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# Effects of Melatonin Against Thioacetamide-Induced Testicular Toxicity in Rats

## Efectos de la Melatonina Contra la Toxicidad Testicular Inducida por Tioacetamida en Ratas

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**SUMMARY:** This study aimed to investigate the changes in testis tissue of thioacetamide-induced rats and the effect of melatonin on these changes. Thirty-five male Wistar Albino rats were divided into five groups. Group I; Control (n=7), Group II; Melatonin (Mel) (10 mg/kg) a single dose (i.p)(n=7), Group III; Thioacetamide (TAA) (300 mg/kg) (i.p) 2 times with 24 hour intervals (n=7), Group IV; TAA (300 mg/kg) was administered at 24-hour intervals, afterwards of 10 mg/kg single dose of Mel (n=7), Group V; Mel was administered 10 mg/kg a single dose 24 hours before the administration of TAA (n=7). Testis was evaluated histologically, immunohistochemically (Heat Shock Proteins (HSP) 70 and 90), blood serum testosterone, total antioxidant status(TAS) and total oxidant status(TOS) in tissue. The tissue sections of Group III decreased seminiferous tubule diameters, and germinal epithelium spills were observed. HSP70 and HSP90 expressions were increased. There wasn't a statistically significant change in testosterone levels among the groups. While TAS levels decreased in Group III compared to control, TOS levels didn't change. HSP70 and HSP90 decreased in groups with Mel-treated. Mel was found to have both protective and therapeutic effects. According to our results, the therapeutic effect of Mel in thioacetamide-induced acute testicular injury is greater than its protective effect.

**KEY WORDS: Thioacetamide; Melatonin; Heat shock proteins; Testis.**

## INTRODUCTION

The male genital system is affected by various diseases such as diabetes (Sönmez *et al*., 2016b), obesity (Jia *et al*., 2018), chemotherapy drugs for cancer (Sönmez *et al*., 2016a). Oxidative stress, hypoxia, toxicity and similar conditions in the tissue caused by diseases trigger testicular damage. Heat shock proteins (HSP) occur in cells exposed to stress factors such as high temperatures (Krawczyk *et al*., 1988). Protein folding and the combined of multimeric structures is performed by proteins called molecular chaperones. Molecular chaperones are generated by HSPs. HSPs are classified according to their molecular weight. HSP70 and HSP90 are members of the HSPs family, make up major groups of molecular chaperons, and occur in protein folding and during times of stress (Craig *et al*., 1993). In addition, these two proteins have been shown to be expressed in diseases such as chronic heat stress (Pei *et al*., 2012), toxicity (Selim *et al*., 2012), cancer (Ren *et al*., 2017), in models such as testicular torsion (Shamsi-Gamchi *et al*., 2018), vasectomy (Du *et al*., 2017). Thioacetamid (TAA) thioacetimidic acid/acetothioamide (CH3CSNH2) is a sulfur-containing compound that is widely used to replace hydrogen sulfide in hospitals and industry (Al-Attar, 2011). TAA is often used to induce liver injury (hepatic necrosis/apoptosis) (Wang *et al*., 2019), hepatic fibrosis (Makled *et al*., 2019), cirrhosis (Keshk *et al*., 2019), hepatocellular carsinoma (Nazmy *et al*., 2019). It is also known to have effects on the cardiovascular system, urinary system and nervous system (Amirtharaj *et al*., 2017; Schyman *et al*., 2018; El Khiat *et al*., 2019). Although TAA is often used to induce liver damage, it causes damage to the testis (Rahman *et al*., 2005; Kang *et al*., 2006). Antioxidant enzyme activities have been reported to be decreased in the testes of thioacetamide-induced cirrhotic rats (Abul *et al*., 2002). Melatonin (Mel) is a hormone that plays a role in the regulation of circadien rhythm. Mel regulates the reduction / oxidation system under stress conditions and has anti-inflammatory, anti-oxidant and anti-apopitotic effects on the biological system (Farhood *et al*., 2019). Mel can be a promising pharmacological agent

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against testicular damage to the reproductive system by reducing the number of oxidative products (Aslankoc & Ozmen, 2019). Mel has been reported to be a cryoprotectant to protect damaged sperm cells during semen cryopreservation (Deng *et al*., 2017). It has also been reported to reduce lipid peroxidation production and protect against testicular tissue damage with its antioxidant properties (Oner-Iyidogan *et al*., 2001). Mel can be an antioxidant suitable to reduce damage caused by oxidative stress within the cell. Therefore, it is important to evaluate the total oxidants and antioxidants in the tissue. Total antioxidant status (TAS) and total oxidant status (TOS) measurements are widely used to determine the total antioxidant and oxidant capacity in tissue (Eraslan *et al*., 2019). We know that HSPs occur if cell function does not continue regularly, and deteriorates for various reasons. Therefore, it is inevitable that TAA-induced oxidative stress in the testis does not activate HSPs. After the detailed literature review, it was concluded that TAA-induced testis injury studies were insufficient. Therefore, we aimed to demonstrate TAA-induced testis injury. We evaluated the testis tissue histologically. We compared the levels of HSP70 and HSP90 expression in testis. In addition, we measured the testosterone, TAS and TOS levels. The present study was aimed to compare the protective and therapeutic effects of melatonin as a potential antioxidant against TAA-induced testis injury.

## MATERIAL AND METHOD

**Chemicals.** Thioacetamide (Sigma-Aldrich, St. Gallen, Switzerland) was used as an inducer of testis damage. Melatonin (Sigma-Aldrich, St. Gallen, Switzerland) was used as an antioxidant substance in the experiment.

**Animals.** Male Wistar rats obtained from the Hakan Çetinsaya Experimental and Clinic Research Center, Erciyes University, Kayseri, Turkey, were used for this study. They were housed in plastic cages in a well-ventilated rat house, allowed ad libitum access to food and water and kept at a 12-h light: dark cycle.

**Ethic Statement.** All the animals received human care according to the standard guidelines. Ethical approval for the study was obtained from Erciyes University Animal Research Local Ethics Committee (date:15.08.2018, no:18/105) and the ethic at regulations were followed in accordance with the national and institutional guidelines.

**Experimental Design.** The rats were randomly assigned to five groups of seven rats per group. Group I: served as Control; Group II: Mel group (10 mg/kg) a single dose intraperitoneally (i.p) on first day; Group III: TAA group (300 mg/kg) 2 times with 24 hour intervals (Shapiro *et al*., 2006); Group IV: TAA (300 mg/kg) + Mel (10 mg/kg) group; TAA 300 mg/kg (i.p) was administered at 24-hour intervals, afterwards at a dose of 10 mg/kg single dose of Mel. Group V: Mel (10 mg/kg) + TAA (300 mg/kg) group; Mel was administered at a dose of 10 mg/kg (i.p) 24 hours before the administration of TAA. After the administration of Mel, TAA was administered at a dose of 300 mg/kg, and 2 doses were given at 24-hour intervals (Sayan *et al*., 2020).

**Histological Examination.** At the end of the experimental period, the animals were killed by decapitation under intraperitoneal ketamine (75 mg/kg) + xylazine (10 mg/kg) anesthesia. After de-capitation, the testis tissues were quickly removed and were fixed in 4 % formaldehyde fixative for histological examination, followed by dehydration and clearing embedded in paraffin. Sections were stained with Hematoxylin-Eosin (H&E), photographs were taken with a light microscope. In addition, Johnsen's testicular biopsy score (JTBS) and mean seminiferous tubule diameter (MSTD) was applied to the sections. According to JTBS, tubular sections are evaluated systematically (given a score from 1 to 10) (Johnsen, 1970). In the study, one hundred tubules were evaluated from each groups. MSTD was measured in micrometers, at 200 magnifications (Analysis LS Research Program). Measurements were performed by two independent histologists and with 100 tubules in each group. Also, the remaining testis tissues were stored at -80 °C for ELISA examination.

**Immunohistochemistry.** To determine the differences in expression of heat shock protein 70, HSP70 (1:100, sc-33575, Santa Cruz Biotechnology, CA, USA) and heat shock protein 90, HSP90 (1:100, PB9635, Boster Biological Technology, Pleasanton, CA) in testis tissue, ?the avidin-biotin-peroxidase method was used for marking. Paraffin sections (5-6 μm) were deparaffinized in xylene. The sections were rehydrated, rinsed in deionized water and antigen retrieval was carried out by microwave treatment in 0.01 M sodium citrate buffer (pH 6.0) at 95 °C for 5 min. The sections were washed with phosphate-buffered saline (PBS) and endogenous peroxidase activity was inhibited by 3 % $H_2O_2$ in methanol for 10 min. The staining kit (Lab Vision, Ultra Vision Detection System Large Volume, Anti-Polyvalent Thermo Scientific HRP) was used for the next stages according to manufacturer instruction. The sections were visualized using 3,3P-diaminobenzidine tetrahydrochloride (DAB) and counterstained with hematoxylin (Karabulut *et al*., 2016). Under the light microscope and images were obtained. A total of 100 different areas were evaluated in each groups using the Image J program.

**Apoptosis (TUNEL).** *In situ* Cell Death Detection Kit Fluorescein Kit (Roche, USA) was used to determine apoptosis in testicular tissue. Testis tissue slides were rehydrated after deparaffinization, they were washed with PBS. Testis slides antigen retrieval was applied. After being washed with PBS three times, the tissues were incubated with a TUNEL reaction mixture in a damp and dark place at 37 °C for 60 minutes. After washing with PBS three times for five minutes, the tissues were contrast colored with 4,6-diamidine-2'-fenilindol. They were all examined with the Olympus BX-51 fluorescent microscope. TUNEL-positive cells in seminiferous tubules of all groups were analyzed by counting (Sönmez *et al*., 2016b).

**ELISA.** Blood samples were taken into empty tubes to obtain serum and were centrifuged at 1,509 g for 10 min. The resulting serums were used for the determination of testosterone using ELISA kits. Sunred Biological rat ELISA kit protocols were used. TAS and TOS levels were measured in testis tissue. For this purpose, testis samples stored at -80 were used. The samples were homogenized on ice and then centrifuged to remove supernatants. These supernatants were used for TAS and TOS, ELISA analysis. Analyzes were made according to the manufacturer's instructions (201-11-5126, DZE201112672, DZE201111669) ELISA kits for testosterone, TAS and TOS, respectively Sunred Biological Technology Co., Ltd., 96 Wells Elisa kit, Shanghai, China).

**Statistical analysis.** The Kolmogorov–Smirnov test was used to identify normal distribution of the data. One-way analysis of variance (ANOVA) and post hoc Tukey test were used to determine the differences between the groups. The nonparametric test (Kruskal-Wallis test) was used for quantitative variables. Results were presented as mean± standard deviation (SD). Values were considered as statistically significant if $p < 0.05$. The Graphpad Prism 7 (Graphpad Software, La Jolla California USA) program was used for the statistical analysis.

## RESULTS

**Histological results.** Testis histomorphology was evaluated in testicular tissue damage TAA-induced. Group I testis tissues showed normal appearance histologically. Interstitium tissue contained the interstitial cells (Leydig cells). Group II, in Mel group, in testis tissues had similar appearance to the control group. In TAA-induced testis tissues, Group III, exhibited rarely atrophic seminiferous tubules. It was observed that the germinal epithelial cells were poured into the lumen in these tubules. The seminiferous tubule epithelium was distributed in most tubules. There was also a decrease in tubule diameter. Group IV testicular tissue sections compared to group III showed a more regular appearance. Group V had a histologic appearance similar to group IV (Fig. 1). In addition, JTBS and MSTD were used to determine testicular tissue injury. JTBS was used to determine injury to the spermatogenic series. According to JTBS scores, Group I, Group II and Group IV were similar. A statistically significant decrease was observed in Group III compared to the other groups. There was also a statistically significant decrease in Group III MSTD. JTBS and MSTD values for all groups are shown in Table I.

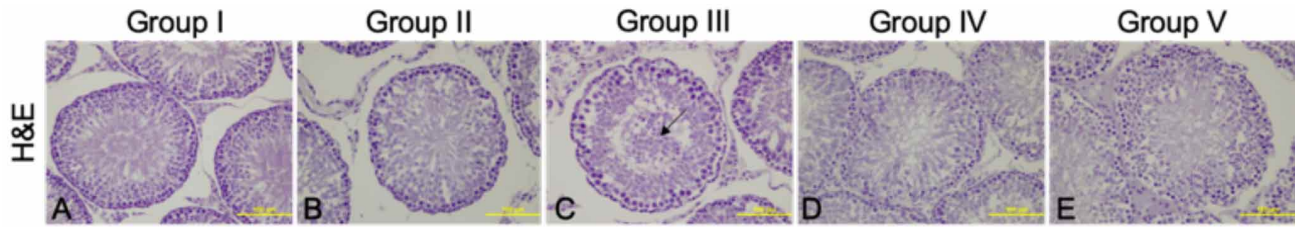


Fig. 1. H&E staining of groups. A, B- normal histological architecture of testis tissue, both control group and Mel, C- Germinal epithelium spilled into the lumen (arrow), D, E- close to normal appearance in TAA+Mel, Mel+TAA, respectively. Scala bar 100 mm.

Table I. Differences in JTBS, MSTD (m) and number of apoptotic cells among groups.

| Groups | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| JTBS | 8.37±0.85 | 8.44±0.87 | 6.09±1.37[a] | 8.11±0.83 | 6.40±1.36[a] |
| MSTD (μ) | 274±27.98 | 267±26.44 | 239.9±30.6[a] | 275±16.26 | 251.4±22.37[a] |
| Apoptotic Cells | 0.14±0.45 | 0.24±0.55 | 0.78±1.16[a] | 0.26±0.69 | 0.32±0.62 |

Values are expressed as mean ± SD. a $p < 0.001$ compared to both the Group I and Group III.
Note: Group I (Control group), Group II (Mel group), Group III (TAA group), Group IV (TAA+Mel), Group V (Mel+TAA).
Abbreviations: Mel; Melatonin, TAA; Thioacetamide, JTBS; Johnsen's testicular biopsy score, MSTD; Mean seminifer tubule diameter.

**Immunohistochemistry results.** HSP70 and HSP90 expression results of the groups are presented in Figure 2. It was observed that both HSP70 and HSP90 expressions in testicular tissue of Group I and Group II were stained in seminiferous tubule epithelium and inter-tubular connective tissue. The staining in connective tissue was determined in interstitial cells and vascular endothelium. HSP70 and HSP90 expressions in Group I were measured as 81.10±4.01 and 79.87±4.40, respectively. In Group II, it was measured that HSP70 was (78.31±4.11) and HSP90 was (78.85±4.19). In Group III testicular tissue, the expressions of HSP70 (91.29±13.00) and HSP90 (101.42±17.81) were increased. The increase in HSP70 and HSP90 in this group was statistically significant. There was a statistically significant decrease in the expressions of HSP70 and HSP90 in both groups IV and V testicular tissues compared to Group III. In Group IV, it was measured that HSP70 was (80.05±4.85) and HSP90 was (81.50±6.40). In Group V, it was measured that HSP70 was (82.95±7.20) and HSP90 was (83.81±4.99). The graphic of the groups is given in Figure 3.

**Apoptotic results.** Apoptotic cells in testicular tissue were determined by TUNEL (Fig. 4). The number of apoptotic cells was significantly increased in Group III. The number of apoptotic cells in Group IV and Group V was similar to the control group, and no statistically significant difference was observed (Table I). TUNEL-positive cell showed with green, fluorescein isothiocyanate (FITC) and nucleus with blue, 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI).

**ELISA results.** Blood serum testosterone level showed no statistically significant difference between the groups. Serum testosterone levels decreased in Group III, but this was not

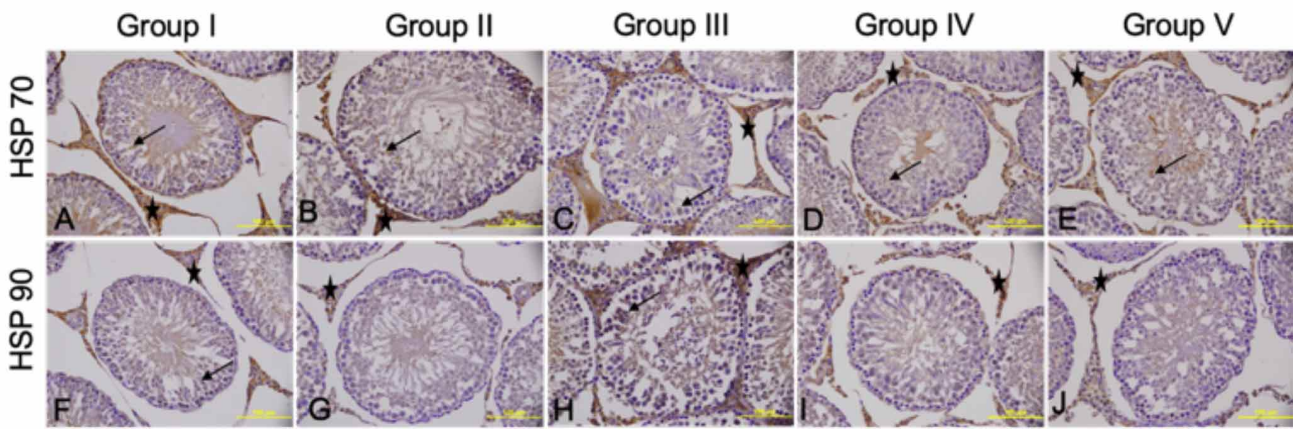


Fig. 2. HSP70 and HSP90 immunohistochemical staining. HSP70 and HSP90 expressions in inter-tubular connective tissue (stars) and seminiferous tubule epithelium (arrows) of the testis among the groups. Scala bar 100 mm.

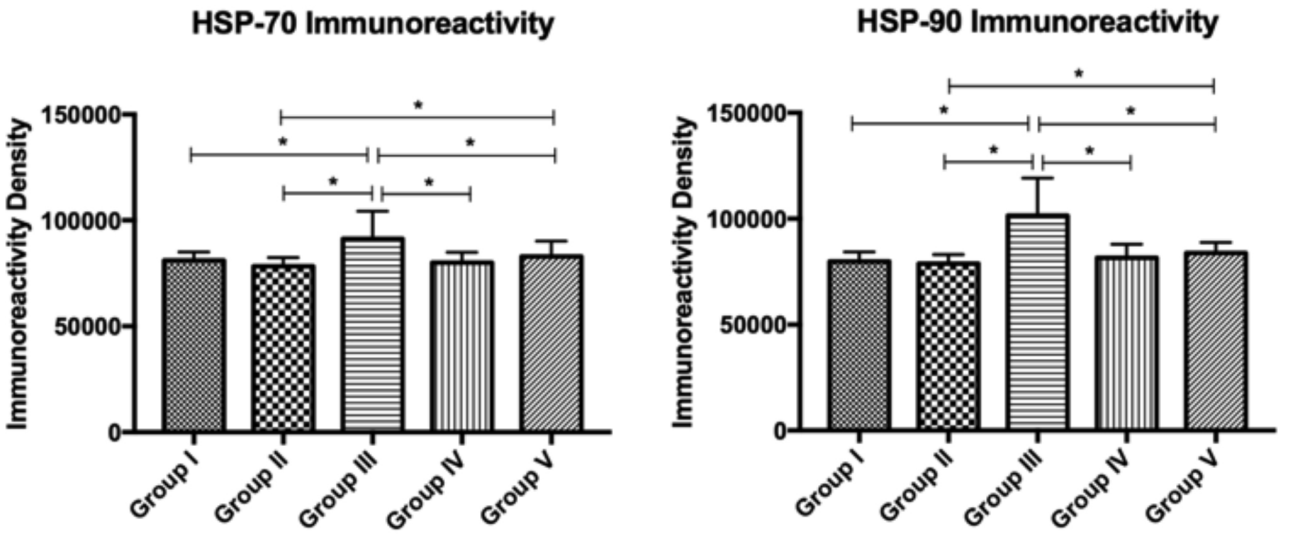


Fig. 3. HSP70 and HSP90 immunreactivity results. Values are presented as means ± SD. * p< 0.001compared to both Group I and Group III.

statistically significant. Group IV testosterone levels were similar to the control group (Table II). There was no statistically significant difference between the groups in TAS and TOS analysis (Table II). TAS was statistically significantly decreased in group III, but it was increased in group IV and V. TOS was decreased in group III, but this decrease was not statistically significant. Melatonin treated groups were similar to the control group.

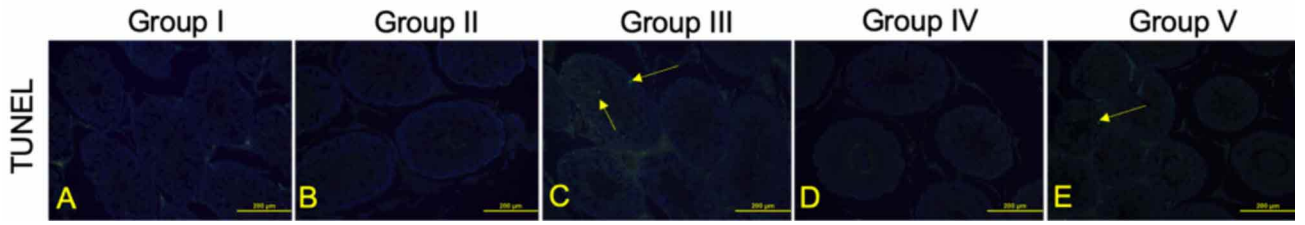


Fig. 4. TUNEL staining of testis among the groups. The pictures are given as FITC+DAPI. TUNEL-positive cells (yellow arrow) were mainly observed in seminiferous tubule epithelium of testis. FITC: fluorescein isothiocyanate, DAPI: 4',6-diamidine-2'-phenylindole dihydrochloride. Scala bar 200 mm.

Table II. Results of blood serum testosterone, TAS and TOS levels of testis tissue obtained by ELISA assay among groups. There was no statistically significant difference between the groups.

| Groups | Group I | Group II | Group III | Group IV | Group V | p |
|---|---|---|---|---|---|---|
| Testosterone (pg/ml) | 407±98.6 | 430.3±69.7 | 333.5±150.2 | 407.9±139.4 | 236.9±132.9 | 0.078 |
| TAS (U/ml) | 7.53±4.75 | 6.80±5.85 | 1.96±0.81[a] | 4.27±2.84 | 4,39±3.29 | 0.091 |
| TOS (nmol/ml) | 1.42±0.30 | 1.41±0.76 | 2.23±1.37 | 1.30±0.80 | 1.42±0.69 | 0.274 |

Values are expressed as mean ± SD. a p< 0.05 compared to both the Group I and Group III.
Note: Group I (Control group), Group II (Mel group), Group III (TAA group), Group IV (TAA+Mel), Group V (Mel+TAA). Abbreviations: Mel; Melatonin, TAA; Thioacetamide, TAS; total antioxidant status, TOS; total oxidant status.

## DISCUSSION

The toxic effect of TAA on the liver is known and often used as an inducing agent in experimental models (Müller *et al*., 1991), however, studies on reproductive biology are limited. In this study, we aimed to demonstrate the injury of TAA to the testis both histologically and HSPs, testosterone, oxidant-antioxidant activities and to investigate the protective and therapeutic effect of melatonin against this injury. Preservation of the structure of spermatogenic germ epithelium is important in the evaluation of testicular tissue damage. We observed that TAA caused significant injury to testicular tissues. According to the JTBS score, we observed impaired spermatogenic germ epithelium in Group III. In this group testis tissue, seminiferous tubule germinal epithelium was scattered, and atrophy was observed in some tubules. Among seminiferous tubules, vascular congestion, edema and vacuolization were reported in the areas close to the capsule (Celik *et al*., 2016). In the groups treated with melatonin (groups IV and V), we observed an improvement in testicular histology. This improvement was statistically significant in Group IV than Group V. MSTD values were also parallel to JTBS score. TAA-induced testis tissues were injured according to MSTD scores.

The testis shows continuous mitotic activity. Therefore, tissue integrity is essential for healthy spermatogenic series. Oxidative stress caused by tissue toxicity causes sperm dysfunction. Reactive oxygen species must be minimal in order to show normal capacitation and acrosome reactions of spermatocytes (Abul *et al*.). HSPs are stress proteins found in all organisms from bacteria to humans (Gao *et al*., 2008). They play a vital role in the realization of cell functions at normal temperature and are induced by various stress factors (Fujisawa *et al*., 2011). HSP 60, 70 and 90 proteins are found in the pre-puberty and puberty rabbit testis (Wu *et al*., 2011), these proteins have been reported to increase after heat chronic stress and di-(2-ethylhexyl) phthalate (DEHP) -induced (Pei *et al*.; Abd El-Fattah *et al*., 2016). HSP70 and HSP90 expressions have been reported to be increased after TAA-induced hepatotoxicity (Andrés *et al*., 2003; Abu-Elsaad *et al*., 2016). However, there is no literature on the increase or decrease of HSPs in TAA-induced testicular injury. In this study, we found that TAA-induced oxidative stress increased HSPs in testis. In addition, the number of apoptotic cells increased significantly in this group. According to these results, we

think that TAA application causes testicular damage, which is mediated by HSP70 and HSP90.

Oxidative stress occurs when there is no balance between oxidants and antioxidants (Birben *et al*., 2012). TAA-induced oxidative stress disrupts this balance, causing tissue injury. In the study, TAA decreased TAS levels, TOS increased slightly, but this increase was not statistically significant in testis tissue. It was reported that TOS level in testicular tissue was increased in cisplatin-induced injury, and there was no change in TAS level. The reason for the absence of changes in the level of TAS may be related to the presence of pro-oxidant molecules (Yucel *et al*., 2019). TAS and TOS levels have been reported to decrease in acrylamide-induced testicular tissue (Erdemli *et al*., 2019). HSP70 and HSP90 expressions were increased in TAA group. This increase may be linked to the increase in TOS. These findings suggest that HSPs may be effective in tissue injury caused by TAA-induced stress. We observed a decrease in testosterone hormone level in Group III, but this was not statistically significant. This may be due to our acute application of TAA. Mel is a hormone secreted primarily from the pineal gland, known to be an endogenous antioxidant (Allegra *et al*., 2003). The antioxidant properties of Mel have been reported to be protective in bisphenol A-induced (Olukole *et al*., 2019), docetaxel-induced (Bas & Naziroglu, 2019), methotrexate-induced (Jahovic *et al*., 2003; Wang *et al*., 2018) testicular tissue damage studies. When histological, immunohistochemistry, apoptosis, TAS and TOS levels findings of our study were evaluated, we observed the protective efficacy of Mel. However, after TAA-treated, our results in Mel (Group V) group were closer to the control group. In Group IV, JTBS scores, MSTD values, apoptotic findings, and testosterone levels were better than in group V. Also, both TAS and TOS levels improved in Group IV and V compared to Group III. However, this improvement was not statistically significant. There was no significant difference between HSP70 and HSP90 expressions in Groups IV and V. These results showed that Mel may have a greater therapeutic effect than protective effect in TAA-induced testicular injury. The presence of oxidative stress in testicular tissue may cause dysfunction and affect spermatozoa production. This is a critical process and can cause infertility when broken. Mel is attached to the outer surface of the phospholipid layer in cells, contacts the free radicals before the membrane and detoxifying them, and in this way, protects the membrane (Lemieux *et al*., 2000; Koziróg *et al*., 2011). Thus, Mel effectively protects the nucleus, organelles and cell membrane against free radicals damage. The continuity of spermatogenesis may be impaired for various reasons. It has been reported that Mel causes a decrease in the number of apoptotic cells and testosterone levels in aged rats (Muratoglu *et al*., 2019). The testicular torsion-detorsion model causes a decrease in seminiferous tubule diameter. It has been reported that Mel tissue and blood glutathione (GSH) and malondialdehyde (MDA) levels applied before testicular torsion-detorsion model were created cause improvement (Semercioz *et al*., 2017). Mel has been reported to improve spermatogenesis in the testis torsion-detorsion model (Mirhoseini *et al*., 2017). According to the above information, it is inevitable that Mel is effective against damage.

In our study, HSP70 and HSP90 expressions were observed both in spermatogenic cell line and in connective tissue between seminiferous tubules. There may be a relationship between decreased JTBS score and MSTD in Group III and increased HSP70 and HSP90 expressions. In addition, decreased TAS and increased TOS levels in Group III indicate that the oxidant-antioxidant balance in the tissue is disrupted. In conclusion, acute administration of TAA causes testicular tissue damage, and Mel has both protective and therapeutic effects in tissue. According to our results, we think that therapeutic effect of Mel is more than its protection. Furthermore, TAA-induced oxidative stress is associated with HSPs, and Mel contributes to recovery in tissue by reducing increased HSP 70 and HSP90 expressions.

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**KARABULUT, D.; AKIN, A. T.; SAYAN, M.; KAYMAK, E.; OZTURK, E. & YAKAN, B.** Efectos de la melatonina contra la toxicidad testicular inducida por tioacetamida en ratas. *Int. J. Morphol., 38(5)*:1455-1462, 2020.

**RESUMEN:** Este estudio tuvo como objetivo investigar los cambios en el tejido testicular de ratas inducidas por tioacetamida y el efecto de la melatonina en estos cambios. Treinta y cinco ratas macho Wistar Albino se dividieron en cinco grupos. Grupo I; Control (n = 7), Grupo II; Melatonina (Mel) (10 mg / kg) una dosis única (i.p) (n = 7), Grupo III; Tioacetamida (TAA) (300 mg / kg) (i.p) 2 veces con intervalos de 24 horas (n = 7), Grupo IV; TAA (300 mg / kg) se administró a intervalos de 24 horas, luego de una dosis única de 10 mg / kg de Mel (n = 7), Grupo V; Mel recibió 10 mg / kg de una dosis única 24 horas antes de la administración de TAA (n = 7). Los testículos se evaluaron histológicamente, inmunohistoquímicamente (proteínas de choque térmico (PCT) 70 y 90), testosterona en suero sanguíneo, estado antioxidante total (EAT) y estado oxidante total (EOT) en el tejido. En secciones de tejido del Grupo III se observó disminución de los diámetros de los túbulos seminíferos y derrames en el epitelio germinal. Se au-

mentaron las expresiones HSP70 y HSP90. No hubo un cambio estadísticamente significativo en los niveles de testosterona entre los grupos. Mientras que los niveles de EAT disminuyeron en el Grupo III en comparación con el control, los niveles de EOT no cambiaron. HSP70 y HSP90 disminuyeron en los grupos tratados con Mel. Se descubrió que Mel tenía efectos protectores y terapéuticos. Según nuestros resultados, el efecto terapéutico de Mel en la lesión testicular aguda inducida por tioacetamida es mayor que su efecto protector.

**PALABRAS CLAVE: Tioacetamida; Melatonina; Proteínas de choque térmico; Testículos.**

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