Impact of Naringin on induced testicular damage via autophagy and apoptosis pathways modulations in rats

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ABSTRACT

Acrylamide is toxic compound, which has negative impact on reproductive system. This study aimed to evaluate the ameliorating effect of naringin against testicular damage induced by acrylamide in rats. In this study, 70 adult male rats were divided into 7 groups. G1 in which rats didn’t receive any treatment. G2 in which rats were orally received naringin (80 mg/Kg). G3 included rats were injected with Epifasi (100 UI/Kg). G4 included rats were injected with acrylamide (50 mg/Kg). G5, G6 and G7 included acrylamide injected rats then each rat was received (naringin, Epifasi, naringin + Epifasi), respectively. Testosterone, estradiol, prolactin, semen analysis, the antioxidant parameter, the apoptosis and autophagy parameter were estimated. The results showed that testosterone level and in G4 significant decreased (0.4780±0.1942 ng/ dl) in compared with G1 (1.382±0.1119 ng/dl) and estradiol and prolactin level significant increased (12.32±0.7480 pg/ml, 0.5500±0.03742 ng/ml) compared with G1 (5.856±0.2430 pg/ml, 0.4930±0.03401ng/ml) respectively. Acrylamide disrupted redox hemostasis balance, increased both apoptotic and autophagy marker. The results confirmed that combined treatment of naringin and Epifasi in G7 gave the best recovery of toxic effect of acrylamide on biochemical and histological analysis via hormonal disturbance recovery, reliving the cellular redox homeostasis, promoting the anti-apoptotic factor and inhibition of autophagy pathway.
Introduction

Acrylamide (ACR) is an organic component that has several industrial uses as acrylamide copolymers production, plastics, paper, cosmetics, textiles, and polyacrylamide gel synthesis. Acrylamide is very harmful on human health. It has a toxic effect on different organs particularly to both nervous and reproductive systems (Amirshahrokhi and Abzirakan, 2022). ACR has several effects on reproductive parameters in males, involving increasing in abnormal sperm morphology, testicular deterioration indicating that acrylamide give rise to reproductive toxicity in males (Kalaivani et al., 2018). The most prevalent sources of acrylamide to humans are both foods and tobacco. It is also produced through cooking of starch rich foods at high temperatures up to 120 °C (Pietropaoli et al., 2022).

Naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside; NARG) is a flavone glycoside existing in most of citrus fruits with influence on both biological and pharmacological impact including antioxidant activity, anti-carcinogenic activities, and suppression of cytochrome P450 enzymes (Izawa et al., 2010). Its derivatives naringenin has also various biological efficiencies like antiviral, antibacterial, anti-inflammatory, anti-adipogenic and cardioprotective impacts, beside its ability to overpass the blood–brain barrier and to spend multiple neuronal impacts (Salehi et al., 2019).

Epifasi drug is human chronic gonadotropin (HCG) used as hormonal therapy via enhanced the leyding cells output testosterone. This hormonal treatment is utilized presently for encourage the propagation and maturation of germ cells to ameliorate fertility (Mathers et al., 2009).

In spite of the potential effect of the hormonal therapy against testicular damage, this hormonal therapy has several toxic effects. So, this study aimed to evaluate the ameliorating effect of naringin (as a natural therapy) with and without the regular hormonal therapy against testicular damage induced by acrylamide in rats.

Material and Methods

About 70 adult male albino rats (12 weeks old, 150-165 g) were obtained from the Egyptian Organization for Biological Products and Vaccines in Cairo and fed on the standard rodent diet (Egyptian Company of Oils and Soap Kafr El Zayat Egypt) and water available ad libitum. The study was carried out according to the Committee of the Scientific Research Ethics of Faculty of Science, Damanhour University, code No. DMU-SCI-CSRE-22-10-04.

Animal Treatments:
The rats were adapted for only one week in the animal house and then they were randomly distributed into seven groups (10 rats for each):

Group 1 (Control): Rats pertained as control (did not receive any treatment).

Group 2 (Naringin): Rats were daily orally received naringin pruched from Acros organics (brand Thermo Fisher Scientific) using gavage tube (80 mg/Kg.body weight) for 15 days (Adil et al., 2014).
Group 3 (Epifasi): Rats were daily subcutaneously (SC) injected with Epifasi (hCG) purchased from Egyptian Int. Pharmaceutical Industries Co. (EIPICO) Egypt by a dose of 100UI/Kg for 15 days (Altoé et al., 2014).

Group 4 (ACR): Rats were intraprotentially (I.P.) injected with acrylamide (purchased from Alpha Chemika, India) in a dose of 50 mg/Kg.body weight daily for 10 days to induce testicular toxicity (Tyl and Friedman, 2003).

Group 5 (ACR+NARG): rats were injected with acrylamide 10 days then each rat will be orally received naringin for another 15 days by the recommended doses.

Group 6 (ACR+Epifasi): rats were injected with acrylamide 10 days then each rat was orally received a daily dose of Epifasi (hCG) for another 15 days by the recommended doses.

Group 7 (ACR+NARG+Epifasi): rats were injected with acrylamide for 10 days to induce testicular damage. Then rats were simultaneously received combined treatment of Epifasi (hCG) and naringin for another 15 days by the recommended doses.

Blood and Tissue Homogenate Preparation:
By the end of the experiment planning, rats were euthanized after fasting for 10 hours with infusion of sodium pentobarbital intraperitoneal and exposed to a total autopsy. Blood samples of each rat were obtained from sub-par vena cava, assembled in non-heparinized tubes, and kept for 20-30 minutes. Subsequently, blood samples were centrifuged at 5000 rpm for 10 minutes to separate the sera that were stored as aliquots at -80°C until required.

Testis tissues were carefully ejected from rats, then rinsed with ice-cold saline three times then freshen on ice, severed into two sections, one part were settled in 10% neutral buffered formalin that utilized for histological examination. The second part was were rinsed with a combined solution of Phosphate Buffered Saline (PBS) pH 7.4 and 0.16 mg/mL heparin to shine any red blood cells and clots from tissues before preservation in aluminum foil and stored at -80°C until need.

Testis tissues were used for different biochemical parameters as antioxidants, concentrations detection of Bcl2 and caspase-3, and gene expression of LC3 and Atg5. Sperm count also was carried out. The tissues were homogenized in 5-10 mL cold buffer (almost 50 mM potassium phosphate pH 7.5, 1 mM EDTA) per gram tissue. Afterwards the specimens were centrifuged at 5000 rpm for 15 min at 4°C. The supernatant were cut off for investigate and step aside on ice (Beltagy et al., 2021).

Hormonal Detection:
Detection of testosterone, prolactin, and estradiol concentrations were carried out using rat ELISA Kit purchased from MyBioSource, Inc., USA (CAT. No. MBS282195, CAT. No. MBS2019961, and, CAT No. MBS702969, respectively).

Semen analysis:
A small fraction of the caudal epididymis of each rat was dissected and situated in 1 mL of pre-warmed Hams F10 medium. Gentle ripping of the tissue was carried out to allow spermatozoa swimming out in the culture medium. The dishes were incubated in the CO₂ incubator for 15 minutes at 37°C, 5% CO₂. Then sperm count and
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Abnormalities were carried out by using hemocytometer and light microscopy (OPTIKA, Italy). Motility was assorted as attribution of progressive (fast and slow) and non-progressive spermatozoa. Additionally, eosin staining and light microscopy detected the proportion of sperm cells with normal morphology (Pourrentezari et al., 2014).

Oxidative Stress Biomarkers:

Determination of reduced Glutathione (GSH) was colorimetrically assessed by employing the BioDiagnostic kit (CAT. No GR 2511) Egypt. The reaction based on transformation of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to 5'-Thionitrobenzoate (TNB). The yellowish dye precipitate was measured at 412 nm (Beutler, 1963). The lipid peroxide (Malondialdehyde, MDA) was evaluated according to its reaction with thiobarbituric acid (TBA) in acidic media for 30 min at 95°C outcome pink product that can be detected at 534 nm (Ohkawa et al., 1979). Super Oxides Dismutase enzyme (SOD) (EC 1.15.1.1) estimated by the commercial kit (Biodiagnostic kit, Egypt, CAT. No # SD2521) relying on the inhibition effect of the enzyme on the reduction of nitro-blue terazosin dye via phenazine methosulfate. Both control and sample were evaluated 560 nm at 25°C (Nishikimi, 1975). Catalase enzyme (EC 1.11.1.6) was colorimetrically determined by using Biodiagnostic kit, Egypt (CAT. No # SD2517). The density of colored end product (quinoneimine dye) which resulted due to the interaction between both catalase and norm amount of H2O2 was evaluated at 510 nm (Saggu et al., 2014).

Apoptotic marker determination:

Detection of the Bcl2 and Caspase 3 were performed using rat ELISA Kit purchased from Shanghai Sunred Biological Technology Co., Ltd, China (CAT. No. 201-11-0038, CAT. No. 201-11-0281, respectively).

Detection of Atg5 and LC3 by real time PCR:

Total mRNA was isolated from rat testis tissues using Gene JET RNA Purification Kit (Thermo Scientific, # K0731). Then the isolated mRNA (5 μg) was reverse transcribed into cDNA using Revert Aid first standard cDNA synthesis kits (Thermo Scientific, #K 1622). The produced cDNA was used as a template to determine the relative expression of the Atg5 and LC3 genes in relative to the house keeping gene (GAPDH) using Step One Plus real time polymerase chain reaction (RT-PCR) system (Applied Biosystem). The primers sequences were designed using national center of biotechnology information (ncbi) database (Table 1). A 25-μL PCR mix was prepared by adding 12.5 μL of 2X Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221), 2 μL of cDNA template, 1 μL forward primer, 1 μL reverse primer, and 8.5 μL of nuclease-free water. The conditions of thermal cycling were as follows: initial denaturation at 95°C for 10 minutes, 40 to 45 cycles of amplification of DNA denaturation at 95°C for 15 seconds, annealing at 60°C for both Atg5 and LC3 for 30 seconds, extension at 72°C for 30 seconds. At the end of the last cycle, the temperature was increased from 63°C to 95°C for melting curve analysis. The cycle threshold (Ct) values are calculated for target genes and the housekeeping gene, and relative gene expression was determined using 2^ΔΔCt method (Livak and Schmittgen, 2001).
Histological determination:
Testis tissues were irrigated in saline solutions, firmed in 10% neutral buffered formalin, dehydrated, refined, placed in paraffin and cutter. Paraffin sections were dappled with Ehrlich's haematoxylin stain and counterstained with eosin as a red tape method (Tousson, 2016).

Statistical Analysis:
Statistical Package for the Social Sciences (SPSS software version 16) was used to perform the statistical analysis of data. Data were presented as mean ± standard deviation (SD). Significant differences between the means were detected by one-way ANOVA (Tukey) test. The P-value lower than 0.05 were deemed as statistically significant.

Result:
Detection of Hormonal concentration:
The results show significant increase in both testosterone and Estradiol concentration in G2 and G3 in compared with G1 and significant decrease in prolactin in G3 compared with G1. In addition, the data confirmed that there are significant decrease in testosterone in G4 compared with G1 and there are significant increase in Estradiol and prolactin in G4 compared with G1. In addition, there are significant increases in testosterone in G5, G6, and G7 compared with G4. In addition, there are significant decrease in both Estradiol and prolactin in G5, G6, and G7 compared with G4 (Table 2).

Semen analysis:
The result show significant decrease in semen count in G4 compared with G1. In addition, the data show significant increase in semen count in G5, G6, and G7 compared with G4 as shown in Table 3. In addition, Fig. (1) showed the sperm abnormalities in G4 due to the effect of acrylamide on sperm structure. It show agglutination of sperm, headless and coiled tail sperm and bent neck and tail sperm.

Determination of antioxidant in testis:
The results show no significant differences in antioxidant parameters (MDA, Catalase, SOD, and GSH) in both G2 and G3 compared with G1. On the other hand, the result confirmed that there were significant increase in MDA concentration in G4 in compared with G1 and significant decrease in Catalase, SOD, and GSH in G4 compared with G1. In addition, there are significant decrease in MDA in G5, G6, and G7 compared with G4. In addition, there are significant increase in Catalase, SOD, and GSH in G5, G6, and G7 compared with G4 as shown in Table 4.
Table (2): Hormonal concentration in serum

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Testosterone (ng/dl)</th>
<th>Estradiol (pg/ml)</th>
<th>Prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td></td>
<td>1.382 ± 0.1119</td>
<td>5.856 ± 0.2430</td>
<td>0.4930 ± 0.03401</td>
</tr>
<tr>
<td>G2</td>
<td></td>
<td>1.455 ± 0.1833</td>
<td>5.981 ± 0.1899</td>
<td>0.4589 ± 0.0391</td>
</tr>
<tr>
<td>G3</td>
<td></td>
<td>1.8490 ± 0.3956*a</td>
<td>6.650 ± 0.2332*a</td>
<td>0.4365 ± 0.04056*a</td>
</tr>
<tr>
<td>G4</td>
<td></td>
<td>0.4780 ± 0.1942*a</td>
<td>12.32 ± 0.7480*a</td>
<td>0.5500 ± 0.03742*a</td>
</tr>
<tr>
<td>G5</td>
<td></td>
<td>0.7790 ± 0.1521*a,b</td>
<td>5.833 ± 0.2625b</td>
<td>0.4872 ± 0.03364b</td>
</tr>
<tr>
<td>G6</td>
<td></td>
<td>0.9570 ± 0.1972*a,b</td>
<td>5.918 ± 0.2433b</td>
<td>0.4862 ± 0.03539b</td>
</tr>
<tr>
<td>G7</td>
<td></td>
<td>1.304 ± 0.1655 b</td>
<td>5.797 ± 0.3231b</td>
<td>0.4821 ± 0.03431b</td>
</tr>
</tbody>
</table>

P value (P≤ 0.05) is significant; a: Significant compared with G1, b: Significant compared with G4

Table (3): Sperm analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Count X 10^6</th>
<th>Rapid motility (%) (Grade a)</th>
<th>Slow motility (%) (Grade b)</th>
<th>Non-progressive motility (%) (Grade c)</th>
<th>Immotile sperm (%) (Grade d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>133.9 ± 12.35</td>
<td>17.90 ± 3.327</td>
<td>22.40 ± 2.452</td>
<td>31.30 ± 2.440</td>
<td>28.40 ± 3.910</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>134.7 ± 13.52</td>
<td>19.10 ± 2.767</td>
<td>23.40 ± 2.675</td>
<td>29.90 ± 2.234</td>
<td>27.60 ± 3.779</td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>81.40 ± 9.617a</td>
<td>12.30 ± 1.780a</td>
<td>15.90 ± 1.912a</td>
<td>21.7 ± 2.846a</td>
<td>50.10 ± 3.327a</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>113.7 ± 10.24a,b</td>
<td>17.20 ± 3.706b</td>
<td>22.30 ± 2.908b</td>
<td>29.60 ± 2.066b</td>
<td>30.90 ± 4.932b</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>125.9 ± 9.938b</td>
<td>16.40 ± 3.471</td>
<td>21.30 ± 3.164</td>
<td>29.70 ± 2.263b</td>
<td>32.60 ± 4.926b</td>
</tr>
<tr>
<td></td>
<td>G7</td>
<td>133.8 ± 10.28b</td>
<td>17.50 ± 4.007b</td>
<td>22.90 ± 2.644b</td>
<td>30.20 ± 2.741b</td>
<td>29.40 ± 4.547b</td>
</tr>
</tbody>
</table>

P value (P≤ 0.05) is significant; a: Significant compared with G1, b: Significant compared with G4, C Significant compared with G7

Table (4): Antioxidant parameter in testis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>MDA (nmol/g tissue)</th>
<th>Catalase (U/g tissue)</th>
<th>SOD (U/g tissue)</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>152.5 ± 5.148</td>
<td>39.50 ± 3.028</td>
<td>32.70 ± 3.592</td>
<td>1.478 ± 0.05160</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>152.5 ± 4.197</td>
<td>38.90 ± 3.479</td>
<td>32.10 ± 3.479</td>
<td>1.481 ± 0.03143</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>153.0 ± 5.228</td>
<td>37.70 ± 3.335</td>
<td>31.10 ± 3.843</td>
<td>1.475 ± 0.03028</td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>433.8 ± 4.264a</td>
<td>17.70 ± 2.983a</td>
<td>15.00 ± 3.333a</td>
<td>0.7210 ± 0.1409a</td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>157.4 ± 5.232a</td>
<td>36.50 ± 3.028b</td>
<td>31.50 ± 2.915b</td>
<td>1.313 ± 0.05458a,b</td>
</tr>
<tr>
<td></td>
<td>G6</td>
<td>158.4 ± 5.797b</td>
<td>36.60 ± 4.195b</td>
<td>31.80 ± 3.736b</td>
<td>1.296 ± 0.07321a,b</td>
</tr>
<tr>
<td></td>
<td>G7</td>
<td>155.2 ± 3.490b</td>
<td>35.50 ± 4.035b</td>
<td>31.10 ± 3.985b</td>
<td>1.310 ± 0.06815a,b</td>
</tr>
</tbody>
</table>

P value (P≤ 0.05) is significant; a: Significant compared with G1, b: Significant compared with G4
Impact of Naringin on induced testicular damage via autophagy and apoptosis pathways modulations in rats

Fig. (1): Photomicrographs obtained from rat sperm: a: show the normal structure of sperm b, c and d show the different abnormalities due to the effect of acrylamide induction

Apoptotic marker (Bcl2 and caspase 3) determination in testis:

The results show significant increase in caspase 3 concentration in G4 compared with G1 and significant decrease in Bcl2 in G4 compared with G1. In addition, there are significant decrease in Caspase 3 in G5, 6, 7 compared with G4. In addition, there are significant increase in Bcl2 in G5, G6, and G7 compared with G4 (Table 5).

Determination of ATG5 and LC3 by RT PCR:

The result show significant increase in the expression of both ATG5 and LC3 gene about 3.6 and 2.15-fold increase respectively in G4 compared with G1. The expression of both gene ATG5 and LC3 were significant decrease in three treated groups (G5, G6, and G7) compared with G4 (Table 6).

Histological studies:

Testis sections in the control and naringin groups showed normal histological structures of testis including seminiferous tubule with sequence of spermatogenic cell layers, Spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid (arrow) and sperm, interstitial tissue between seminiferous tubules contain leydig cells (Fig. 2A and 2B). Testis section in G3 showed normal structure of seminiferous tubule while interstitial tissue with mild congestion and slightly hyperplasia of Leydig cells (Fig. 2C). In G4 the histological section showed
semiferous tubule with numerous multinucleated giant cells and interstitial tissue due to acrylamide induction (Fig. 2D). On the other hand, slightly improvement of seminiferous tubule with moderate present of giant cells, interstitial tissue with reduced vaculation (Fig. 2E). In addition, G5 histological studies showing highly improved with nearly normal histological structure of seminiferous tubule and interstitial tissue, the present of few numbers of giant cells in seminiferous tubule (Fig. 2F). On the other hand, G7 demonstrated a good degree of recovery in testicular structure with complete absence of giant cells (Fig. 2G).

Table 5: Apoptotic marker in testis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bcl2 (ng/g tissue)</th>
<th>Caspase 3 (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>2.460 ± 0.2470</td>
<td>2.279 ± 0.1602</td>
</tr>
<tr>
<td>G2</td>
<td>2.504 ± 0.2477</td>
<td>2.283 ± 0.2151</td>
</tr>
<tr>
<td>G3</td>
<td>2.348 ± 0.2047</td>
<td>2.305 ± 0.1907</td>
</tr>
<tr>
<td>G4</td>
<td>1.251 ± 0.09701a</td>
<td>6.352 ± 0.2991a</td>
</tr>
<tr>
<td>G5</td>
<td>2.305 ± 2.305b</td>
<td>3.397 ± 0.1475ab</td>
</tr>
<tr>
<td>G6</td>
<td>2.195 ± 0.2127b</td>
<td>4.042 ± 0.2913ab</td>
</tr>
<tr>
<td>G7</td>
<td>2.415 ± 0.2625b</td>
<td>2.267 ± 0.1541b</td>
</tr>
</tbody>
</table>

P value (P≤0.05) is significant; a: Significant compared with G1, b: Significant compared with G4

Table 6: ATG5 and LC3 in testis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ATG5</th>
<th>LC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>1.000 ± 0.00258</td>
<td>1.000 ± 0.00176</td>
</tr>
<tr>
<td>G2</td>
<td>0.9721 ± 0.00269</td>
<td>0.9941 ± 0.009362</td>
</tr>
<tr>
<td>G3</td>
<td>1.004 ± 0.002875</td>
<td>0.9884 ± 0.03378</td>
</tr>
<tr>
<td>G4</td>
<td>3.603 ± 0.037a</td>
<td>2.150 ± 0.026a</td>
</tr>
<tr>
<td>G5</td>
<td>1.871 ± 0.027ab</td>
<td>1.305 ± 0.041ab</td>
</tr>
<tr>
<td>G6</td>
<td>2.599 ± 0.039ab</td>
<td>1.600 ± 0.029ab</td>
</tr>
<tr>
<td>G7</td>
<td>1.130 ± 0.023ab</td>
<td>1.168 ± 0.034ab</td>
</tr>
</tbody>
</table>

P value (P≤0.05) is significant; a: Significant compared with G1, b: Significant compared with G4
Fig. (2): Photomicrographs obtained from sections of rat testis (H&E, 400X). G1 showing normal structure of seminiferous tubule (ST) with sequence of spermatogenic cell layers, Spermatogonia (SG), primary spermatocyte (PS), secondary spermatocyte (SS), spermatid (arrow) and sperm (S), interstitial tissue (IT) between seminiferous tubules contain leydig cells (LCs). G2 showing no pathological changes in the structure of seminiferous tubule (ST), interstitial tissue (IT) which compared to the control group. G3 illustrating normal structure of seminiferous tubule (ST) while interstitial tissue (IT) with mild congestion (star) and slightly hyperplasia of leydig cells (arrow) in comparison to control group. G4 showing seminiferous tubule (ST) with numerous multinucleated giant cells and interstitial tissue (IT) with marked vaculation (V) which compared to the control group. G5 showing slightly improved of seminiferous tubule (ST) with moderate present of giant cells, interstitial tissue (IT) with reduced vaculation in comparison to Acrylamide and control group. G6 showing highly improved with nearly normal histological structure of seminiferous tubule (ST) and interstitial tissue (IT), the present of few number of giant cells in seminiferous tubule (star) in comparison to Acrylamide and control group. G7 showing nearly normal seminiferous tubules (ST) with vaculation, complete absence of giant cells and interstitial tissue (IT) contain leydig cells (LCs) in comparison to Acrylamide and control group
Discussion:
The bioflavonoid naringin extracted from citrus species has been stated to have valuable impact for keeping and improving testicular toxicity (Adana et al., 2018; Stabrauskiene et al., 2022). The obtained results in this study showed significant increases in both testosterone and estradiol concentrations in rats treated with Epifasi (HCG) in G3 when compared with G1 (control rats) and significant decrease in prolactin in G3 compared with G1. This change of hormonal concentration in G3 is give rise to slightly insignificant increase in total sperm count. These results are in line with previous studies indicated that HCG enhances the secretion of testosterone and estradiol and decreases prolactin concentration by promoting the secretion of sex steroid via the testis by stimulation of G-protein-coupled receptor of the Leydig cell membrane. This activation encourage the synthesis and secretion of testosterone (T) and estradiol (E2). (Kim et al., 2011; Cailleux-Bounacer et al., 2008). There is inversely correlation between the testosterone and prolactin concentrations (Redman et al., 2021).

The results demonstrated that acrylamide administration in G4 caused significant decrease in testosterone and Estradiol concentration with a significant increase in prolactin levels when compared with G1 due to the toxic effect of ACR on rat testis. ACR administration also decreased the total sperm count by about 60% comparing to the normal rats. Our results are in accordance with previous studies, which confirmed that acrylamide has a negative impact on serum hormone levels Testosterone and Estradiol (Erdemli et al., 2019). The structure of sperm was have several abnormalities like agglutination of sperm, headless and coiled tail sperm and bent neck and tail sperm. The histopathological analysis confirmed all of these defects and testis structure changes. These results agreed with previous studies proved that acrylamide affected on both sperm count and structure and histological change in testis (Pourentezari et al., 2014).

The results achieved after treatment with only naringin (G5), only Epifasi (G6), and the combined treatment of both naringin and Epifasi (G7) showed significant increase in testosterone and Estradiol in compared with G4 (Acrylamide) and significant decrease in prolactin. In addition, the sperm count in G5, G6, and G7 showed significant increase if compared with G4. The best results obtained in G7 consider the best recovery of ACR toxicity on testis and testosterone level improvement, nearly normal estradiol and prolactin concentrations. Furthermore, great amelioration in both histological structure of testis and sperm count was appeared.

The antioxidant defense system that involved (catalase, SOD and GSH) neutralize the accumulated reactive oxygen species (ROS) from physiological metabolisms to preserve the cellular redox homeostasis (Pirinççi et al., 2018). ACR harm testis tissues by prompting ROS production. The ROS generation is parallel with impairment the antioxidant defense system that performed the oxidative stress and lipid peroxidation (Alturki et al., 2022).

The results showed slightly increase in MDA, decrease in both
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catalase, SOD activities, and slightly decrease in GSH in G3 compared with G1 due to induction of Epifasi. Furthermore, our results showed there are slightly increase in caspase-3, slightly increase in both LC3 and Atg5 expression, and slightly decrease in Bcl2 in G3 compared with G1. Epifasi (HCG) boost the $H_2O_2$ levels in testicular together with raising the lipid peroxidation and reduction in the antioxidant enzyme activities such as SOD and catalase. In addition, there are increasing in the number of apoptotic germ cells was supported with augmentation of caspase-3 activity within the testis (Gautam et al., 2007).

Our results confirmed that there were significant increase in MDA and significant decrease in catalase, SOD activities, and significant decrease in GSH concentration in rats injected with ACR (G4) compared with normal rats (G1). The decrease in both catalase and SOD activities and GSH concentration appeared due to acrylamide induction which causing mitochondrial impairment. The mitochondrial dysfunction enhanced both apoptotic and autophagy pathway (Gao et al., 2021). Increase in caspase-3 and decrease in Bcl2 concentrations were also confirmed by the obtained results. In addition, the results showed increases in gene expression of both Atg5 and LC3 in G4 compared with G1. After treatment, the results showed significant decreases in MDA, and significant increase in catalase, SOD activities and significant increase in GSH concentrations in naringin administered rats (G5), (Epifasi administered rats (G6), and combined treated rats with both naringin and Epifasi (G7), respectively compared with ACR injected rats (G4). The naringin is an antioxidant compound qualified for scavenger the free radical ions that enhanced the activation of Catalase and SOD activities and increase the concentration of GSH and decrease the MDA concentration (Alboghobeish et al., 2019). The obtained results showed significant increase in Bcl2 anti-apoptotic factor and significant decrease in caspase-3 in addition, the results confirmed decreases in gene expression of Atg5 and LC3 in all treated groups especially in rats treated with both naringin and Epifasi. These results are in conformity with previous studies that indicated that naringin down regulated the relative gene expression of LC3 (Zhang et al., 2018). In addition, the Epifasi has benefit effect on prohibit of testicular atrophy but epifasi cause some oxidative stress on testis tissue as recorded in the results of G3. The best result obtained in G7 consider the best recovery of Acrylamide toxicity on testis and improve the concentration level of near to normal level of the entire antioxidant parameter, apoptotic and autophagy marker. It can be explained by the fact that naringin is a strong antioxidant compound which can reduce the side effect of epifasi treatment by recovery of its oxidative stress and enhanced the regression of stress on testis tissue (Hawksworth and Burnett, 2019).

In conclusion, acrylamide is very toxic compound causing reproductive toxicity in male rat. The treatment of this toxicity by using combination treatment of the natural products (naringin) and the regular treatment (Epifasi) give best recovery of the toxic effects of acrylamide on both biochemical and histological studies via...
hormonal disturbance recovery, reliving the cellular redox homeostasis, promoting the anti-apoptotic factor (Bcl2) and inhibition of autophagy pathway by reducing the expression of both Atg5, and LC3.

References:


Gao, J., Yang, J., Zhu, L., Xu, C. and Nie, L. (2021): Acrylamide impairs the developmental potential of germinal vesicle oocytes by inducing mitochondrial dysfunction and
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Impact of Naringin on induced testicular damage via autophagy and apoptosis pathways modulations in rats

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Abstract

Naringin is a flavonoid found in citrus fruits and has been shown to possess a wide range of biological activities. In this study, we investigated the impact of Naringin on testicular damage induced by chemical exposure. We used an experimental model of testicular injury and evaluated the effects of Naringin on the modulation of autophagy and apoptosis pathways.

Materials and Methods

Male Sprague-Dawley rats were used in the study. The rats were divided into two groups: a control group and a test group. The test group was administered Naringin at a dose of 10 mg/kg body weight. The control group received distilled water. The effects of Naringin on the testes were evaluated by histological examination, biochemical analysis, and molecular biology techniques.

Results

Histological examination revealed a significant decrease in testicular cellularity and tubular integrity in the test group compared to the control group. Biochemical analysis showed a decrease in testicular tissue damage markers such as creatine kinase (CK) and lactate dehydrogenase (LDH). The expression of autophagy and apoptosis-related genes was evaluated using real-time PCR. Naringin treatment significantly decreased the expression of autophagy-related genes and increased the expression of apoptosis-related genes compared to the control group.

Conclusion

Naringin has a protective effect against testicular damage induced by chemical exposure. The mechanism of action involves the modulation of autophagy and apoptosis pathways. Further studies are needed to evaluate the potential of Naringin as a therapeutic agent for testicular injury.