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RESEARCH ARTICLE
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Acrylamide-Induced Testicular Toxicity and Oxidative Stress in Male Albino Rats: Potential Protection by Natural Antioxidant-Naringin

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ABSTRACT

Acrylamide (ACR), being one of the major public health concerns, is produced during frying and baking of different foods and is widely used in various sectors such as research laboratories and cosmetics. This study aimed to induce testicular toxicity of acrylamide and to investigate a possible protective effect of natural antioxidant naringin in male albino rats. A total of twenty-five adult albino rats, aged 12-14 weeks, were used. Acrylamide was injected intraperitoneally at a dose of 30mg/kg daily for 20 days to induce testicular toxicity. Naringin was administered at doses of 75 and 100mg/kg by oral gavage daily for 20 days. Light microscopy examination showed necrotic spermatids, oedema, severe thinning and degeneration of wall of seminiferous tubules and necrotic spermatids in the lumen of seminiferous tubules. Comet assay undertaken on testicular cells showed genotoxicity in the form of comet cells in acrylamide treated group. Likely, acrylamide-treated rats gavaged with naringin showed a reduction in DNA damage. Antioxidative and oxidative stress profile in testicular tissues of rats showed a significant increase in (ROS and TBARS) in acrylamide-treated rats with lower values of antioxidant enzymes (SOD, POD, GSH, and CAT) compared to normal rats. The supplementation with naringin partially mitigates the induction of inflammation and oxidative stress. Together, Naringin, particularly at higher doses, could potentially offer a protective mechanism against ACR-mediated testicular toxicity. In conclusion, at the used dose, ACR caused toxic effects in male rats that can be reduced by concomitant treatment with a higher dose of naringin.

Keywords: Acrylamide, Albino rats, Naringin, Testes, Oxidative stress, Histopathology.

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INTRODUCTION

Acrylamide (ACR) is frequently and persistently used in different industries including textile, printing, cosmetics, treatment of water systems, research laboratories and even in cosmetics across the globe (Tepe & Çebi, 2019; Kito et al., 2020; Yildirim et al., 2024). ACR is not produced naturally and is a colorless, crystalline, odorless highly toxic chemical which is produced during frying, cooking, roasting and baking of different food and food products such as carbohydrate- protein rich foods chips, bread, meat, cereals, roasted coffee and French fries at a temperature >120°C (Ahmed et al., 2022; Yildirim et al., 2024). ACR is passively entered into the body via ingestion of ACR-contaminated food and food products, air, and direct contact (Abdel-Daim et al., 2015; Eisenbrand, 2020; Yildirim et al., 2024). Various published studies have indicated that ACR induces different deleterious effects, including disruption of male and female endocrine functions, genotoxicity, neurotoxicity, cancer, and testicular toxicity in target and nontarget exposed organisms via damage to proteins, DNA, and normal physiological functions of the cells mediated by rapid and over-generation of free radicals (Khalil et al., 2014; Yilmaz et al., 2017; Rajeh & Al-Shehri, 2019; Elblehi et al., 2020; Gelen et al., 2022; Seify et al., 2024). It has been recorded that ACR causes testicular and nephrotoxicity and reduces the reproductive functions (Wang et al., 2010; Camacho et al., 2012; Sengul et al., 2021; Üremiş et al., 2024) in terms of low amount of sperm, atrophy and degeneration of seminiferous tubules, apoptosis, and induction of abnormalities in sperm (Kaçar et al., 2018).

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Earlier studies have found that ACR reduces glutathione levels and enhances lipid peroxidation in testicular tissues, leading to an imbalance between oxidants and antioxidants and causing infertility (Radad et al., 2020; Tekin & Çelebi, 2022; Naseer et al., 2025). Studies have indicated that the use of plant-based natural medicinal products is more common than that of artificial products to lower oxidative stress and explore antioxidant properties worldwide (Gelen et al., 2022; Tekin & Çelebi, 2022; Masood et al., 2025). Moreover, it is tempting to speculate that various substances derived from natural herbal products have attracted attention due to their antioxidant potential for therapeutic and chemotherapeutic uses (Gül et al., 2021; Gelen et al., 2022). Various studies have shown that antioxidant supplementation is vital for optimal fertility, spermatogenesis, and other biological functions, including immune modulation (Omar, 2016; Sadek et al., 2016).

Citrus fruits containing high bioflavonoid compounds are recognized as an enriched source of antioxidants because of their promising and broad biological functions and as a therapeutic potential in a variety of health disorders. Reports have indicated that naringin plays an important role in various inflammatory diseases, including pulmonary fibrosis, chronic obstructive pulmonary disease, asthma, cystic fibrosis, and lung cancer (Shilpa et al., 2023). Naringin inhibits the pro-inflammatory cytokine response caused by lipopolysaccharide in both macrophages and other blood cells. However, there has been minimal effort to examine species differences and the significance of studies on mammals and fish (Shilpa et al., 2023). Naringin has been shown to have antifibrotic properties (Shirani et al., 2020). Various studies have indicated that naringenin has antioxidant properties and inhibits abnormal apoptosis and cytotoxicity by modulating cytochrome P450 activity (Heidary Moghaddam et al., 2020). Moreover, studies have shown that naringenin is very effective in repairing DNA damage (He and Zhang, 2023). In spite to of low bioavailability, naringenin has several beneficial biological effects like anti-inflammatory and antioxidant properties (He and Zhang, 2023). Scant information is available on the effects of naringenin on ACR-induced oxidative stress and testicular toxicity in male albino rats. Therefore, this study was executed to determine the antioxidant potential of naringin against ACR-induced testicular toxicity.

MATERIALS & METHODS

General Materials

The chemicals and reagents used in this experimental study were of analytical grade and procured from Sigma-Aldrich (USA) and Merck (Germany). Acrylamide (99.9%) was obtained from Sigma Aldrich (USA). Naringin tablets in a concentration of 500mg/capsule were purchased from Swanson (USA) and dissolved in water to prepare the required concentrations.

Methods

Animals and Husbandry

A total of twenty-five adult male albino rats, active, aged (12-14 weeks), and free of any obvious ailment were purchased from a local laboratory animal house. The rats were kept in wire cages in typical laboratory conditions, with temperatures between 26-28℃. Each rat had full access to feed containing approximately 23% protein and to fresh water throughout the experiment. All procedures involving the use of laboratory animals were carried out in strict compliance with the guidelines established by the National Institutes of Health regarding the Care and Use of Laboratory Animals.

Experimental Design

Following a two-week acclimatization period, 25 male albino rats were divided into four groups of 5 rats each. The rats in each group were kept in separate wire cages under controlled conditions. Groups were as follows: Control group, group for acrylamide alone (30mg/kg body weight), group injected with acrylamide +75mg/kg naringin, and group injected with acrylamide +100 mg/kg naringin. Acrylamide was given via intraperitoneal injection at a dose of 30mg/kg daily for 20 days, while naringin was given via oral gavage at two doses of 75mg/kg and 100mg/kg body weight daily for 20 days.

Sample Collection

At the end of the experiment, all albino rats were weighed before scarification. Then were euthanized using chloroform via inhalation in a closed chamber to ensure little distress. After that, all the rats were dissected immediately for the separation of testicular tissues. After dissection, the testes were quickly removed, dried, and weighed separately. Then, they were rinsed with phosphate-buffered saline (PBS) and preserved in 10% neutral-buffered formalin for histological examination.

Tissue Preparation

Testes from each rat were carefully collected from both treated and untreated groups to measure oxidative stress and antioxidant defense biomarkers. After collection, each testis was placed in a clean Petri dish and minced, and a homogenate was prepared. After homogenization, the samples were centrifuged at 5000rpm for 10min to remove cellular debris. The supernatants were then gently extracted and stored at -4°C for further analysis (Wang et al., 2022).

Biochemical Analysis

Oxidative stress and antioxidant profile, including reduced glutathione (Jollow et al., 1974; Raza et al., 2022), thiobarbituric acid reactive substances (Chance & Maehly, 1955), and reactive oxygen species (Hayashi et al., 2007; Akram et al., 2021), were measured. Catalase and superoxide dismutase (Kakkar et al., 1984) and peroxidase (Iqbal et al., 1996) were also recorded in testicular tissues. The absorbance measurements for POD, SOD, and CAT were measured at 470 nm, 560 nm, and 240 nm, respectively, while ROS and TBARS were detected at 505 and 532nm using a UV-spectrophotometer.

Single Cell Electrophoresis (COMET Assay)

The estimation of DNA damage in isolated testicular cells was determined using earlier well-established

protocol (Hussain et al., 2021; Raza et al., 2022). The testicular tissue was removed immediately after dissection and triturated or homogenized to isolate cells from each rat. Briefly, 1.0% low-melting-point agarose (LMPA) and 0.9% normal-melting-point agarose (NMPA) were prepared separately using deionized water and were placed at 60˚C. After that, smears of NMPA (75µL) were prepared on frosted glass. These prepared slides were kept on ice for solidification. After that, a second layer (90µL) of LMPA diluted with isolated testicular cells was applied to a coverslip. These prepared slides were again placed on ice for solidification for 5min. After solidification, 75µL (0.5% LMPA) was spread onto the second layer, and a cover slip was placed on the third layer. After 15min of solidification, all prepared slides were placed in a fresh, cold lysing solution for 5 h at 4°C. After that, these slides were subjected to electrophoresis at 4°C for 25min at 25V. Finally, the slides were neutralized with a Tris-HCl buffer (pH 7.5) and stained with ethidium bromide. The slides were examined under a fluorescent microscope. In the comet assay, 500 cells were counted from each rat, and the percentage was calculated for comparison.

Morphometric Studies on Seminiferous Tubule Diameter

To evaluate the effect of ACR on seminiferous tubule diameters, and whether naringin had an opposing action on them or not, 5 different fields of the testes of each rat in each experimental group(n=5), were evaluated under low power (4X), using light microscopy. Measurement of tubule diameter was performed using Image J (National Institute of Mental Health, Bethesda, MD). Then, the diameters of 100 seminiferous tubules for each rat in each group were calculated (Rehan et al., 2014; Hussain et al., 2018).

Histopathological Examination

For histopathological observation, testes from each experimental rat reared in all groups were collected during the necropsy. Each harvested organ was placed in a 10% formaldehyde solution for histological analysis (Hussain et al., 2019; Hussain et al., 2022; Raza et al., 2022). Thin microscopic sections (4-5µm thick) were cut with the help of a rotary microtome, processed, and finally stained with hematoxylin and eosin (H&E) using standard histological procedures (Jabeen et al., 2024). Microscopic investigations of various sections of testes were made using a light microscope (Nikon Eclipse 80i, Nikon Co., Tokyo, Japan) to observe any histological changes (Rani et al., 2023; Ullah et al., 2023).

Statistical Analysis

Data on oxidative stress, DNA damage, diameter of seminiferous tubules, and frequency of seminiferous tubules were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics software (version 20). Tukey's post hoc test was used to determine the significant difference at $P<0.05$.

RESULTS

General Observation

All the rats in the control group remained active and healthy and showed no abnormal signs. The rats exposed to acrylamide showed watery fecal contents, hair loss, and were lethargic. No deaths were reported among all rats.

Effect on Body Mass, Absolute and Relative Weight of Testes of Male Albino Rats

At the end of the experiment, a significant reduction in the rat's body weight of the acrylamide-treated group compared to the control group was reported. Furthermore, a significant increase in absolute and relative testes weight in rats treated with acrylamide was observed compared to the control group. No significant effect on these parameters was noted in rat's gavaged with naringin (Table 1).

Effect on Antioxidative and Oxidative Stress Profile in Testicular Tissues

Naringin supplementation in rats partially reversed the toxic effects of acrylamide. In the control group, all parameters remained within normal ranges because the rats were not exposed to any treatment. However, rats in the acrylamide-treated group indicated severe inflammation and induction of oxidative stress in terms of a significant increase in concentration of ROS and TBARS, while a significant reduction in concentration of antioxidant enzymes like SOD, POD, GSH, and CAT was observed compared to the control groups. The results showed that naringin supplementation significantly mitigated acrylamide-induced inflammation and oxidative stress. In rats treated with higher doses of naringin, a significant reduction in ROS and TBARS levels was reported. Furthermore, a significant increase in antioxidant enzyme concentrations (SOD, POD, CAT, and GSH) was reported compared with the ACR group. However, at the lower dose of naringin (75mg/kg), significant partial reductions in ROS and TBARS, and significant partial increases in the contents of different antioxidant enzymes in testicular tissues were recorded compared with the higher dose of naringin. When naringin, a flavonoid with potential protective effects, was administered along with acrylamide, the alterations in the testicular parameters were partially ameliorated to varying degrees, with a more apparent positive effect seen with the higher dose rather than with the lower dose of naringin (Table 2).

Table 1: Effect of ACR on rat's body mass, absolute and relative testis weights

Parameters	Groups/Treatments			
	Control group	ACR 30mg/kg	ACR+ 75mg/kg naringin	ACR+ 100 mg/kg naringin
Body weight	235.7±3.4	221.2±2.8*	227.4±1.2	230.5±1.3
Absolute weight of testes	2.23±0.31	2.89±0.11*	2.47±0.10	2.39±0.11
Relative weight of testes	0.98±0.07	1.32±0.03*	1.10±0.04	1.03±0.04

Values (mean+SD) bearing asterisks in a row indicate a significant difference at $P<0.05$.

Table 2: Antioxidative and oxidative stress profile in testicular tissues of male albino rats

Parameters	Groups/Treatments			
	Control group	ACR 30mg/kg	ACR+ 75mg/kg naringin	ACR+ 100 mg/kg naringin
Antioxidant Enzymes				
Superoxide (units/mg protein)	0.63±0.08	0.34±0.07*	0.43±0.08*	0.51±0.06*
Catalase (units/min)	0.81±0.09	0.55±0.07*	0.61±0.05*	0.69±0.05*
Peroxidase (units/min)	0.33±0.08	0.19±0.07*	0.22±0.05*	0.27±0.05*
GSH (mmol-g^{-1} tissue)	1.23±0.13	0.73±0.10*	0.83±0.08*	0.97±0.05*
Oxidative biomarkers				
ROS (optical density)	0.23±0.06	0.43±0.05*	0.37±0.05*	0.32±0.06*
TBARS (nmol/TBARS formed/mg protein/min)	0.39±0.07	0.59±0.05*	0.51±0.05*	0.47±0.05*

Values (mean+SD) bearing asterisks in a row indicate a significant difference at P<0.05.

Table 3: Seminiferous tubule diameter, percentile rate of seminiferous tubules exhibiting normal spermatozoa, and frequency of DNA damage in testes of treated and untreated male albino rats

	GROUPS/TREATMENT		
Parameters			
Control group	ACR alone	ACR+75mg/kg naringin	ACR+100 mg/kg naringin
Diameter of seminiferous tubule (µm)			
198.4±4.3	162.5±3.7*	174.7±2.2*	182.3±2.14*
*Percentile rate of seminiferous tubules with *normal spermatogenesis (%)*			
98.3 ±1.5	68.6 ±2.54*	76.9±1.9*	84.9±1.7*
DNA damage (%) in isolated cells of testes			
2.3±1.07	9.7±1.4*	7.2±1.1*	4.5±1.3*

Values (mean+SD) bearing asterisks in a row indicate a significant difference at P<0.05.

Effect on Frequency of Seminiferous Tubules with Normal Spermatogenesis

The results on the frequency of seminiferous tubules with normal spermatogenesis indicated a significantly lower percentile of seminiferous tubules with normal spermatogenesis in the group of rats treated with acrylamide alone compared to the control group. The supplementation of naringin at two different doses significantly reduced the severity of pathological ailments, with a significant increase in seminiferous tubules containing normal spermatogenesis compared to the ACR-treated group (Table 3). More significant increase was noted with the higher dose of naringin (Table 3).

Effect on Seminiferous Tubule Diameter

Results on measurement of seminiferous tubules diameter indicated significantly lower values in rats treated with ACR alone in comparison to untreated male albino rats. The results of naringenin supplementation at different doses partially increased the diameter of the seminiferous tubules (Table 2). The higher dose of naringin induced a more significant increase in the diameter of the seminiferous tubules in comparison to the lower dose of naringin.

Effect on DNA Damage or Comet Cells

The results of genotoxic analysis indicated a significant increase in DNA damage in isolated cells from the testicular tissues of male albino rats treated with acrylamide compared to control rats. The results on supplementation of naringin partially ameliorated the induction of inflammation and oxidative stress in term of lower frequency of DNA damage in reproductive organs of rats of treated with ACR along with different doses of naringin (Table 2; Fig. 1). With the higher dose of naringin, it induced more significant reduction value in comet cells in comparison to the lower dose of naringin, indicated more protection.

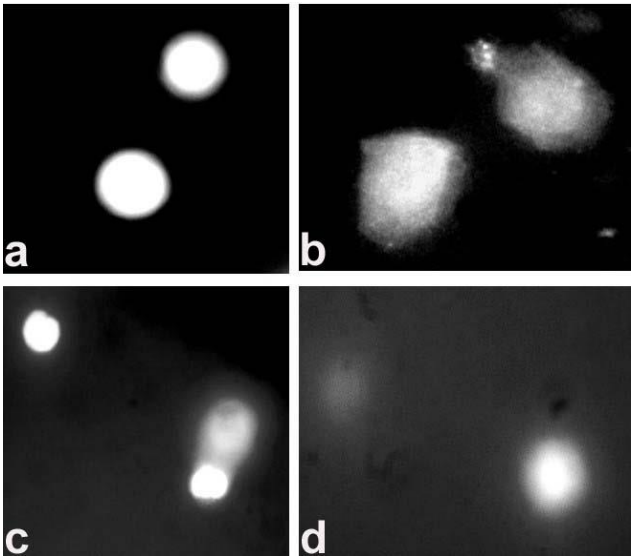

Fig. 1: Photomicrograph showing DNA damages fluorescing around the nucleus or making a tail moment in isolated cells of testicular tissues of male albino rats exposed to acrylamide alone and in combination with naringin a) normal/intact cells with DNA material from control rat; b) exhibiting severe DNA damage from ACR treated rats; c) moderate DNA damages from rats treated with 75mg naringin and d) mild DNA damages from rats treated with 100mg naringin 600X; Ethidium bromide stain.

Effect on the Histological Appearance of the Rat Testis

At the microscopic level, severe histoarchitectural ailments, including necrosis and detachment of germinal epithelium, arrest of the process of spermatogenesis, vacuolations, necrotic spermatids, edema, severe thinning, and degeneration of the wall of seminiferous tubules. An admixture of necrotic spermatids and inflammatory material in the lumen of seminiferous tubules and the presence of necrotic cells in interstitial spaces of seminiferous tubules with apparent atrophy of Leydig cells were noted in ACR-treated rats (Table 4; Fig. 2). Moderate to severe histopathological changes were observed in rats treated with 75mg/kg naringin. At the same time, mild to moderate pathological changes were observed in rats treated with 100mg/kg of naringin, indicating greater protection.

Table 4: Severity of different testicular lesions in the testicular tissues of male albino rats.

Lesions	Groups/Treatments			
	Control group	ACR alone	ACR+ 75mg/kg naringin	ACR+ 100 mg/kg naringin
Necrosis of spermatids	-	++++	+++	++
Increased weight of testes	-	++++	+++	++
Decreased size of testes	-	++++	+++	++
Reduced volume of testes	-	+++	++	++
Presence of inter tubular sloughed cells	-	++++	+++	++
Detachment of germinal epithelium	-	++++	++++	+++
Thinning of germinal epithelium	-	++++	+++	++
Admixture of necrotic cells in lumen of seminiferous tubules	-	++++	+++	++
Depletion of germ cells in epithelium of seminiferous tubules	-	++++	+++	+++
Inflammatory processes	-	+++	+++	+++
Depletion of germ cells in the epithelium of the seminiferous tubules	-	++++	+++	+++
Inflammatory processes	-	+++	+++	+++
Necrosis of Sertoli cells and spermatogonia	-	+++	+++	+++
Arrest of the process of spermatogenesis	-	+++	++	++
Reduction in the diameter of the seminiferous tubules	-	+++	++	++
Reduced frequency of seminiferous tubules with normal cells	-	++++	+++	++
Vacuolation in the epithelium of the seminiferous tubules	-	++++	+++	++

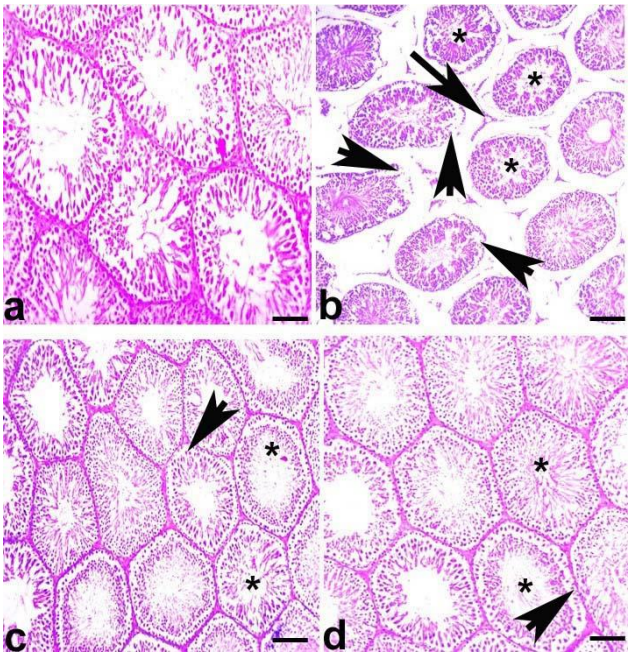

Fig. 2: Photomicrograph of various sections of testes of male albino rats showing a) normal histological pattern from control rat; b) showing severe microscopic lesions like edema, necrosis of spermatids, arrest of process of spermatogenesis (arrow heads), sloughed cells (arrow), vacuolation and detachment of germinal epithelium from ACR treated rat; c) showing moderate histopathological ailments necrosis of spermatids, admixture of necrotic cells in lumen of seminiferous tubule (*) from rat treated with 75mg/kg naringin and d) showing mild histopathological ailments including necrosis of spermatids, admixture of necrotic cells in lumen of seminiferous tubule (*) from rat treated with 100mg/kg naringins. 400X; H&E Stain.

DISCUSSION

In the current study, the potential role of the antioxidant naringin in protecting against ACR-induced testicular toxicity following sub-chronic exposure to ACR was evaluated in albino rats. Concomitant treatment of naringin with ACR was effective in preventing ACR-mediated oxidative stress in both tested doses of naringin. In addition, naringin improved the histological appearance of the testis and reduced DNA damage in testicular tissue. It caused a significant increase in seminiferous tubule diameter and the percentage rate of seminiferous tubules with normal spermatogenesis. The concentrations of different antioxidant enzymes were significantly depleted in the testicular tissues of rats in acrylamide-treated groups, suggesting that acrylamide exposure might have induced oxidative stress in the rats (Dobrovolsky et al., 2016). The reduction in antioxidant enzymes in the testes of rats in this study indicates suppression of the immune system, as immune cells play crucial roles in immune responses and defense mechanisms. Conversely, the increase in ROS and TBARS suggests an inflammatory response or an attempt of the rats' immune system to combat the toxic effects of acrylamide. However, co-treatment with different concentrations of naringin mitigated these adverse effects. The groups treated with higher concentrations of naringin exhibited a significant improvement in the antioxidative enzymes both in testicular tissues of rats, indicating the protective potential of naringin against acrylamide-induced testicular toxicity (Sabik et al., 2011; Eisenbrand, 2020). The ameliorative effects of naringin on testicular tissues can be attributed to its antioxidant and anti-inflammatory properties (Sabik et al., 2011; Abdel-Daim et al., 2015; Eisenbrand, 2020; Moradi et al., 2025). Naringin may have neutralized the reactive oxidative species generated by acrylamide exposure, thereby preventing oxidative stress and damage to testicular and immune cells. Additionally, the anti-inflammatory effects of naringin may have modulated the inflammatory response, leading to the normalization of rats' defense mechanisms. Additionally, the reduced glutathione content, an important endogenous antioxidant, was significantly reduced, further compromising the antioxidant capacity of blood (Abdel-Daim et al., 2015; Eisenbrand, 2020; Yildirim et al., 2024). Furthermore, the reduced glutathione content was also partially recovered in these groups, suggesting that naringin helped to maintain the antioxidant defense system. Overall, these findings suggested that acrylamide exposure induces oxidative stress in the testicular tissues of male albino rats, disrupting the antioxidant defense system and increasing reactive oxygen species and lipid peroxidation products (Kermani-Alghoraishi et al., 2010; Lebda et al., 2014; Dobrovolsky et al., 2016). However, co-treatment with naringin, particularly at higher concentrations, exhibited a protective effect by mitigating oxidative stress and restoring antioxidant enzyme activities in testicular tissues. It has been recorded that naringin

inhibits the transport of different proteins (multidrug resistance protein), transport of organic anion polypeptide isoforms, sulfotransferase, and cytochrome isoenzymes, leading to reversal of toxic effects (Egert and Rimbach 2011; Sharma et al., 2021). This reduction in antioxidant enzyme activities suggests impaired antioxidant defense mechanisms leading to oxidative stress. The severe to very severe histopathological lesions like necrosis of spermatids, vacuolation in epithelium of seminiferous tubules, arrest of process of spermatogenesis, inflammatory processes, depletion of germ cells in epithelium of seminiferous tubules, admixture of necrotic cells in the lumen of seminiferous tubules, detachment of germinal epithelium, presence of inter tubular sloughed cells and thinning of germinal epithelium observed in male albino rats exposed to acrylamide could be related to over release of pro-inflammatory and apoptotic biomarkers resulting to induction of oxidative stress, apoptosis and testicular inflammation (Morris et al., 2006; Kim et al., 2015; de Conti et al., 2019). Moreover, the microscopic alterations in the testes of male albino rats might also be related to damage to the plasma membrane, resulting from increased free-radical release, thereby disrupting normal mitochondrial function. Previously, testicular lesions, including epithelial vacuolization, epithelial detachment, and sloughing of germinal cells due to toxic effects, have also been observed in mice. It has also been reported that acrylamide caused histopathological lesions in testicular organs due to overproduction of reactive oxygen species, leading to inflammation, oxidant injury, and cell death (Clewell et al., 2010; Zeng et al., 2018). The significantly increased weigh of testes might also be related to inflammation as indicated by higher contents of ROS and TBARS in testicular tissues of male albino rats treated with acrylamide (Kim et al., 2015; de Conti et al., 2019). Remarkably, co-treatment of naringin with acrylamide ameliorated these histopathological changes in a dose-dependent manner. In rats treated with (75mg/kg naringin) and (100mg/kg naringin), the damage in the tissue was moderately reduced compared to the acrylamide-treated rats. The supplementation of naringin might have prevented or reduced tissue damage caused by acrylamide-induced toxicity (Kandhare et al., 2016; Zeng et al., 2020). These findings demonstrated the severe histopathological consequences of acrylamide exposure in the testicular organs of male albino rats and highlighted the remarkable potential of naringin to mitigate tissue-level damage. The dose-dependent amelioration of histopathological alterations by naringin suggested its therapeutic value in developing strategies to mitigate the toxic effects of various chemicals, including acrylamide, and in promoting tissue integrity. The lower oxidative stress profile and microscopic alterations might be related to the inhibition of xanthine oxidase, which enhances antioxidant potential and lowers protein and lipid oxidation in rats due to naringin supplementation (Kandhare et al., 2016; Goodarzi-Boroojeni et al., 2018). Previously, the histopathological alterations in different visceral organs of rats, including testes, treated with acrylamide could be due to inflammatory responses and increased oxidative stress. The significantly increased frequency of DNA damage could also be linked to the induction of oxidative stress in the testicular tissues of treated albino rats. The histopathological changes and genotoxic effects could also be related to the depletion of antioxidant enzymes in multiple tissues, leading to disorders in cellular signaling pathways. Significantly increased DNA damage may result from acrylamide's ability to react with essential enzymes, hemoglobin, and DNA (Sabik et al., 2011; Abdel-Daim et al., 2015; Eisenbrand, 2020).

Conclusion

The findings of this experimental research indicated the toxic effects of acrylamide in male albino rats, including elevated levels of oxidative stress biomarkers, reduced levels of antioxidant enzymes, and the induction of microscopic lesions in the testes. The quantity of reduced glutathione was also significantly reduced suggesting a weak antioxidant defense mechanism in rats. The supplementation of higher doses of naringin progressively and partially reversed the toxic effects of acrylamide in male albino rats, demonstrating that naringin had a dose-dependent protective effect against acrylamide-induced testicular toxicity. The result of the current study is mainly relevant to human health, hence the suggested testicular and genotoxic risk of dietary acrylamide may be reduced by concomitant intake of naringin, which is present naturally in citrus fruits. Further investigations are needed to study the precise mechanism of this protection at the CYP2E1 level in the liver.

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Data Availability: Not applicable.

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