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Research Article

Taify Pomegranate Juice Extract Abrogates Testicular Dysfunction Induced by Acrylamide: Role of Inflammatory, Antioxidants, and Oxidative Stress Biomarkers

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High altitude (HA) poses various dangers to living organisms, the most serious of which are oxidative stress and its accompanying metabolic problems, as well as hypoxia and its associated metabolic abnormalities. Acrylamide is a poisonous chemical produced under the oxidative stress as a result of intracellular reactive oxygen species formation and toxicity. Heating carbohydrates-rich meals give acrylamide that is widely used in the industry. The precise mechanism of acrylamide toxicity is unknown. The current study aimed to examine the impacts of Taify pomegranate juice (TPJ) from Taif area (*Punica granatum L.*) on acrylamide-induced testicular stress and dysfunction. Twenty four male of adult Wistar rats, were divided into four groups: Group 1 was a negative-control received only saline; Group 2 was a positive-control that received orally acrylamide (20 mg/kg/bw) for consecutive 4 weeks; and Group 3 received TPJ; 2 mL/kg/bw, orally for 4 weeks. Rats in Group 4 received pomegranate juice and acrylamide as described in Groups 2 and 3, with the TPJ delivered 1 week before the acrylamide. Acrylamide elevated serum levels of inflammatory cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α)). Moreover, acrylamide decreased concentrations of follicle stimulating hormone (FSH), leutinizing hormone (LH), and testosterone and altered semen characteristics (decreased sperm viability and concentration, and increased sperm abnormalities). Acrylamide increased malondialdehyde levels (MDA), while it decreased antioxidant activities (superoxide dismutase; SOD and reduced glutathione; GSH). The inflammation associated cytokines were restored by TPJ administration to acrylamide rats. GSH and SOD were significantly recovered to near control levels in TPJ plus acrylamide group compared to acrylamide-administered rats. TPJ preadministration to rats restored semen profiles, alteration in testis pathology, and normalized the changes on the male reproductive hormones affected by acrylamide. Furthermore, the TPJ reversed the upregulation in caspases-3 and the decrease in B-cell lymphoma-2 (Bcl-2) gene expressions affected by acrylamide, with significant upregulation of hemoxygenase-1 (HO-1) and nuclear factor erythroid-2 (Nrf2) mRNA expression. These findings collectively revealed that TPJ possesses anti-inflammatory, potent antioxidant, and antiapoptotic effect against acrylamide-induced testicular damage.

1. Introduction

Acrylamide is a molecule formed by a natural chemical interaction between sugars and asparagine, an amino acid, in plant-based meals such as potatoes and cereal grains [1]. Acrylamide is produced during heating off some culinary processes as baking, roasting, and frying [1, 2]. High doses of acrylamide caused cancer in laboratory animals during research experiments, although the quantities of acrylamide employed in these studies were far higher than those seen in

human meals. Because of its potential to harm human health, the Food and Drug Administration monitors levels of this contaminant in the specific foods. The International Societies of Cancer reported that acrylamide is a carcinogenic agent in 1994 [3]. Acrylamide is a serious threat to human health [1, 4–8]. Various studies reported the negative impact for acrylamide on health through the generation of oxidative stress together with neurotoxicity, reproductive, and hepatorenal toxicity [1, 3, 5, 9, 10]. It decreases the antioxidant capacity through its deleterious impacts on the endogenous antioxidant enzymes [11, 12]. Acrylamide increases the production of inflammatory cytokines [12, 13] and induces mitochondrial and caspase-dependent apoptosis [14, 15]. Recent studies demonstrated that acrylamide is genotoxic [16]. Acrylamide accumulates in the testis, disrupts testicular functions and reproductive health [17, 18]. Chronic acrylamide exposure in male rodents resulted in testicular cytotoxicity as evidenced by atrophy of the seminiferous tubules, and apoptosis. Low-sperm viability and concentration, and impairment in spermatogenesis have all been reported in the acrylamide toxicity [19]. Acrylamide-induced reproductive dysfunction was associated with a decrease in reproductive hormones due to Leydig cell dysfunction [18].

The mutagenicity of germ cells, transmitted mutations, embryo loss rates whether before and after implantation, and other harmful effects of acrylamide on the reproductive system have been thoroughly examined [20]. Recent investigations have shown that acrylamide binds to spermatid protamines and may cause dominant lethality for gonadal cells and sperm morphologic defects, although it has no influence on the concentration and motility of epididymal sperm [21]. According to studies, even the smallest amount of exposure to acrylamide lowers sperm reserves associated to spermatogenesis and sex steroid transfer as well as testicular gene expression in the cauda epididymis [22]. It has also been demonstrated that rat seminiferous tubules exposed to acrylamide developed histological abnormalities [23].

Taif is located in the cost area of Saudi Arabia, with elevations ranging from 1,300 to 2,300 m above sea level. Taif area location makes it ideal for a wide range of biodiversity for the different plant species [24]. The pomegranate grown in Taif has special commercial value, cultural, and nutritional importance [25]. The elevated location of Taif makes its pomegranate as a potential and specific antioxidants properties. Pomegranate juice is an essential fruit extract with a wide range of impacts among which is antioxidant and antioxidative stress activity against organs dysfunction [26, 27], antimutagenic [28], antiviral [29], antiobesity [25, 30], amelioration of neurodegenerative diseases [31]. Moreover, pomegranate peel shows antioxidant and antimicrobial activity [27, 32]. In parallel, reports confirmed that seed extract of pomegranate had antioxidants impact [33–35, 36].

Till now, no direct study about the protective effects of Taify pomegranate against testicular dysfunction due to stress and environmental toxicants occurred at high altitude (HA). Therefore, in the present study, we aimed to examine the impacts of acrylamide administration on oxidant levels/antioxidant activities at proteins and gene levels, changes in reproductive hormone profiles and testis histopathology, and apoptosis-associated genes and possible protection by pomegranate juice were completely outlined.

2. Materials and Methods

2.1. Chemicals and Kits. Abcam Company provided testosterone kits (Cat # ab108666, Tokyo; Japan). SYBR-Green Master Mix, Oligo-dT, and Qiazol reagent were from QIAGEN (Valencia, USA). Acrylamide was provided by Sigma Aldrich Company; Memphis; USA. The kits of antioxidants (superoxide dismutase; SOD, reduced glutathione; GSH, and malondialdehyde; MDA) were from Biodiagnostic Company (Giza, Egypt).

2.2. Plant Extracts Preparation. Pomegranate fruits from Taif were harvested from natural habitat of Taif region. To obtain solely the juice components, whole seeds were gathered from well matured fruits. The juice was extracted through hand squeezing using cheesecloth. Taify pomegranate juice (TPJ) was filtered by passing through 0.22 μm filter mesh and given to animals in fresh state as described before [26].

2.3. Experimental Design, Sampling, and Handling. Twenty-four male of adult Westar rats weighting 180 g and 2 months age from King Fahd Institute, King Abdel-Aziz University, were used for this study. The animals were maintained at room temperature with 12 hr dark day light at 24°C on Taif University. After adaptation for 2 weeks, rats were subdivided into four groups each with six rats. The ethical committee of Taif University has approved the usage and regulation applied for this study for ethical code number 42-0081. Control group (CNT) administered saline only; the TPJ was orally administered TPJ (2 mL/kg/bw) for 4 weeks [26]; acrylamide group (ACR) received ACR (20 mg/kg/bw) orally and daily for 4 weeks [27]. Rats of protective group (TPJ + ACR) received same alone treatments explained on TPJ and ACR groups. After 28 days of treatment and after sedation of rats with isoflurane, they were killed, and decapitated after isoflurane inhalation. Rats were then decapitated for tissue sampling. Serum extraction was performed on clotted blood samples after isoflurane inhalation (2.5%–3%) [37]. Blood samples (5 mL) were obtained from the eyes, kept for complete clotting at room temperature. Serum about (2 mL) was taken after centrifugation at 3,000 rpm for 6 min. Serum was stored at −30°C for future serum chemistry and hormones assessments. Before rinsing in cold saline, testicular tissues were cleansed and sliced. Samples from testes were kept in Qiazol for one part for total RNA extraction and quantitative real-time PCR (qRT-PCR)), and the second part reserved in 10% neutral formalin solution for histopathology. Some parts of serum analysis and gene expression are repeated three times per each treatment and described in tables and figure legends. The detailed design of experiments are listed in Figure 1.

2.4. Antioxidants and Reproductive Hormones Assessments. A colorimetric spectrophotometer assessed serum antioxidants biomarkers (SOD, CAT. No. SD 25 21; GSH, CAT. No. GR 25 11, and MDA, CAT. No. MD 25 29) based on the manufacturer's guidelines. Testosterone was tested using

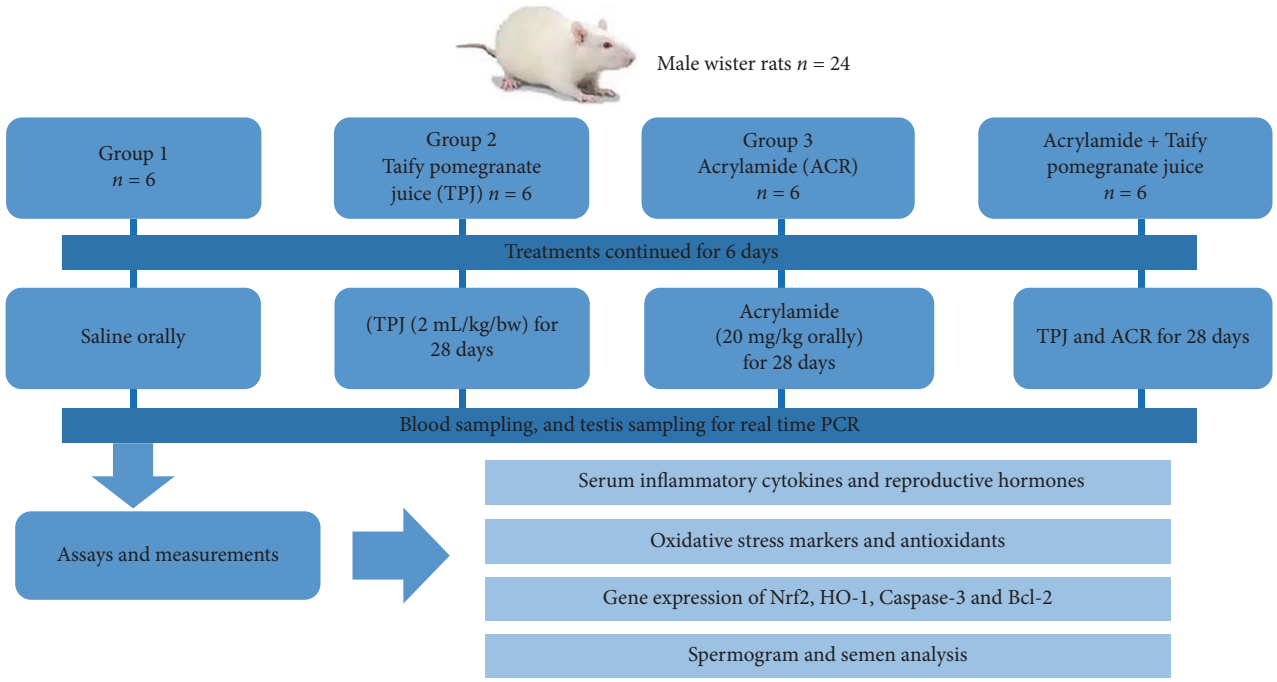

Figure 1: Experimental design of current study.

the commercial kits bought from *Clini Lab*., Cairo, Egypt. Follicle-stimulating hormone (LS-F6305, follicle stimulating hormone (FSH)), luteinizing hormone (ab235648; leutinizing hormone (LH)), and Rat Testosterone ELISA Kit (ab285350) were from abcam company (152 Grove Street Waltham, MA 02453, USA). The vendors' instructions for each kit were strictly followed uELISA machine from Bio-Rad Company, USA.

2.5. Measurements of Inflammatory Cytokines. Interleukin-1 (IL-1 and IL-6), and tumor necrosis factor alpha (TNF-α) were measure with specific spectrophotometric colorimetric ELISA kits from abcam (ab255730, ab100768, and ab46070, respectively) according to the kits' instructions. ELISA reader was used for showing the data changes and calculated it as completely explained in each instruction manual.

2.6. Semen Characteristics. As recently stated by Solimanet al. [38], the caude epididymis were incised and squeezed to discharge sperm in wet glass slide. One drop of sperm were mixed with 20 mL of 2.9% sodium citrate. The percentage of movable spermatozoa was examined in four different microscopic fields at 400x magnification [39]. The sperms concentrations were determined with a Neubauer hemocytometer with a magnification of 40. Seminal smears were stained with eosin/nigrosin to check sperm viability [40].

2.7. Quantitative Real Time PCR and Gene Expression. RNA from testes was extracted, purified, and measured at 260/280 nm. The QuantiTect reverse transcription kit employed a two-step technique to synthesize cDNA from a total of 2 μg of RNA. A random primer hexamer was used to amplify the genes of interest. For cDNA amplification, the SYBR-Green master (Catalog number: 4368577) mix was used. SYBR Green master mix (Thermo scientific, USA) was employed as a template for qRT-PCR amplification of cDNA from testis. Macrogen Company (Seoul, South Korea) synthesized the primers. Primers for qPCR amplification are listed in Table 1. The CFX96 Touch RT-PCR (Bio-Rad, USA) was used to analyze the collected qPCR data using the $^{2-\Delta\Delta}$CT technique. As a control, the gene β-actin was compared as internal standard and matched with examined genes under analysis. Values of comparative-cycle threshold (CT) were used to assess the mRNA expression and intensity studied genes.

2.8. Testicular Histopathology. Tissues from the testicles were preserved by immersion in 10% neutral buffered formalin. After being cleared in Ultra Clear^{TM} clearing agent, impregnated in methanol, and blocked in paraffin wax, the fixed specimens were rinsed in distilled water and dehydrated in ethyl alcohol. The tissue was then sectioned at a thickness of 4 μm. [41]. H&E stains were used to stain the testicular sections, then mounted in Canada Balsam and viewed under a light microscope for any histological alterations induced by acrylamide [42]. The stained sections were examined microscopically and then underwent multiparametric quantitative morphometric and lesion scoring as assessed previously [43]. All measurements were performed using AmScope software ToupView, AmScope, USA, and the results were shown in percentages (means $\pm$ SE) for four different slides per each group.

2.9. Statistical Analysis. The obtained data are presented as means $\pm$ SEM using SPSS software for Windows (version 26, IBM Analytics, NY, USA), with one-way ANOVA and Dunnett's post hoc descriptive test. The statistical

Table 1: Primer sequences of gene expression in testis of rats.

Primer sequence	Direction	Gene	Accession number
TAACCGGGTGCGGTAGAGTA	Antisense	Caspase-3	NM 012922
GAGCTTGGAACGCGAAGAAA	Sense		
TGACATCTCCCTGTTGACGC	Antisense	Bcl-2	NM_016993
ACTCTTCAGGGATGGGGTGA	Sense		
TGTCCTGCTGTATGCTGCTT	Antisense	Nrf2	NM_031789.2
TTGTAGATGACCATGAGTCGC	Sense		
ATGTGCCAGGCATCTCCTTC	Antisense	HO-1	NM_012580.2
GTAAATGCAGTGTTGGCCCC	Sense		
CGCAGCTCAGTAACAGTCCG	Antisense	β-actin	NM 031144
AGGAGTACGATGAGTCCGGC	Sense		

Table 2: Effects of *TPJ* against acrylamide-induced oxidative stress biomarkers in rats.

Groups	GSH (U/mL)	SOD (U/mL)	MDA (nmol/mL)
Control	13.2 ± 1.3	18.1 ± 0.9	10.2 ± 1.2
TPJ	14.6 ± 1.7	20.8 ± 1.8	9.8 ± 0.9
Acrylamide	$8.9 \pm 1.7^{*}$	$10.1 \pm 1.3^{*}$	$42.7 \pm 5.8^{*}$
TPJ + Acrylamide	$12.9 \pm 1.1^{\#}$	$17.6 \pm 1.5^{\#}$	$22.9 \pm 2.7^{\#}$

Note: Values are presented as means ± standard error for six rats per group. Values are considered significant at $^{*}P < 0.05$ relative to control and TPJ, and at $^{\#}P < 0.05$ versus acrylamide group. TPJ, Taify pomegranate juice.

Table 3: Effects of TPJ against acrylamide-altered inflammatory cytokines levels.

	Control	TPJ	Acrylamide	TPJ + acrylamide
TNF-α	200.2 ± 15.0	194 ± 12.0	$483.4 \pm 28.1^{*}$	$301.1 \pm 13.1^{\#}$
IL-1β	160.7 ± 17.1	171.2 ± 16.9	$411.0 \pm 33.0^{*}$	$274.4 \pm 16.4^{\#}$
IL-6	156.6 ± 14.1	163.7 ± 17.3	$389.8 \pm 30.5^{*}$	$260.4 \pm 26.1^{\#}$

Note: Values are presented as means ± standard error for six rats per group. Values are considered significant at $^{*}P < 0.05$ relative to control and TPJ, and at $^{\#}P < 0.05$ versus acrylamide group. TPJ, Taify pomegranate juice. Each samples are measured on triplicates to confirm the obtained results.

significance level was $P < 0.05$ value between groups. Prior to data analysis, the homogeneity of the variances for the groups and populations was subsequently tested by Levene's test by the SPSS program.

3. Results

3.1. Boosting Effects of TPJ against Acrylamide-Induced Changes on Oxidative Stress. As seen in Table 2, and compared with control rats, acrylamide-administered rats showed a significant rise in MDA levels as well as a clear decreases in SOD and GSH levels. All oxidative stress biomarkers, except MDA, were shown to be reduced in acrylamide-treated rats, including SOD and GSH (Table 2). Pomegranate juice pretreatment inhibited the increase in MDA and restored altered oxidative stress markers such as GSH and SOD (Tables 2).

3.2. Protective Effects of TPJ against Acrylamide-Induced Changes on Inflammatory Cytokines Secretions. As seen in Table 3 acrylamide increased inflammation associated markers, as there were an increase in serum levels of IL-1β, TNF-α, and IL-6. When animals preadministered by TPJ, there were restorning and normalization in the levels of IL-1β, TNF-α, and IL-6 (Table 3).

3.3. Impacts of TPJ on Acrylamide-Induced Changes on Sperm Characteristics and Reproductive Hormones. When the control compared with TPJ groups, the acrylamide-administered group showed low-sperm number, motility, and viability, as well as elevation on the sperm abnormalities and deformities (Table 4). Preadministration of TPJ restored significantly the percentage of motility, concentration, and viabilities between groups. Compared to other groups, acrylamide administration decreased serum levels of LH, FSH, and testosterone (Table 4). Protective group, TPJ restored significantly the decrease reported for FSH, LH, and testosterone group, Table 5.

3.4. Mitigating Effects of TPJ on Acrylamide-Altered Testicular Gene Expressions. The mRNA expression of enzymes incorporated in oxidative stress of testis was quantified using qRT-PCR. The expression of nuclear factor erythroid-2 (Nrf2) and hemoxygenase-1 (HO-1) was reduced in rats given acrylamide (Figure 2). When TPJ was preadministered to acrylamide-treated rats, these oxidative stress indicators were elevated and normalized.

In addition, Figure 3 demonstrates that acrylamide-treated groups exhibit significant upregulation in the expression of caspase-3 gene, which is downregulated in TPJ + acrylamide-administered rats. In the same figure (Figure 3), a significant decrease in B-cell lymphoma-2 (Bcl-2) gene expression in the acrylamide-treated rats. Preadministration of TPJ to acrylamide-administered rats, restored the down expression of Bcl-2 gene (Figure 3).

3.5. Impacts of TPJ against Acrylamide-Induced Changes on Testis Histology. As shown in Figures 4(a) and 4(b), testis of control and TPJ was consisted of numerous seminephrous tubules which was lined by the series of spermatogenic cells (S) at different stages of maturation with normal

Table 4: The effects of *TPJ* against acrylamide induce changes on sperm parameters rats.

	Motility (%)	Concentration (×1,000)	Viability (%)	Abnormal morphology (%)
Control	61.3 ± 5.2	40.0 ± 4.0	62.0 ± 7.3	13.2 ± 2.4
TPJ	70.1 ± 8.4	43.1 ± 8.8	68.0 ± 7.2	9.9 ± 0.8
Acrylamide	$29.8 \pm 5.8^{*}$	$18.2 \pm 4.23^{*}$	$31.6 \pm 6.3^{*}$	$37.1 \pm 3.5^{*}$
TPJ + Acrylamide	$49.3 \pm 4.6^{\#}$	$36.5 \pm 6.2^{\#}$	$49.5 \pm 3.8^{\#}$	$16.1 \pm 2.4^{\#}$

Note: Values are presented as means ± standard error for six rats per group. Values are considered significant at $^{*}P < 0.05$ relative to control and TPJ, and at $^{\#}P < 0.05$ versus acrylamide group. TPJ, Taify pomegranate juice.

Table 5: Effects of TPJ on acrylamide induced changes in reproductive hormones.

	FSH (ng/mL)	LH (mIU/mL)	Testosterone (pg/mlL)
Control	6.7 ± 0.6	26.1 ± 1.1	4.6 ± 0.6
TPJ	7.1 ± 0.3	27.3 ± 0.7	5.5 ± 0.5
Acrylamide	$3.4 \pm 0.1^{*}$	$13.1 \pm 2.4^{*}$	$2.1 \pm 0.2^{*}$
TPJ + Acrylamide	$5.7 \pm 1.4^{\#}$	$22.4 \pm 1.9^{\#}$	$3.9 \pm 0.11^{\#}$

Note: Values are means ± standard error (SEM) for six different rats per treatment. Values are presented as means ± standard error for six rats per group. Values are considered significant at $^{*}P < 0.05$ relative to control and TPJ, and at $^{\#}P < 0.05$ versus acrylamide group. TPJ, Taify pomegranate juice; LH, Luteinizing hormone; FSH, Follicle stimulating hormone. Each samples are measured on triplicates to confirm the obtained results.

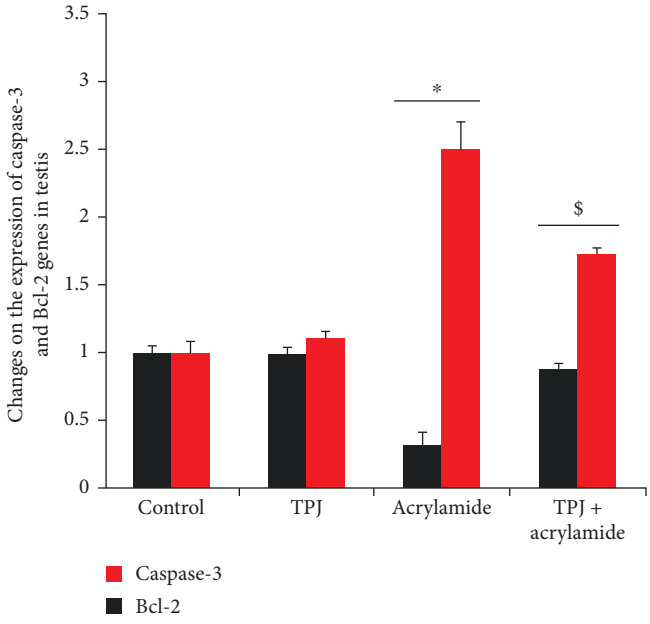

Figure 2: Quantification of mRNA expression of HO-1and Nrf2 genes. The graphic presentations of examined genes were based on qRT-PCR analysis for HO-1and Nrf2 after normalization with β-actin in different groups. $^{*}P < 0.05$ versus the control and TPJ groups; $^{\$}P < 0.05$ versus acrylamide-administered group. Gene expression was carried out on triplicate to confirm reliability of obtained results.

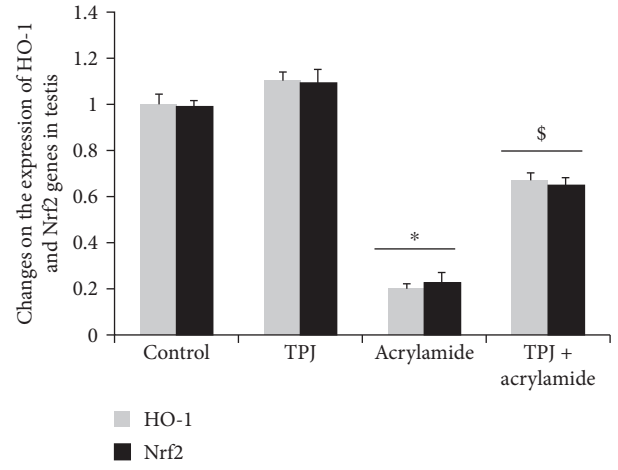

Figure 3: Quantification of mRNA expression of caspase-3 and Bcl-2 genes. The graphic presentations of examined genes were based on qRT-PCR analysis for caspase-3 and Bcl-2 after normalization with β-actin in different groups. $^{*}P < 0.05$ versus the control and TPJ groups; $^{\$}P < 0.05$ versus acrylamide-administered group. Gene expression was carried out on triplicate to confirm reliability of obtained results.

spermatozoa (Z). The spermatozoa was numerous and occupied the lumen of the tubules. In acrylamide injected rats, the seminephrous tubules showed odema (O) and degeneration (d) in the primary and secondary spermatocytes. There was a vaculation in some cells (V) were seen in some seminephrous tubules (Figure 4(c)). In the protective group, the testicular tissues showed a progressive improvement. Thickening in the interstitial CT under the seminiferous tubules basement is deceased but still persist (Figure 4(c)). The degree and lesion scoring of affected testis are induced by acrylamide and possible protection by TPJ is listed in Table 6.

4. Discussion

The present study confirmed that TPJ is effective to counter act acrylamide induced testicular oxidative stress. TPJ increased the antioxidants activity and showed anti-inflammatory impacts. Moreover, it increased semen quality and improved its characteristics and restored the decrease in the reproductive hormone levels. It upregulated Nrf2 and HO-1 expression as a biomarkers for oxidative stress and controlled apoptosis associated genes (caspase-3 and Bcl-2).

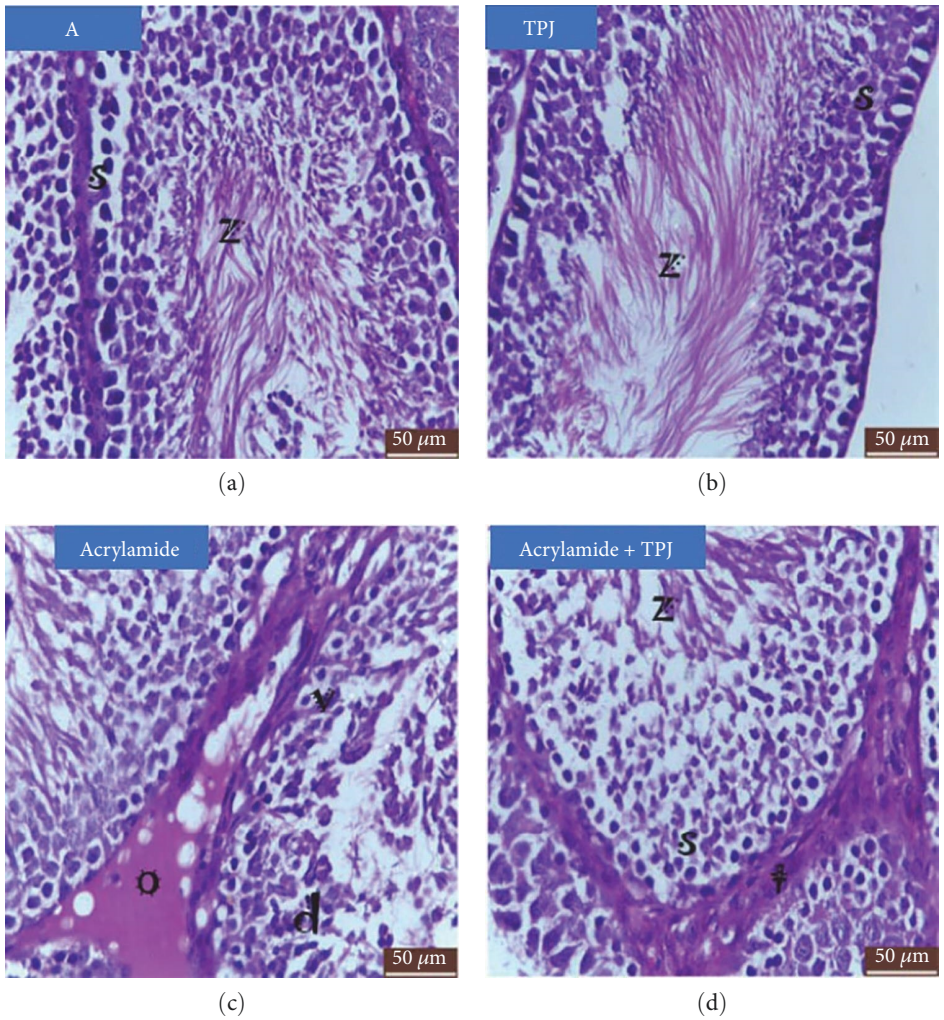

Figure 4: Testis histopathology after acrylamide and TPJ administration in rats. (a, b) testis of control and TPJ with numerous seminephrous tubules and intact spermatogenic cells (S) with normal spermatozoa (Z). (c) Acrylamide group show odema of seminephrous tubules (O) and degeneration (d) in the primary and secondary spermatocytes, and vaculation in some cells (V). (d) The protective group showing progressive improvement in the degenerative changes reported in acrylamide group. Stain H&E, bar = 50 μm.

Table 6: Morphometric analysis and lesion scoring in the testicular tissues of rats in response to acrylamide treatment and protection by TPJ.

Lesion	Control	TPJ	Acrylamide	TPJ + ACR
Mean diameter of ST	200.5 ± 3.01^{a}	293.7 ± 5.1^{a}	131.1 ± 3.9^{a}	2280.6 ± 2.1^{a}
Numbers of STs/10x	24.2 ± 0.9^{a}	25 ± 0.2^{a}	13.5 ± 0.4^{a}	27.7 ± 0.5^{a}
Height of germinal epithelium	70.2 ± 3.5^{a}	67.6 ± 1.6^{a}	31.01 ± 2.3^{b}	71.9 ± 2.7^{c}
Spermatid retention	0^{a}	0^{a}	2.1 ± 0.3^{b}	1.1 ± 0.2^{c}
Giant cell formation	0^{a}	0^{a}	0.9 ± 0.1^{b}	0.2 ± 0.003^{c}
Interstitial inflammatory infiltrate	0^{a}	0^{a}	2.5 ± 0.2^{b}	0.9 ± 0.2^{c}
Interstitial edema	0^{a}	0^{a}	5.4 ± 1.2^{b}	3 ± 0.3^{c}
Interstitial congestion	0^{a}	0^{a}	6 ± 0.9^{b}	$4 \pm .6^{c}$
Germ-cell vacuolation	0^{a}	0^{a}	10.8 ± 1.6^{b}	6.7 ± 1.2^{c}
Germ-cell exfoliation	0^{a}	0^{a}	9.1 ± 0.9^{b}	5.6 ± 0.9^{c}
Germ-cell depletion	0^{a}	0^{a}	6.8 ± 1.1^{b}	2.6 ± 0.2^{c}
Germ-cell necrosis	0^{a}	0^{a}	2.9 ± 0.3^{b}	0.9 ± 0.1^{c}
Sperm stasis	0^{a}	0^{a}	2.7 ± 0.5^{b}	1.1 ± 0.4^{c}

Note: Values are mean ± SE for three slides/group. Means within the same row (in each parameter) carrying different superscripts are significantly different at $P < 0.05$. TPJ, Taify pomegranate juice.

Plants that grown at HA respond by creating more antioxidants than plants growing at lower altitude [44, 45]. *Scrophularia striata* grown at 600 m above sea level showed high levels of total phenolics together with high-antioxidant activity [46]. HA was found to be adversely associated with tannin concentration and positively related to flavonoids, total phenolics contents, rutin, and antioxidant activity of some plants grown at HA [47, 48]. The phytochemical composition of plants is mostly affected by their elevation above sea level and altitude. Plants grown at HA showed high-biological functions because they produce more bioactive chemicals such as polyphenols and flavonoids Serafini and Peluso [49]. This explains the potential effect for pomegranate as nutritional source with more antioxidant, anti-inflammatory, antimicrobial, and antiproliferative properties [50, 51].

SOD, and GSH as endogenous antioxidant enzymes are affected by oxidative stress via binding the Nrf2 transcriptional factor with antioxidant-responsive-elements in the promoter areas. Nrf2 controls antioxidant responsive elements-regulated genes. Nrf2 is moved to the nucleus to stimulate the transcription and expression of HO-1 [52]. These explains the down expression and secretion reported by the acrylamide groups and the potential ameliorative effect of TPJ. TPJ is power antioxidant factor because of high contents of polyphenols and flavonoids [26, 53]. In a previous investigation, acrylamide decreased caspase-3 activity, and male reproductive hormones and were ameliorated by crocin [20].

A variety of harmful agents were tested to see if they could be mitigated by antioxidant molecules, which are widely utilized as nutrients. Here pomegranate juice confirmed this hypothesis as it showed ameliorative impacts against acrylamide induced testicular dysfunction and testicular inflammation. Inflammation occurred when a physical, chemical, or biological material enters our bodies [54]. Continuous or chronic inflammation, on the other hand, is hazardous since it contributes to male infertility development [55, 56]. Several pro-inflammatory transcription of some mediators or cytokines (IL-1, TNF-α, and IL-6) are supported by NF-κB; central regulator of inflammation [57, 58]. These mediators play major functions in regulation of spermatogenesis, control normal fertility, testicular steroidogenesis, and semen maturation [59, 60]. Acrylamide administration stimulated the secretion of IL-1β, TNF-α, and IL-6 reported in this study and TPJ administration controlled the oxidative stress in testis and prevented extension of acrylamide effects to maintain normal tests function. The adverse effects of acrylamide on inflammatory cytokines were described in part in the other studies [61, 62].

Flavonoids may prevent cell death and DNA damage because they reduce oxidative stress and inflammation. We found that mRNA expression levels of caspase-3 was lower in the flavonoid-supplemented animals than in the acryamide-intoxicated animals, while the mRNA expression level of Bcl-2 was higher. Previous research demonstrated that ROS and inflammatory stimuli might cause not only apoptosis and DNA damage, but also the synthesis and release of other mediators. In order to prevent and treat organ damage caused by acrylamide, it was necessary to target Bcl-2 and oxidative stress biomarkers such as Nrf2 and HO-1. Our research verified this notion by showing that flavonoids dramatically decreased levels of Nrf2 and HO-1. Supplementation with pomegranate juice may modify oxidative stress, inflammation, and cell apoptosis, which may reduce the severity of organ injuries and function failure.

Caspase-3 and Bcl-2, both are the primary apoptosis regulator genes [63]. Both inflammation and oxidative stress activate the expression of Caspase-3 and a Bcl-2 gene family member [64]. Caspase-3 activation and upregulation inhibit Bcl-2 activity and consequently apoptosis. Caspase-3 upregulation and activation reduce Bcl-2 function that result in apoptosis [65]. The current investigation confirmed that acrylamide-regulated caspase-3 and Bcl-2 expression (upregulation and downregulation consequently). These findings are consistent with those of others [65, 66], who found that testicular damage is related with increased germ cell apoptosis. TPJ was found to have an abrogative impact against these acrylamide-induced adverse effects.

5. Conclusion

TPJ showed positive antioxidants, anti-inflammatory, and antioxidative stress impacts against acrylamide-induced testicular dysfunction through normalizing decreased antioxidants, decreased male reproductive hormones and semen parameters, and controlling the gene expression of genes associated with oxidative stress and apoptosis.

Abbreviations

ACR: Acrylamide
Bcl-2: B cell lymphoma-2
CNT: Control
FSH: Follicle stimulating hormone
GSH: Reduced glutathione
HA: High altitude
HO-1: Hemoxygenase-1
IL-1b: Interleukin-1
LH: Leutinizing hormone
MDA: Malondialdehyde
Nrf2: Nuclear factor erythroid-2
qRT-PCR: Quantitive real time PCR
SOD: Superoxide dismutase
TNF-α: Tumor necrosis factor alpha
TPJ: Taify pomegranate juice.

Data Availability

Data supporting this research article are available upon request.

Ethical Approval

All experimental procedures were carried out under National Institutes of Health Guidelines for the care and use of laboratory animals, and all procedures designed to minimize the suffering of animals were followed.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] J.-Y. Han and C.-W. Zhang, "Toxicity study for acrylamide," *Wei Sheng Yan Jiu*, vol. 35, no. 4, pp. 513–515, 2006.

[2] M. Kopanska, R. Muchacka, J. Czech, M. Batoryna, and G. Formicki, "Acrylamide toxicity and cholinergic nervous system," *Journal of Physiology and Pharmacology*, vol. 69, no. 6, 2018.

[3] A. Koszucka, A. Nowak, I. Nowak, and I. Motyl, "Acrylamide in human diet, its metabolism, toxicity, inactivation and the associated European Union legal regulations in food industry," *Critical Reviews in Food Science and Nutrition*, vol. 60, no. 10, pp. 1677–1692, 2020.

[4] M. Huang, J. Jiao, J. Wang, Z. Xia, and Y. Zhang, "Characterization of acrylamide-induced oxidative stress and cardiovascular toxicity in zebrafish embryos," *Journal of Hazardous Materials*, vol. 347, pp. 451–460, 2018.

[5] T. Imai and T. Kitahashi, "A 13-week toxicity study of acrylamide administered in drinking water to hamsters," *Journal of Applied Toxicology*, vol. 34, no. 1, pp. 57–65, 2014.

[6] Y. Komoike and M. Matsuoka, "*In vitro* and *in vivo* studies of oxidative stress responses against acrylamide toxicity in zebrafish," *Journal of Hazardous Materials*, vol. 365, pp. 430–439, 2019.

[7] L. Rifai and F. A. Saleh, "A review on acrylamide in food: occurrence, toxicity, and mitigation strategies," *International Journal of Toxicology*, vol. 39, no. 2, pp. 93–102, 2020.

[8] J. Tabeshpour, S. Mehri, K. Abnous, and H. Hosseinzadeh, "Role of oxidative stress, MAPKinase and apoptosis pathways in the protective effects of thymoquinone against acrylamide-induced central nervous system toxicity in rat," *Neurochemical Research*, vol. 45, pp. 254–267, 2020.

[9] Z. Lin, P. Luo, D. Huang, Y. Wu, F. Li, and H. Liu, "Multi-omics based strategy for toxicity analysis of acrylamide in *Saccharomyces cerevisiae* model," *Chemico-Biological Interactions*, vol. 349, Article ID 109682, 2021.

[10] S. M. Murray, B. M. Waddell, and C.-W. Wu, "Neuron-specific toxicity of chronic acrylamide exposure in *C. elegans*," *Neurotoxicology and Teratology*, vol. 77, Article ID 106848, 2020.

[11] M. Goudarzi, M. A. Mombeini, I. Fatemi et al., "Neuroprotective effects of ellagic acid against acrylamide-induced neurotoxicity in rats," *Neurological Research*, vol. 41, no. 5, pp. 419–428, 2019.

[12] J. Guo, X. Cao, X. Hu, S. Li, and J. Wang, "The anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on acrylamide-induced neurotoxicity in rats," *BMC Pharmacology and Toxicology*, vol. 21, Article ID 62, 2020.

[13] F. Zhu, J. Wang, J. Jiao, and Y. Zhang, "Exposure to acrylamide induces skeletal developmental toxicity in zebrafish and rat embryos," *Environmental Pollution*, vol. 271, Article ID 116395, 2021.

[14] L. Deng, M. Zhao, Y. Cui et al., "Acrylamide induces intrinsic apoptosis and inhibits protective autophagy *via* the ROS mediated mitochondrial dysfunction pathway in U87-MG cells," *Drug and Chemical Toxicology*, vol. 45, no. 6, pp. 2601–2612, 2022.

[15] S. G. Kunnel, S. Subramanya, P. Satapathy, I. Sahoo, and F. Zameer, "Acrylamide induced toxicity and the propensity of phytochemicals in amelioration: a review," *Central Nervous System Agents in Medicinal Chemistry*, vol. 19, no. 2, pp. 100–113, 2019.

[16] V. N. Dobrovolsky, M. Monserrat Pacheco-Martinez, L. Patrice McDaniel, M. G. Pearce, and W. Ding, "In vivo genotoxicity assessment of acrylamide and glycidyl methacrylate," *Food and Chemical Toxicology*, vol. 87, pp. 120–127, 2016.

[17] H.-J. Yang, S.-H. Lee, Y. Jin et al., "Toxicological effects of acrylamide on rat testicular gene expression profile," *Reproductive Toxicology*, vol. 19, no. 4, pp. 527–534, 2005.

[18] N. Yildizbayrak and M. Erkan, "Acrylamide disrupts the steroidogenic pathway in leydig cells: possible mechanism of action," *Toxicological & Environmental Chemistry*, vol. 100, no. 2, pp. 235–246, 2018.

[19] E.-T. Wang, D.-Y. Chen, H.-Y. Liu, H.-Y. Yan, and Y. Yuan, "Protective effect of allicin against glycidamide-induced toxicity in male and female mice," *General Physiology and Biophysics*, vol. 34, no. 2, pp. 177–187, 2015.

[20] M. Gül, E. K. Kuştepe, M. E. Erdemli et al., "Protective effects of crocin on acrylamide-induced testis damage," *Andrologia*, vol. 53, no. 9, Article ID e14176, 2021.

[21] J. Favor and M. D. Shelby, "Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure," *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, vol. 580, no. 1-2, pp. 21–30, 2005.

[22] S. Kaçar, V. Şahintürk, B. Can, and A. Musmul, "L-cysteine partially protects against acrylamide-induced testicular toxicity," *Balkan Medical Journal*, vol. 35, no. 4, pp. 311–319, 2018.

[23] H. Wang, J.-Y. Ge, Z.-Q. Zhou, Z.-C. Wang, and F.-X. Shi, "Oral acrylamide affects the development and reproductive performance of male rats," *Zhonghua Nan Ke Xue*, vol. 13, no. 6, pp. 492–497, 2007.

[24] Y. M. Al-Sodany, S. A. Bazaid, and H. A. Mosallam, "Medicinal plants in Saudi Arabia: I. Sarrwat mountains at Taif, KSA," *Academic Journal of Plant Sciences*, vol. 6, no. 4, pp. 134–145, 2013.

[25] M. M. Ahmed, E.-S. A. Samir, A. M. El-Shehawi, and M. E. Alkafafy, "Anti-obesity effects of Taif and Egyptian pomegranates: molecular study," *Bioscience, Biotechnology, and Biochemistry*, vol. 79, no. 4, pp. 598–609, 2015.

[26] A. M. El-Shehawi, S. Sayed, M. M. Hassan et al., "Taify pomegranate juice (TPJ) abrogates acrylamide-induced oxidative stress through the regulation of antioxidant activity, inflammation, and apoptosis-associated genes," *Frontiers in Veterinary Science*, vol. 9, Article ID 833605, 2022.

[27] S. Sayed, S. S. Alotaibi, A. M. El-Shehawi et al., "The anti-inflammatory, anti-apoptotic, and antioxidant effects of a pomegranate-peel extract against acrylamide-induced hepatotoxicity in rats," *Life*, vol. 12, no. 2, Article ID 224, 2022.

[28] M. Cano-Lamadrid, F. C. Marhuenda-Egea, F. Hernández, E. C. Rosas-Burgos, A. Burgos-Hernández, and A. A. Carbonell-Barrachina, "Biological activity of conventional and organic pomegranate juices: antioxidant and antimutagenic potential," *Plant Foods for Human Nutrition*, vol. 71, pp. 375–380, 2016.

[29] T. S. Salles, M. D. F. Meneses, L. A. Caldas et al., "Virucidal and antiviral activities of pomegranate (*Punica granatum*)

extract against the mosquito-borne Mayaro virus," *Parasites & Vectors*, vol. 14, Article ID 443, 2021.

[30] H. Song, X. Shen, Q. Chu, and X. Zheng, "Pomegranate fruit pulp polyphenols reduce diet-induced obesity with modulation of gut microbiota in mice," *Journal of the Science of Food and Agriculture*, vol. 102, no. 5, pp. 1968–1977, 2022.

[31] M. Kujawska, M. Jourdes, L. Witucki et al., "Pomegranate juice ameliorates dopamine release and behavioral deficits in a rat model of Parkinson's disease," *Brain Sciences*, vol. 11, no. 9, Article ID 1127, 2021.

[32] B. Jacob, N. M. Sureshbabu, M. Ranjan, A. Ranganath, and R. Siddique, "The antimicrobial effect of pomegranate peel extract versus chlorhexidine in high caries risk individuals using quantitative real-time polymerase chain reaction: a randomized triple-blind controlled clinical trial," *International Journal of Dentistry*, vol. 2021, Article ID 5563945, 14 pages, 2021.

[33] Z. Derakhshan, M. Ferrante, M. Tadi et al., "Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds," *Food and Chemical Toxicology*, vol. 114, pp. 108–111, 2018.

[34] L. Fernandes, J. A. C. Pereira1, I. Lopéz-Cortés, D. M. Salazar, and E. C. D. Ramalhosa, "Physicochemical changes and antioxidant activity of juice, skin, pellicle and seed of pomegranate (cv. Mollar de Elche) at different stages of ripening," *Food Technology and Biotechnology*, vol. 53, no. 4, pp. 397–406, 2015.

[35] T. Kaseke, U. L. Opara, and O. A. Fawole, "Effect of blanching pomegranate seeds on physicochemical attributes, bioactive compounds and antioxidant activity of extracted oil," *Molecules*, vol. 25, no. 11, Article ID 2554, 2020.

[36] T. Kaseke, U. L. Opara, and O. A. Fawole, "Effects of enzymatic pretreatment of seeds on the physicochemical properties, bioactive compounds, and antioxidant activity of pomegranate seed oil," *Molecules*, vol. 26, no. 15, Article ID 4575, 2021.

[37] A. Bhatia, P. P. Saikia, B. Dkhar, and H. Pyngrope, "Anesthesia protocol for ear surgery in Wistar rats (animal research)," *Animal Models and Experimental Medicine*, vol. 5, no. 2, pp. 183–188, 2022.

[38] M. M. Soliman, A. Aldhahrani, A. Gaber et al., "Impacts of n-acetyl cysteine on gibberellic acid-induced testicular dysfunction through regulation of inflammatory cytokines, steroid and antioxidant activity," *Andrologia*, vol. 53, no. 5, Article ID e14036, 2021.

[39] M. Sönmez, G. Türk, and A. Yüce, "The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats," *Theriogenology*, vol. 63, no. 7, pp. 2063–2072, 2005.

[40] R. P. Amann, "Use of animal models for detecting specific alterations in reproduction," *Fundamental and Applied Toxicology*, vol. 2, no. 1, pp. 13–26, 1982.

[41] D. L. Sterchi, "Molecular pathology—in situ hybridization," in *Theory and Practice of Histological Techniques*, pp. 537–558, Churchill Livingstone, 6th edition, 2008.

[42] M. Slaoui and L. Fiette, "Histopathology procedures: from tissue sampling to histopathological evaluation," in *Drug Safety Evaluation*, J. C. Gautier, Ed., pp. 69–82, Humana Press, 2011.

[43] A. Behairy, N. I. El-Sharkawy, T. M. Saber et al., "The modulatory role of vitamin C in boldenone undecylenate induced testicular oxidative damage and androgen receptor dysregulation in adult male rats," *Antioxidants*, vol. 9, no. 11, Article ID 1053, 2020.

[44] A. Kirsch, "Successful treatment of metastatic malignant melanoma with *Viscum album* extract (Iscador® M)," *The Journal of Alternative and Complementary Medicine*, vol. 13, no. 4, pp. 443–446, 2007.

[45] K. L. Rebeiro, "Occupational therapy and the multidisciplinary team," *Psychiatric Services*, vol. 53, no. 6, Article ID 767, 2002.

[46] Z. Zargoosh, M. Ghavam, G. Bacchetta, and A. Tavili, "Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata* boiss," *Scientific Reports*, vol. 9, Article ID 16021, 2019.

[47] R. Kaundal, M. Kumar, S. Kumar, D. Singh, and D. Kumar, "Polyphenolic profiling, antioxidant, and antimicrobial activities revealed the quality and adaptive behavior of viola species, a dietary spice in the himalayas," *Molecules*, vol. 27, no. 12, Article ID 3867, 2022.

[48] W. Liu, D. Yin, N. Li et al., "Influence of environmental factors on the active substance production and antioxidant activity in potentilla fruticosa L. and its quality assessment," *Scientific Reports*, vol. 6, Article ID 28591, 2016.

[49] M. Serafini and I. Peluso, "Functional foods for health: the interrelated antioxidant and anti-inflammatory role of fruits, vegetables, herbs, spices and cocoa in humans," *Current Pharmaceutical Design*, vol. 22, no. 44, pp. 6701–6715, 2016.

[50] M. Abedini, N. Ramezani-Jolfaie, H. Ghasemi-Tehrani, M. J. Tarrahi, and R. Amani, "The effect of concentrated pomegranate juice on biomarkers of inflammation, oxidative stress, and sex hormones in overweight and obese women with polycystic ovary syndrome: a randomized controlled trial," *Phytotherapy Research*, vol. 37, no. 6, pp. 2255–2261, 2023.

[51] A. Zarfeshany, S. Asgary, and S. H. Javanmard, "Potent health effects of pomegranate," *Advanced Biomedical Research*, vol. 3, Article ID 100, 2014.

[52] S. Inouye, Y. Hatori, T. Kubo, S. Saito, H. Kitamura, and R. Akagi, "NRF2 and HSF1 coordinately regulate heme oxygenase-1 expression," *Biochemical and Biophysical Research Communications*, vol. 506, no. 1, pp. 7–11, 2018.

[53] V. Vučić, M. Grabež, A. Trchounian, and A. Arsić, "Composition and potential health benefits of pomegranate: a review," *Current Pharmaceutical Design*, vol. 25, no. 16, pp. 1817–1827, 2019.

[54] J. H. Park, B. M. Lee, and H. S. Kim, "Potential protective roles of curcumin against cadmium-induced toxicity and oxidative stress," *Journal of Toxicology and Environmental Health, Part B*, vol. 24, no. 3, pp. 95–118, 2021.

[55] M. P. Hedger, "Immunophysiology and pathology of inflammation in the testis and epididymis," *Journal of Andrology*, vol. 32, no. 6, pp. 625–640, 2011.

[56] T. Khamis, A. A. Hegazy, S. S. A. El-Fatah et al., "Hesperidin mitigates cyclophosphamide-induced testicular dysfunction via altering the hypothalamic pituitary gonadal axis and testicular steroidogenesis, inflammation, and apoptosis in male rats," *Pharmaceuticals*, vol. 16, no. 2, Article ID 301, 2023.

[57] Y. Temel, S. Kucukler, S. Yıldırım, C. Caglayan, and F. M. Kandemir, "Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress, inflammation, and apoptosis," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 393, pp. 325–337, 2020.

[58] S. Zhang, C. Jiang, H. Liu et al., "Fluoride-elicited developmental testicular toxicity in rats: roles of endoplasmic reticulum stress and inflammatory response," *Toxicology and Applied Pharmacology*, vol. 271, no. 2, pp. 206–215, 2013.
[59] D. B. Hales, T. Diemer, and K. H. Hales, "Role of cytokines in testicular function," *Endocrine*, vol. 10, pp. 201–217, 1999.
[60] X. Pan, X. Wu, D. Yan, C. Peng, C. Rao, and H. Yan, "Acrylamide-induced oxidative stress and inflammatory response are alleviated by N-acetylcysteine in PC12 cells: Involvement of the crosstalk between Nrf2 and NF-κB pathways regulated by MAPKs," *Toxicology Letters*, vol. 288, pp. 55–64, 2018.
[61] K. Amirshahrokhi and A. Abzirakan, "Carvedilol attenuates acrylamide-induced brain damage through inhibition of oxidative, inflammatory, and apoptotic mediators," *Iranian Journal of Basic Medical Sciences*, vol. 25, no. 1, pp. 60–67, 2022.
[62] E. Tomaszewska, S. Muszynski, I. Swietlicka et al., "Prenatal acrylamide exposure results in time-dependent changes in liver function and basal hematological, and oxidative parameters in weaned Wistar rats," *Scientific Reports*, vol. 12, Article ID 14882, 2022.
[63] D.-O. Moon, S.-Y. Park, M.-S. Heo et al., "Key regulators in bee venom-induced apoptosis are Bcl-2 and caspase-3 in human leukemic U937 cells through downregulation of ERK and Akt," *International Immunopharmacology*, vol. 6, no. 12, pp. 1796–1807, 2006.
[64] X.-X. Wang, B. Zhang, R. Xia, and Q.-Y. Jia, "Inflammation, apoptosis and autophagy as critical players in vascular dementia," *European Review for Medical and Pharmacological Sciences*, vol. 24, no. 18, pp. 9601–9614, 2020.
[65] F. Eslami, M. Mahdavi, E. Babaei et al., "Down-regulation of survivin and Bcl-2 concomitant with the activation of caspase-3 as a mechanism of apoptotic death in KG1a and K562 cells upon exposure to a derivative from ciprofloxacin family," *Toxicology and Applied Pharmacology*, vol. 409, Article ID 115331, 2020.
[66] R. Bayatmakoo, N. Rashtchizadeh, P. Yaghmaei, M. Farhoudi, and P. Karimi, "Atorvastatin inhibits cholesterol-induced caspase-3 cleavage through down-regulation of p38 and up-regulation of Bcl-2 in the rat carotid artery," *Cardiovascular Journal of Africa*, vol. 28, no. 5, pp. 298–303, 2017.