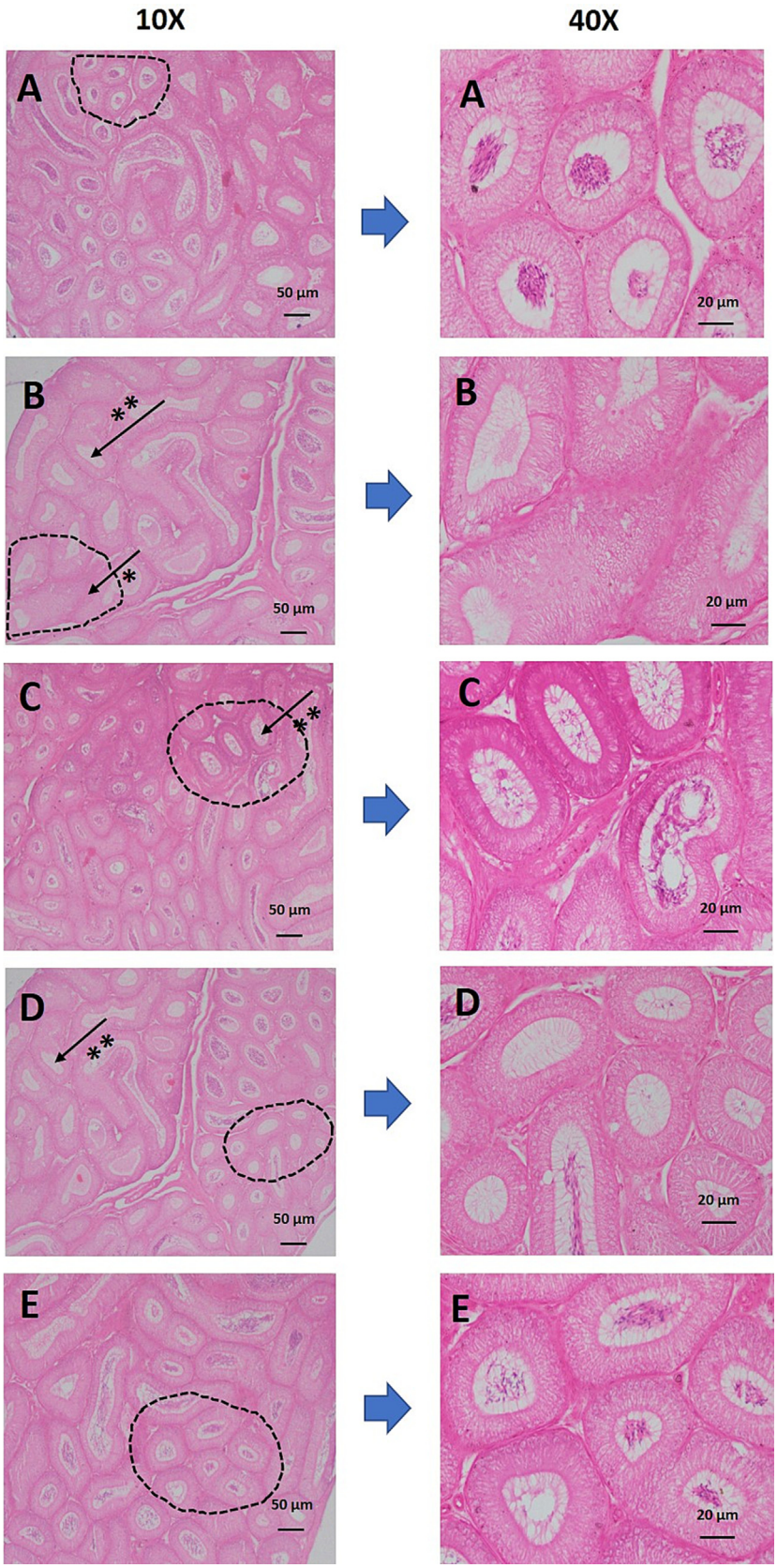

Fig. 8. Effect of vitamin C and 5-ASA on histostructure of epididymis against ACR-toxicity in mice. Mice of different groups (n = 10) were treated with either of ACR (Group B), or ACR and vitamin C (Group C) or ACR and 5-ASA (Group D) or ACR, vitamin C and 5-ASA (Group E). Control (Group A) means the mice were kept as untreated with any drugs. The slides were stained with Hematoxylin and Eosin, and the structures were studied at both 10X (Left column) and 40X (Right column) objectives. Here, arrow with * indicates "tubular atrophy with disarrangement of lining epithelial structure; arrow with ** indicates lack of sperm within the tubules.".

C and 5-ASA can be compared with the findings that reported a protective effect of two antioxidants on the histology of epididymis (Aghaie et al., 2016). The results revealed that vitamin C and 5-ASA may exert their antioxidative property to prevent ACR-induced reproductive damage and their combined application is highly recommended.

5. Conclusion

ACR exposure can reduce a male mouse's capacity to reproduce, which may be explained by a decrease in testosterone. It has an impact on the germ cells, which impairs spermatogenesis process and decreases sperm concentration, sperm motility percentages, and increases the proportion of defective sperm. While, combined treatment with vitamin C and 5-ASA may play a worthwhile role in the restoration of these changes resulting in the protection of male mouse fertility from ACR toxicity.

Authors contribution statement

MEJB performed the research work, analyzed the research data, and wrote the first draft of the manuscript, AS helped in staining of sperm, MGH, and MKI critically revised the draft and helped in the analysis of data, FYB assisted in studying sperm morphology, MAHNAK assisted in research planning and histopathological study, and SA designed layout and performed the research work, finalized the manuscript and supervised the entire experiment.

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.

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References

Aghaie, S., Nikzad, H., Mahabadi, J.A., Taghizadeh, M., Azami-Tameh, A., Taherian, A., Sajjadian, S.M.S., Kamani, M., 2016. Protective effect of combined pumpkin seed and ginger extracts on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with cyclophosphamide. Anat. Sci. Int. 91 (4), 382–390. https://doi.org/10.1007/s12565-015-0314-x.

Akter, M. T., Bhuiyan, M. E. J., Sujan, K. M., Haque, Z., & Akter, S. (2021). *Investigation of growth performance , lipid profile , and liver histotexure of mice treated with butyric acid. September*. https://doi.org/10.5455/jabet.2021.d140.

ALKarim, S., ElAssouli, S., Ali, S., Ayuob, N., & ElAssouli, Z. (2015). Effects of low dose acrylamide on the rat reproductive organs structure, fertility and gene integrity. *Asian Pacific Journal of Reproduction*, *4*(3), 179–187. https://doi.org/10.1016/j.apjr.2015.05.001

Alorabi, M., Mohammed, D.S., Mostafa-Hedeab, G., El-Sherbeni, S.A., Negm, W.A., Mohammed, A.I.A., Al-Kuraishy, H.M., Nasreldin, N., Alotaibi, S.S., Lawal, B., Batiha, G.E.S., Conte-Junior, C.A., 2022. Combination treatment of omega-3 fatty acids and vitamin C exhibited promising therapeutic effect against oxidative impairment of the liver in methotrexate-intoxicated mice. Biomed. Res. Int. 2022. https://doi.org/10.1155/2022/4122166.

Angulo, C., Maldonado, R., Pulgar, E., Mancilla, H., Córdova, A., Villarroel, F., Castro, M.A., Concha, I.I., 2011. Vitamin C and oxidative stress in the seminiferous epithelium. Biol. Res. 44 (2), 169–180. https://doi.org/10.4067/S0716-97602011000200009.

Banchroft, J., Stevens, A., & Turner, D. (1996). *Theory and practice of histological techniques* (8th ed.).

Baskaran, S., Finelli, R., Agarwal, A., Henkel, R., 2021. Reactive oxygen species in male reproduction: A boon or a bane? Andrologia 53 (1). https://doi.org/10.1111/and.13577.

Camacho, L., Latendresse, J.R., Muskhelishvili, L., Patton, R., Bowyer, J.F., Thomas, M., Doerge, D.R., 2012. Effects of acrylamide exposure on serum hormones, gene expression, cell proliferation, and histopathology in male reproductive tissues of Fischer 344 rats. Toxicol. Lett. 211 (2), 135–143. https://doi.org/10.1016/j.toxlet.2012.03.007.

Christina, O.U., Daniel, U.O., 2017. Effects of acrylamide on the reproductive hormones and sperm quality in male rats. Int. J. Sci. Res. (IJSR) 6 (7), 5–8. https://doi.org/10.21275/art20174640.

DaSari, S. S., Ganjayi, M. S. M., GonuGuntla, S., Kothapalli, S. R., KonDa, P. Y., baSha, S. S., KutaGolla, P., & MeriGa, B. (2018). Evaluation Of Biomarkers Distress In Acrylamide-Induced Hepatic And Nephrotoxicity Of Albino Wistar Rat. *Advances in Animal and Veterinary Sciences*, *6*(10), 427–435. https://doi.org/10.17582/journal.aavs/2018/6.10.427.435

Dcunha, R., Hussein, R.S., Ananda, H., Kumari, S., Adiga, S.K., Kannan, N., Zhao, Y., Kalthur, G., 2022. Current insights and latest updates in sperm motility and associated applications in assisted reproduction. Reprod. Sci. 29 (1), 7–25. https://doi.org/10.1007/s43032-020-00408-y.

Dirican, E.K., Kalender, Y., 2012. Dichlorvos-induced testicular toxicity in male rats and the protective role of vitamins C and E. Exp. Toxicol. Pathol. 64 (7–8), 821–830. https://doi.org/10.1016/j.etp.2011.03.002.

El Kotb, S.M., El-ghazouly, D.E., Ameen, O., 2020. The potential cytoprotective effect of Vitamin C and Vitamin E on monosodium glutamate-induced testicular toxicity in rats. Alexand. J. Med. 56 (1), 134–147. https://doi.org/10.1080/20905068.2020.1804311.

Elbashir, A.A., Omar, M.M.A., Ibrahim, W.A.W., Schmitz, O.J., Aboul-Enein, H.Y., 2014. Acrylamide analysis in food by liquid chromatographic and gas chromatographic methods. Crit. Rev. Anal. Chem. 44 (2), 107–141. https://doi.org/10.1080/10408347.2013.829388.

Fang, J., Liang, C.L., Jia, X.D., Li, N., 2014. Immunotoxicity of acrylamide in female BALB/c mice. Biomed. Environ. Sci. 27 (6), 401–409. https://doi.org/10.3967/bes2014.069.

Farag, O.M., Abd-Elsalam, R.M., El Badawy, S.A., Ogaly, H.A., Alsherbiny, M.A., Ahmed, K.A., 2021. Portulaca oleracea seeds' extract alleviates acrylamide-induced testicular dysfunction by promoting oxidative status and steroidogenic pathway in rats. BMC Complement. Med. Ther. 21 (1), 1–15. https://doi.org/10.1186/s12906-021-03286-2.

Farjana, M., Moni, A., Sohag, A.A.M., Hasan, A., Hannan, M.A., Hossain, M.G., Uddin, M.J., 2020. Repositioning vitamin c as a promising option to alleviate complications associated with COVID-19. Infect. Chemother. 52 (4), 461–477. https://doi.org/10.3947/IC.2020.52.4.461.

Fiedan, I.O., Ahmed, E.A., Omar, H.E.M., 2015. Acrylamide induced testicular toxic ity in rats : Protective effect of garlic oil abstract histopathological results. Biomark. J. 1, 1–8. https://doi.org/10.21767/2472-1646.100005.

Hamdy, S.M., Bakeer, H.M., Eskander, E.F., Sayed, O.N., 2012. Effect of acrylamide on some hormones and endocrine tissues in male rats. Hum. Exp. Toxicol. 31 (5), 483–491. https://doi.org/10.1177/0960327111417267.

Hasan, I., Rubayet Jahan, M., Nabiul Islam, M., Rafiqul Islam, M., 2021. Effect of 2400 MHz mobile phone radiation exposure on the behavior and hippocampus morphology in Swiss mouse model: Effect of 4G mobile phone radiation on the brain. Saudi J. Biol. Sci. https://doi.org/10.1016/j.sjbs.2021.08.063.

Hassan, S.M., Mady, M.E., Cartwright, A.L., Sabri, H.M., Mobarak, M.S., 2003. Effect of acetyl salicylic acid in drinking water on reproductive performance of Japanese quail (Coturnix coturnix japonica). Poult. Sci. 82 (7), 1174–1180. https://doi.org/10.1093/ps/82.7.1174.

Ibrahim, R.M., Nawar, I., Yousef, M.I., El-Sayed, M.I., Hassanein, A., 2019. Protective role of natural antioxidants against the formation and harmful effects of acrylamide in food. Trends Appl. Sci. Res. 14 (1), 41–55. https://doi.org/10.3923/tasr.2019.41.55.

Kalaivani, M., Saleena, U. V., Kamath Katapadi, K. G., Praveen Kumar, Y., Nayak, D., & Nilima. (2018). Effect of acrylamide ingestion on reproductive organs of adult male wistar rats. *Journal of Clinical and Diagnostic Research*, *12*(11). https://doi.org/10.7860/JCDR/2018/38170.12364

Kermani-Alghoraishi, M., Anvari, M., Talebi, A.R., Amini-Rad, O., Ghahramani, R., Miresmaili, S.M., 2010. The effects of acrylamide on sperm parameters and membrane integrity of epididymal spermatozoa in mice. Eur. J. Obstet. Gynecol. Reprod. Biol. 153 (1), 52–55. https://doi.org/10.1016/j.ejogrb.2010.07.008.

Lüke, L., Campbell, P., Sánchez, M.V., Nachman, M.W., Roldan, E.R.S., 2014. Sexual selection on protamine and transition nuclear protein expression in mouse species. Proc. R. Soc. B Biol. Sci. 281 (1783). https://doi.org/10.1098/rspb.2013.3359.

Ma, Y., Shi, J., Zheng, M., Liu, J., Tian, S., He, X., Zhang, D., Li, G., Zhu, J., 2011. Toxicological effects of acrylamide on the reproductive system of weaning male rats. Toxicol. Ind. Health 27 (7), 617–627. https://doi.org/10.1177/0748233710394235.

Mansur, M. A. Al, Alam, M. G. S., Jha, P. K., Rimon, M. A., Naher, N., & Bari, F. Y. (2018). Conception rate following intra-cervical artificial insemination using frozen semen at field level in indigenous sheep of Bangladesh. *Asian Journal of Medical and Biological Research*, *4*(1), 55–62. https://doi.org/10.3329/ajmbr.v4i1.36822

Matoso, V., Bargi-Souza, P., Ivanski, F., Romano, M.A., Romano, R.M., 2019. Acrylamide: A review about its toxic effects in the light of Developmental Origin of Health and Disease (DOHaD) concept. Food Chem. 283, 422–430. https://doi.org/10.1016/j.foodchem.2019.01.054.

Mehni, N.M., Ketabchi, A.A., Hosseini, E., 2014. Combination effect of pentoxifylline and L-carnitine on idiopathic oligoasthenoteratozoospermia. Iran. J. Reprod. Med. 12 (12), 817–824.

Obstr, C., Dis, P., 2018. Journal of the COPD foundation review chronic obstructive pulmonary diseases : Antioxidants and chronic obstructive pulmonary disease. J. COPD Found. Rev. 5 (4), 277–288.

Omidi, Z., Piravar, Z., Ramezani, M., 2020. The effect of acrylamide on mitochondrial membrane potential and glutathione extraction in human spermatozoa: A laboratory study. Int. J. Reprod. BioMed. 18 (10), 855–864. https://doi.org/10.18502/ijrm.v13i10.7770.

Ommurugan, B., Rao, V., Tilak, A., Bairy, K.L., 2018. Effect of Vitamin C and atorvastatin on male fertility in rats subjected to forced swimming stress. Int. J. Infertil. Fetal Med. 9 (3), 27–31. https://doi.org/10.5005/jp-journals-10016-1171.

Park, Y. E., & Kim, T. O. (2020). *Sexual Dysfunction and Fertility Problems in Men with Inflammatory Bowel Disease*. *38*(3), 285–297.

Pourentezari, M., Talebi, A., Abbasi, A., Khalili, M.A., Mangoli, E., Anvari, M., 2014. Effects of acrylamide on sperm parameters, chromatin quality, and the level of blood testosterone in mice. Iran. J. Reprod. Med. 12 (5), 335–342.

Rahayu, I., Usman, E., Reza, M., 2019. Effect of vitamin C on testosterone level, sperm count and sperm morphology in gentamicin-induced Wistar rats. Int. J. Res. Med. Sci. 7 (2), 451. https://doi.org/10.18203/2320-6012.ijrms20190352.

Rajeh, N.A., Al-Dhaheri, N.M., 2017. Antioxidant effect of vitamin E and 5-aminosalicylic acid on acrylamide induced kidney injury in rats. Saudi Med. J. 38 (2), 132–137. https://doi.org/10.15537/smj.2017.2.16049.

Rajeh, N.A., Khayyat, D., 2017. Effect of the combined administration of vitamin-E and 5-aminosalicylic acid on acrylamide-induced testicular toxicity. J. Taibah Univ. Med. Sci. 12 (5), 445–454. https://doi.org/10.1016/j.jtumed.2017.03.005.

Rajeh, N. A. (2020). *Acrylamide Induced Toxicity in Seminal Vesicles and its Amelioration by Vitamin E Supplementation : A Histological Study*. 0–5. https://doi.org/10.31031/DCMP.2020.02.000528.

Rifai, L., Saleh, F.A., 2020. A review on acrylamide in food: Occurrence, toxicity, and mitigation strategies. Int. J. Toxicol. 39 (2), 93–102. https://doi.org/10.1177/1091581820902405.

Rima, U.K., Bari, A.S.M., Hossain, M.Z., Khan, M.A.H., 2018. Plasmid DNA vaccine coding eight repeats of gonadotrophin-releasing hormone induced atrophy of prostate in male mice. Prostate Int. 6 (4), 151–156. https://doi.org/10.1016/j.prnil.2018.01.001.

Saalu, L.C., Kpela, T., Benebo, A.S., Oyewopo, A.O., Anifowope, E.O., Oguntola, J.A., 2010. The dose-dependent testiculoprotective and testiculotoxic potentials of Telfairia occidentalis Hook f. leaves extract in rat. Int. J. Appl. Res. Nat. Prod. 3 (3), 27–38.

Sadeghzadeh, F., Mehranjani, M.S., Mahmoodi, M., 2019. Vitamin C ameliorates the adverse effects of dexamethasone on sperm motility, testosterone level, and spermatogenesis indexes in mice. Hum. Exp. Toxicol. 38 (4), 409–418. https://doi.org/10.1177/0960327118816137.

Sujan, K., Miah, M., Mustari, A., Islam, M., 2021. Beneficial effects of black seed oil on blood, hormone profile, sperm parameters and histotexture of testis of male mice exposed to bisphenol-A. J. Bangladesh Agric. Univ., 1 https://doi.org/10.5455/jbau.68360.

Talukder, S., Sarker, S., Hossain, M.A., Khan, M.A.H., Hannan, M.A., Islam, M.T., 2014. Evaluation of fertility disrupting potentials of Abrus precatorius seed extracts in male rats for arresting spermatogenesis and suppressed fertility in vivo. Pakistan Vet. J. 34 (1), 18–23.

Tyl, R.W., Friedman, M.A., 2003. Effects of acrylamide on rodent reproductive performance. Reprod. Toxicol. 17 (1), 1–13. https://doi.org/10.1016/S0890-6238(02)00078-3.

Vijayprasad, S., Ghongane, B.B., Nayak, B.B., 2014. Effect of vitamin C on male fertility in rats subjected to forced swimming stress. J. Clin. Diagn. Res. 8 (7), 6–9. https://doi.org/10.7860/JCDR/2014/8432.4622.

Visvanathan R, K. T. (2014). Acrylamide in Food Products: A Review. *Journal of Food Processing & Technology*, *05*(07). https://doi.org/10.4172/2157-7110.1000344

Yan, Y., Ren, F., Wang, P., Sun, Y., Xing, J., 2019. Synthesis and evaluation of a prodrug of 5-aminosalicylic acid for the treatment of ulcerative colitis. Iran. J. Basic Med. Sci. 22 (12), 1452–1461. https://doi.org/10.22038/ijbms.2019.13991.

Zamani, E., Shokrzadeh, M., Modanloo, M., Shaki, F., 2018. In vitro study towards role of acrylamide-induced genotoxicity in human lymphocytes and the protective effect of L-Carnitine. Braz. Arch. Biol. Technol. 61, 1–10. https://doi.org/10.1590/1678-4324-20181600685.

Zhang, X., Chen, F., Huang, Z., 2009. Apoptosis induced by acrylamide is suppressed in a 21.5% fat diet through caspase-3-independent pathway in mice testis Mice testis. Toxicol. Mech. Methods 19 (3), 219–224. https://doi.org/10.1080/15376510802499048.

Zhao, M., Zhang, B., Deng, L., 2022. The mechanism of acrylamide-induced neurotoxicity: Current status and future perspectives. Front. Nutr. 9 (March), 1–15. https://doi.org/10.3389/fnut.2022.859189.