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Research Article
Effect of Taxifolin on Acrylamide-Related Oxidative Ovarian Damage, Infertility and Intrauterine Growth Retardation in Female Rats

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Abstract

Background and Objective: Acrylamide (AA) is a toxin that can cause reproductive organ toxicity and infertility through oxidative stress. This study aimed to investigate the effect of taxifolin, an antioxidant flavonoid, against acrylamide-induced infertility and intrauterine growth disorder in rats. **Materials and Methods:** Thirty-six rats were randomly divided into HG, ACR and TACR groups. The TACR group was given orally 50 mg kg^{-1} of taxifolin. One hour later, 20 mg kg^{-1} acrylamide was administered orally to the ACR and TACR groups. This procedure was repeated once a day for 30 days. Afterward, 6 rats in the groups were euthanized with 50 mg kg^{-1} thiopental sodium. The removed ovaries were analyzed biochemically and histopathologically. Others were kept in the laboratory with male rats for 2 months for breeding. Rats that did not become pregnant and did not give birth during this period were considered infertile. Intrauterine growth retardation was assessed by the weight of the offspring. **Results:** Taxifolin decreased the levels of malondialdehyde (MDA), total oxidant system (TOS) and oxidative stress index (OSI) that increased with acrylamide in ovarian tissues while preventing the decrease in Total Glutathione (tGSH) and total antioxidant system (TAS) levels. In the histopathological examination, it was observed that the damage in the ACR group decreased with the application of taxifolin. In addition, it was determined that it significantly prevented infertility and the decrease the birth weight of the offspring. **Conclusion:** Taxifolin may be a potential therapeutic strategy to prevent acrylamide-induced ovarian damage, infertility and intrauterine growth retardation.

Key words: Acrylamide, infertility, ovarian damage, rats, taxifolin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acrylamide (AA, $CH_2 = CHCONH_2$) is a colorless and odorless toxin utilized in various industries, including water refinery, cosmetics products and paper manufacture[1,2]. The AA is also produced from foods with high-temperature processing and low protein content[3]. The AA is genotoxic, carcinogenic, neurotoxic and has toxic effects on reproduction and development[4]. In a study on female rats, the administration of AA caused cystic changes in the ovary and has been linked with degenerative effects on zona pellucida, granulosa cells and oocytes. It has been reported that AA has negative effects on reproductive organ structure and fertility[5]. According to literature, exposure to AA toxicity from foodborne is increasing day by day and there are opinions that the parallel increase in infertility may be related to AA toxicity[6]. Due to its easy solubility in water, AA can easily pass from the placenta to the fetus and it is stated that it can affect the development of fetuses negatively in the intrauterine period[7]. The AA has been shown to cause reproductive organ toxicity and infertility through oxidative stress[8]. Ovarian and oocyte growth retardation was observed in mice exposed to AA[9]. Excessive production of reactive oxygen species (ROS) is held responsible for the pathogenesis of these pathologies[9,10]. Erdemli *et al.*[6] suggested that one of the major components of ovarian damage and infertility is the increase in oxidant levels such as malondialdehyde (MDA), total oxidant system (TOS) and nitric oxide (NO) in the ovarian tissue and the decrease in antioxidant levels such as reduced glutathione, superoxide dismutase (SOD), catalase (CAT) and total antioxidant system (TAS).

Taxifolin (dihydroquercetin) is an antioxidant flavonoid whose effect we will test against AA-related oxidative ovarian damage, infertility and intrauterine growth disorder in this study[11]. Flavonoids, which are abundant in many plants, including vegetables and fruits, attract attention due to their health promotion and disease prevention effects[12,13]. Studies have shown that taxifolin inhibits ROS production with antioxidant activity[14]. In addition, taxifolin has been reported to have anti-inflammatory, antibacterial and anticancer effects. In the literature, there is no information on the protective effect of taxifolin against AA-induced oxidative ovarian damage, infertility and intrauterine growth retardation. Therefore, current study aims to investigate the effect of taxifolin against possible infertility and intrauterine growth retardation due to AA administration in rats and evaluate the ovarian tissues biochemically and histopathologically.

MATERIALS AND METHODS

Study area: This study was carried out at Atatürk University Medical Experimental Application and Research Center between April to August, 2020.

Animals: Thirty-six albino Wistar female rats, which were taken from Ataturk University Medical Experimental Application and Research Center and weighing between 250-265 g, were used. Animals were kept in a light-regulated (12 hrs light/dark) laboratory environment at 22°C and fed unrestricted. The applications to be made in the study were approved by the Local Animal Experiments Ethics Committee (Approval number: 2020, 3/50).

Chemicals: Taxifolin was obtained from Evalar (Russia), AA from Sigma–Aldrich (USA) and thiopental sodium from IE ULAGAY (Turkey).

Animal groups: Rats were divided into healthy (HG), AA administered (ACR) and taxifolin+AA administered (TACR) groups.

Experimental procedure: Animal cages were moved to the vaginal smear room each morning throughout the experiment. For vaginal smear, a sample was taken with a pipette filled with 10 µL NaCl 0.9%. Samples were examined under a light microscope at 10× and 40× magnifications. Three types of cells were screened: Epithelial cells (round and nucleated), cornified cells (irregular without nuclei) and leukocytes (small round cells). Their ratios were used to determine the phases of the estrous cycle[15].

Within the scope of the experiment, the TACR (n = 12) rat group was given 50 mg kg^{-1} of taxifolin by oral gavage into their stomach[16,17]. The same volume of distilled water was applied to the ACR (n = 12) and HG (n = 12) groups by gavage. One hour after, AA (20 mg kg^{-1}) was given orally to the TACR and ACR groups[18]. These practices continued once a day for 30 days. At the end of one month, six rats from each group were sacrificed with 50 mg kg^{-1} thiopental sodium and ovarian tissues were examined biochemically and histologically. Other animals were placed in the same environment with male rats for two months to evaluate fertility. During this period, the pregnant rats were placed in separate cages and housed individually. Rats that did not become pregnant and did not give birth within two months were considered sterile. Weights of the born pups were measured to evaluate the intrauterine developmental disorder. By analyzing the obtained data, the groups were compared with each other.

Biochemical analyses

Preparation of samples: Tissue samples (0.2 g) were taken from each rat. For the analysis of MDA level, 1.15% potassium chloride solution was used and for Total Glutathione (tGSH) measurement, it was made up to 2 mL in phosphate buffer with pH = 7.5 and homogenized in ice. The mixtures were then centrifuged (10.000 rpm, 15 min, +4°C) and analysis was made from the supernatant.

MDA and tGSH analysis: The method described by Ohkawa *et al.* was used for MDA (µmol/g protein) analysis[19]. The method described by Sedlak and Lindsay was used to determine the amount of GSH (nmol/g protein)[20].

TOS and TAS analysis: The method developed by Erel[21,22] and commercial kits (Rel Assay Diagnostics, Turkey) were used to determine TOS (µmol H2O2 Equiv./L) and TAS (mmol Trolox Equiv./L) levels in ovarian tissue. The formula "oxidative stress index (OSI)=TOS/10xTAS" was used for the OSI.

Histopathological analysis: Ovarian tissues removed from rats were placed in buffered 10% formalin solution and fixed. It was then washed for 24 hrs and purified from water by passing through alcohol series. It was embedded in paraffin after treatment with xylol. Sections of 5 µm were taken from paraffin blocks, stained with Hematoxylin-Eosin and evaluated with light microscope. The examination was semi-quantitative and scored between 0-3: Absence (0), mild (1), moderate (2) and severe (3).

Statistical analysis: The IBM SPSS 22.0 was used for analysis and GraphPad Prism 9 program was used for drawing graphics. The $p<0.05$ was considered statistically significant. One-way ANOVA was used for the numerical data obtained from the experiments and Tukey HSD was used as a *post hoc* test. Results were expressed as "Mean value±standard deviation" (X±SD). Kruskal-Wallis test was preferred because the histopathological data were ordinal. *Post hoc* evaluation was also done with Dunn's Test. Histopathological data were expressed as median (minimum-maximum).

RESULTS

MDA and tGSH analysis: As seen in Fig. 1a and Table 1, AA administration significantly increased ovarian MDA levels in the ACR group compared to the HG group ($p<0.001$). Taxifolin decreased MDA levels in the TACR group compared to the ACR group ($p<0.001$). The MDA levels of HG and TACR groups were close to each other ($p = 0.058$).

In addition, the ACR group had lower tGSH levels than the HG group ($p<0.001$). When TACR was compared with ACR, tGSH levels were found to be statistically significantly higher ($p<0.001$) (Fig. 1b, Table 1).

TOS, TAS and OSI analysis: As seen in Fig. 2(a-c) and Table 1, AA administration significantly increased ovarian TOS and OSI levels in the ACR group compared to the HG group ($p< 0.001$). The TOS and OSI levels in the TACR group were lower than the ACR group, the difference was statistically significant

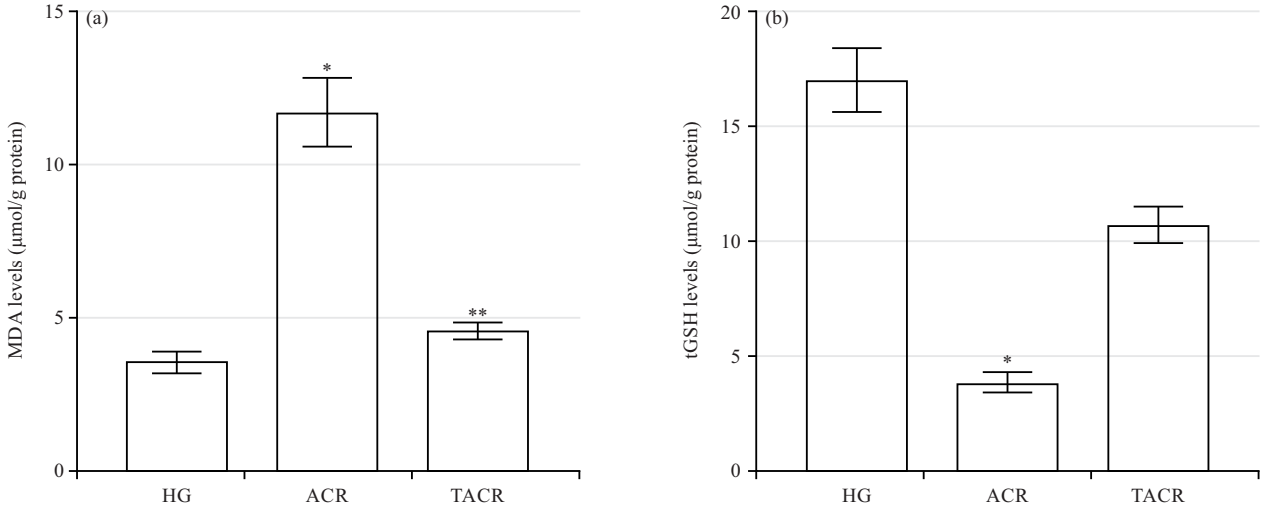

Fig. 1(a-b): MDA and tGSH levels in the ovarian tissue of experimental groups

Bars are mean±SD, *$p<0.001$ according to HG and TACR groups, **$p>0.05$ according to HG, MDA: Malondialdehyde, tGSH: Total glutathione, HG: Healthy group, ACR: Acrylamide group and TACR: Taxifolin+acrylamide group

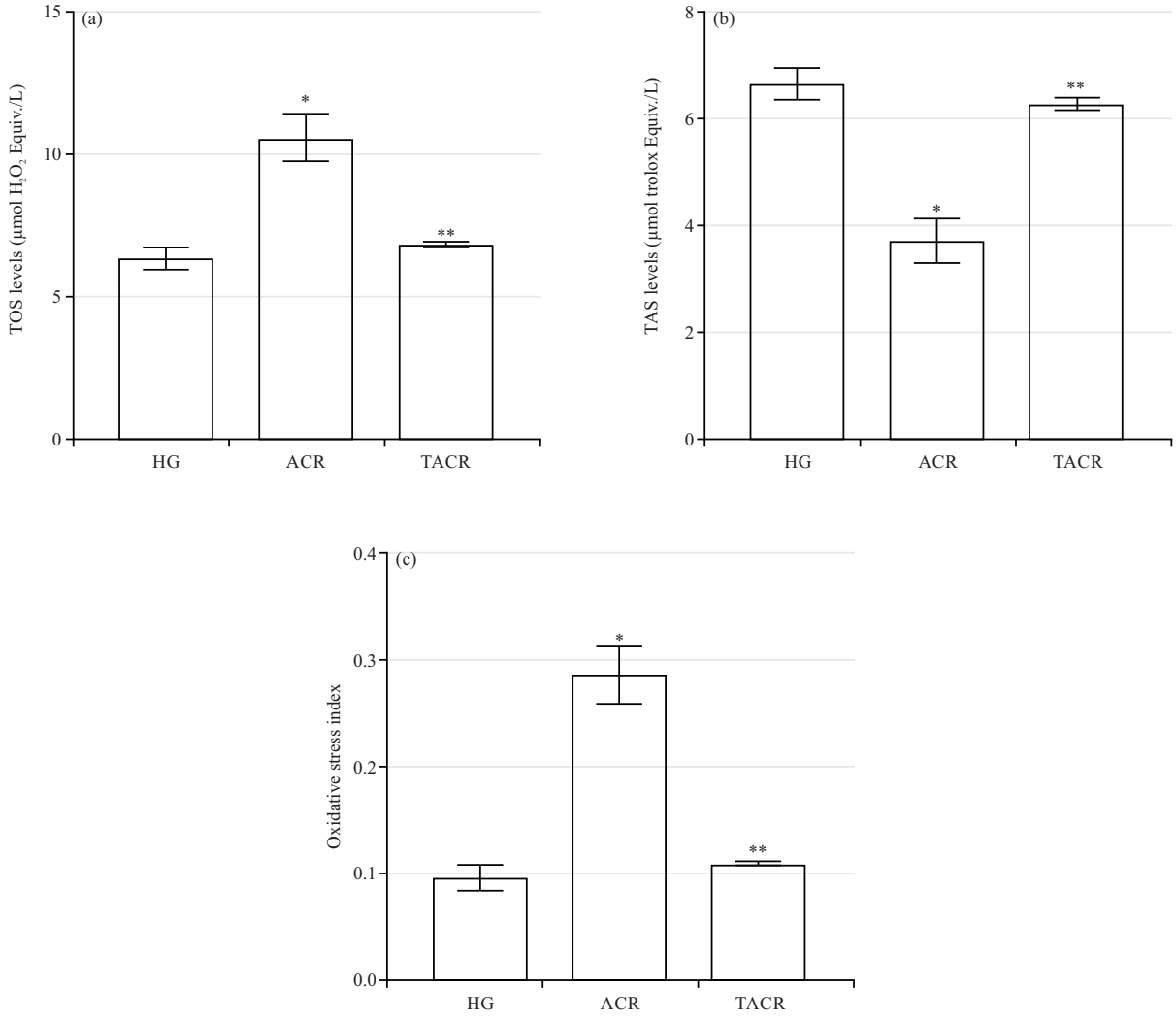

Fig. 2(a-c): TOS, TAS and OSI levels in the ovarian tissue of experimental groups

Bars are mean±SD, *p<0.001 according to HG and TACR groups, **p>0.05 according to HG, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, HG: Healthy group, ACR: Acrylamide group and TACR: Taxifolin+acrylamide group

Table 1: Biochemical analysis results of ovarian tissue and the p-values of *post hoc* comparisons between experimental groups

Biochemical parameter	Mean±standard deviation			p-values		
	HG	ACR	TACR	HG vs. ACR	HG vs. TACR	ACR vs. TACR
MDA	3.567±0.344	13.400±4.220	4.583±0.279	<0.001	0.058	<0.001
tGSH	17.050±1.366	3.850±0.437	10.752±0.784	<0.001	<0.001	<0.001
TOS	6.345±0.390	10.611±0.825	6.849±0.097	<0.001	0.265	<0.001
TAS	6.661±0.311	3.722±0.311	6.276±0.109	<0.001	0.108	<0.001
OSI	0.096±0.012	0.287±0.026	0.109±0.001	<0.001	0.407	<0.001

HG: Healthy group, ACR: Acrylamide group, TACR: Taxifolin+acrylamide group, MDA: Malondialdehyde, tGSH: Total glutathione, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, biochemical results are given as mean±standard deviation, one-way ANOVA test was used for statistical evaluation and Tukey HSD was used as *post hoc* test

(p<0.001). The TACR group and HG group TOS and OSI data were close to each other (p>0.05). The TAS levels were lower in the ACR group than in the HG group (p<0.001). In addition, taxifolin increased TAS levels statistically significantly compared to the ACR group (p<0.001). For TAS, the TACR and HG groups were similar (p = 0.108).

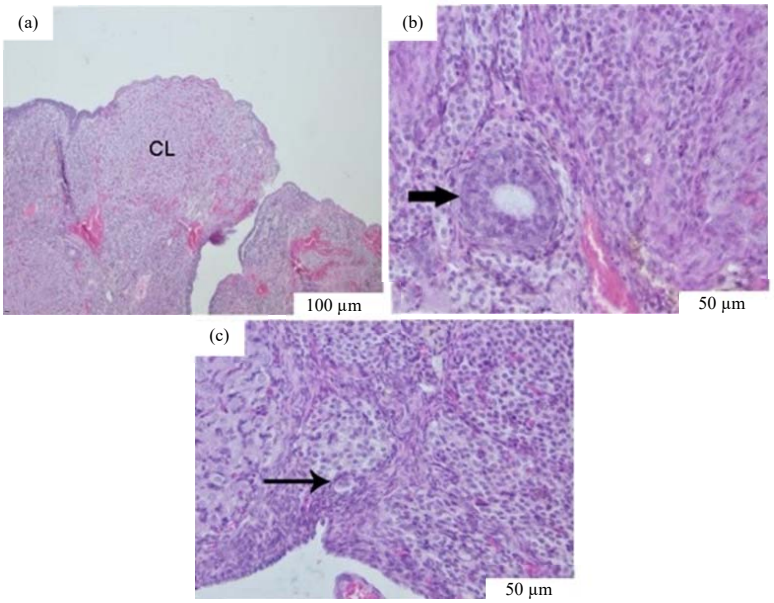

Fig. 3(a-c): Normal histological appearance of the healthy group (HG), (a) Corpus luteum (CL)-10×, (b) Primary follicle (arrow)-20× and (c) Primordial follicle (thin arrow)-20× and H&E

Table 2: Histopathological analysis of ovarian tissues in study groups

	Histopathological grading Median (minimum-maximum)		
Histopathological damage	HG	ACR	TACR
Degeneration in primary/secondary follicles	0(0-1)	3(2-3)*	1(1-2)**
Vacuolization	0(0-1)	3(2-3)*	1(1-2)**
Degeneration in primordial follicles	0(0-1)	2(2-3)*	1(1-2)**

*p<0.001 according to HG group, **p>0.05 according to HG group, HG: Healthy group, ACR: Acrylamide group, TACR: Taxifolin+acrylamide group, Kruskal-Wallis test was used for statistical evaluation and afterward, Dunn's Test were used as *post hoc* test

Table 3: Comparison of the experimental groups in terms of reproductive results

	Non-infertile rats		Infertile rats		Sex of offspring		Birth weight (g)
Group	n	%	n	%	Female	Male	Mean±standard deviation
HG	6	100	-	-	21	19	4.99±0.23
ACR	2	33.3	4	66.7	6	5	2.76±0.09*
TACR	4	66.7	2	33.3	13	11	4.95±0.11**

*p<0.001 according to HG and TACR groups, **p = 0.687 according to HG, HG: Healthy group, ACR: Acrylamide group, TACR: Taxifolin+acrylamide group, n: Number of animals, one-way ANOVA test was used for statistical evaluation and Tukey HSD was used as *post hoc* test

Histopathological evaluation: The histopathological effect of taxifolin on AA-related ovarian damage can be seen in Table 2. The ovarian tissues of the rats in the HG group had a normal histological structure. When the ovarian sections of the HG group were evaluated, the normal histological structure was observed (Fig. 3). In the ACR group, degeneration and vacuolization in primary and secondary follicles were severe and degeneration in primordial follicles was moderate, these histopathological findings were found to be mild in the TACR group (Fig. 4(a-d)).

Reproduction test results: Table 3 showed that after a two-month waiting period, all six female rats in the HG group gave birth (100%). Four of the six rats in the ACR group (66.7%) did not give birth within two months and were termed sterile, whereas the other two (33.3%) did. In the same environment, four (66.7%) of six rats in the TACR group treated with taxifolin gave birth, while two (33.3 %) rats who did not give birth during this period were termed infertile.

The average weight of the pups in the HG group was 4.99 g and the total number of offspring was 40. The average

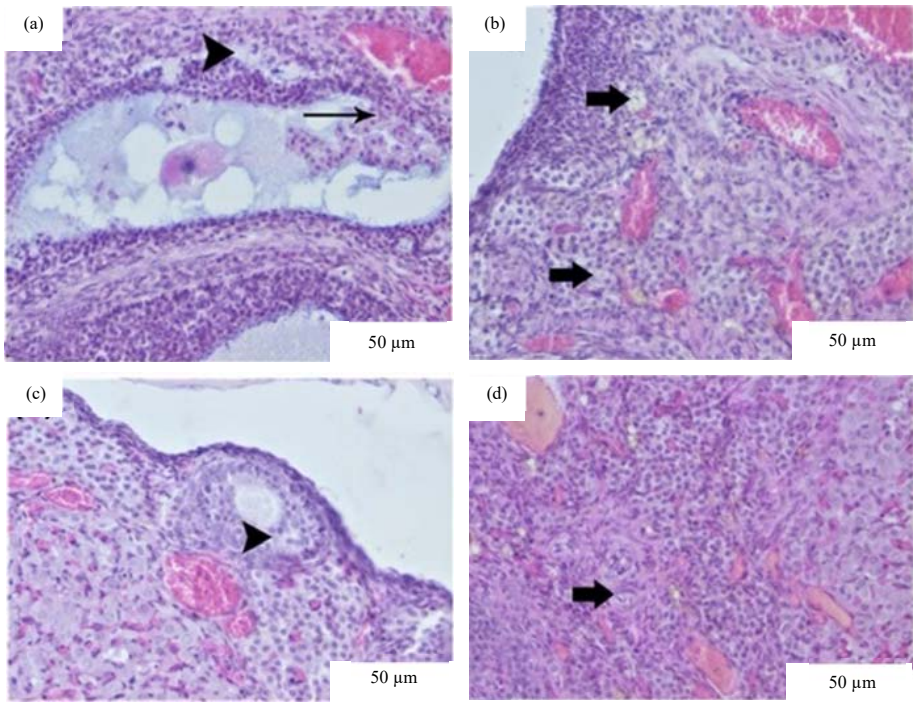

Fig. 4(a-d): a-b: Histopathological appearance of acrylamide group (ACR), (a) Severe vacuolization (arrowhead) and follicular degeneration (thin arrow), (b) Moderate primordial follicular degeneration (arrows), c-d: Histopathological appearance of taxifolin+acrylamide administered group (TACR), (c) Mild follicular vacuolization (arrowhead) and (d) Mild degeneration of primordial follicles (arrow)-20×, H&E

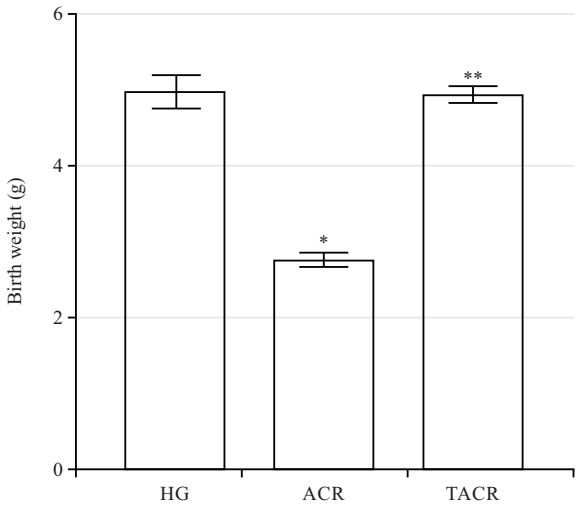

Fig. 5: Birth weights of puppies born in the study groups

Bars are mean±SD, *p<0.001 according to HG and TACR groups, **p>0.05 according to HG, HG: Healthy group, ACR: Acrylamide group and TACR: Taxifolin+acrylamide group

weight of 11 puppies born in the ACR group was 2.80 g, but the average weight of 24 puppies born in the TACR group was 4.90 g (Table 3). There was no difference in offspring weight between the TACR and HG groups (p = 0.687). The pup weights in the ACR group were statistically significantly lower than those born in the HG and TACR groups (p<0.001) (Fig. 5).

DISCUSSION

This study investigated the effects of taxifolin against possible infertility, intrauterine growth retardation and ovarian damage due to AA administration in female rats. Current experimental results showed that oxidative stress was induced in the ovarian tissue of female rats administered AA orally. It was determined that AA-related oxidative damage was significantly reduced in the ovarian tissue of the animal group treated with taxifolin.

In daily life, pregnant women are also unwittingly exposed to certain levels of AA[23]. Recently, intensive research has been carried out to reveal the damage that AA may cause to fetuses[6]. Studies have shown that AA causes oxidative stress in organs and tissues and the severity of stress increases even more at high doses[24]. As is known, the balance between the formation and destruction of oxidants is important for the normal continuation of biological processes[25]. In current study, in accordance with the literature, it was observed that the amount of MDA in the ovarian tissues of animals increased significantly in the AA-administered group compared to the healthy and taxifolin-treated groups. The literature states that MDA is the end product of lipid peroxidation (LPO) and the increase in its production is an indicator of oxidative stress[26]. In this study, TOS and OSI levels were also evaluated to support the development of oxidative stress. The TOS is used to evaluate the total oxidative effects of various oxidants[21]. Current biochemical test results also showed that the amount of TOS and OSI in the ovarian tissues of animals was also increased in the AA-administered group compared to the HG and TACR groups.

In order to maintain tissue integrity and functions at normal levels, over-produced ROSs are neutralized by GSH and other enzymatic and non-enzymatic antioxidant systems[27,28]. If antioxidants are insufficient to neutralize oxidants, the balance between oxidants/antioxidants will be upset in favor of oxidants. The fact that the tGSH level, an indicator of decreased antioxidant defense in the ovarian tissue of the group exposed to AA, is lower than that of the healthy group is compatible with the literature. In other studies, supporting our experimental results, it was emphasized that AA reduced the amount of tGSH in the ovarian tissue[5,29]. In addition, in this study, TAS levels were determined to support the antioxidant status. The TAS levels were found to be lower in the ACR group compared to the other groups. The TAS shows the cumulative antioxidant effect of different antioxidants[30]. Current experimental results and literature[6] indicated that oxidative damage plays an important role in AA-related ovarian toxicity.

It has been reported in many previous scientific studies that taxifolin, the effect of which was tested in this study, is a powerful antioxidant agent[11,14]. In an experimental study, it was determined that taxifolin was effective against ovarian damage and reproductive problems due to oxidative stress induced by antipsychotic drugs and it was shown that taxifolin significantly inhibited the increase in MDA and the decrease in tGSH in the ovarian tissues[31]. In the group treated with taxifolin, MDA, TOS, TAS and OSI levels were almost the same as in the healthy control group, consistent with the literature. These results showed that taxifolin significantly inhibited AA-related oxidative ovarian damage in ovarian tissue.

In the ACR group, degeneration and vacuolization in primary and secondary follicles were severe and degeneration in primordial follicles was moderate. In previous studies, cystic formation, vacuolation and degenerative changes in follicles were observed in the ovaries of female rats fed AA[5,32]. In this study, it was determined that the damage was significantly reduced in the taxifolin added group. In a study by Zhao *et al.*[33] taxifolin attenuated streptozocin-induced diabetes-related kidney damage in rats. In another study, it was stated that histopathological damage caused by oxidative stress and inflammation in rat optic nerve tissues with cisplatin was prevented by taxifolin[34]. These findings are consistent with both our biochemical results and the literature.

Previous studies have shown that AA changes the antioxidant/oxidant balance in favor of oxidants and causes oxidative stress in placental tissue, while taxifolin protects the ovary from oxidative damage[35].

In addition, AA has also been found to pass into the placenta and human breast milk in humans. Therefore, dietary exposures to AA are likely to begin at the embryonic stage and continue throughout life[36]. In a study done, showed that although AA did not significantly affect mating performance, pregnancy rates and offspring size in rats, it significantly reduced offspring body weight and weight gain[36]. In another study by Duan *et al.*[10], the offspring sizes of mice exposed to AA were shown to be significantly smaller than control mice. In current study, the offspring weights of AA-administered rats were significantly smaller than control group rats, consistent with the literature. With the application of taxifolin, puppies weight approached the values of the healthy group. These results were compatible with the toxicity of AA, especially on growth and development and the knowledge that oxidative stress causes a decrease in birth weight and that an increase in birth weight occurs with antioxidant therapy[6].

CONCLUSION

As AA increased oxidants and decreased antioxidants in ovarian tissue. Taxifolin prevented the increase of AA-induced oxidants and the decrease of antioxidants in ovarian tissue. In

addition, taxifolin was beneficial in preserving the histological structure of the ovarian tissue. Taxifolin also reduced AA-induced infertility and reproductive dysfunction. The results of this experiment suggested that taxifolin may be beneficial in preventing AA-induced reproductive dysfunction, infertility and adverse effects on the fetus.

SIGNIFICANCE STATEMENT

Exposure to acrylamide toxicity is increasing every day and it is thought that the increase in infertility may be related to acrylamide toxicity. It is also stated that it can negatively affect the development of fetuses in the intrauterine period. Therefore, besides reducing acrylamide exposure, it is also important to prevent the toxic effects of exposure. Our study shows that taxifolin shows promise in preventing acrylamide-induced ovarian damage and adverse effects on reproductive functions.

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