ISTANBUL UNIVERSITY PRESS

N-Acetylcysteine Improves Acrylamide-Induced Changes in Ovarian Tissue and Serum Levels of Pituitary-Ovarian Axis Hormones in Adult Rats

Marziyeh Naimi[1,2] (ID), **Mehrdad Shariati[3]** (ID), **Syrus Naimi[4]** (ID),
Mohammad Amin Edalatmanesh[2] (ID)

[1]Islamic Azad University, Fars Science and Research Branch, Department of Biology, Fars, Iran
[2]Islamic Azad University, Shiraz Branch, Department of Biology, Shiraz, Iran
[3]Islamic Azad University, Kazerun Branch, Department of Biology, Kazerun, Iran
[4]Islamic Azad University, Kazerun Branch, Department of Genetics, Kazerun, Iran

ORCID IDs of the authors: M.N. 0000-0003-0014-9924; M.S. 0000-0001-7360-0208; S.N. 0000-0003-4557-1336;
M.A.E. 0000-0002-7936-1145

ABSTRACT

Objective: The purpose of this study was to evaluate the protective effect of N-acetylcystein (NAC) on ovarian tissue and serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone in Acrylamide (AA)-treated adult rats.

Materials and Methods: Forty-two adult female Wistar rats were randomly assigned into 7 groups of 6 including: a control group without treatment, a placebo group received distilled water intraperitoneally, an AA group received 50 mg/kg by oral gavage, a NAC group received 40 mg/kg intraperitoneally, an AA+NAC10, AA+NAC20 and an AA+NAC40 groups received 10, 20 and 40 mg/kg of NAC intraperitoneally, respectively and then also received 50 mg/kg AA by oral gavage for 28 days. Serum levels of FSH, LH, estradiol and progesterone were measured by radioimmunoassay method and ovarian tissue was evaluated histopathologically.

Results: Administrating AA alone decreased the number of ovarian follicles, corpus luteum and the levels of FSH, estradiol and progesterone, while increased the number of atretic follicles and LH level compared to the control, placebo and NAC groups (p<0.05). The administration of NAC alone had no effect on the number of ovarian follicles, corpus luteum and the level of hormones compared to the control and placebo groups (p>0.05). Following AA+NAC20 and AA+NAC40 administration and not AA+NAC10, the number of ovarian follicles, corpus luteum and also the levels of FSH, estradiol and progesterone increased, while the number of atretic follicles and LH level decreased (p<0.05), which was in a dose-dependent manner compared to the AA group.

Conclusion: NAC could recover the AA female rat reproductive toxicity in a dose-dependent manner, and improved folliculogenesis.

Keywords: Acrylamide, n-acetylcystein, ovary, estradiol, rat

INTRODUCTION

Acrylamide (AA) also known as acrylic acid amide or propanamide is an unsaturated amide with the chemical formula $CH_2=CH-CONH_2$, which exists in two mono- and polymeric forms (1). The monomeric AA is mutagenic and tumorigenic in human and laboratory animals, but the toxic effects of its polymeric form have not yet been recognized (2). AA is produced in a large amount in starchy foods cooked at high-temperature (120℃) such as potato, corn, and wheat, which is due to the presence of asparagine amino acid (150-4000 µg/kg) and in foods containing protein in an average amount (5-50 µg/kg) and it has raised a general concern in societies that consume a large amount of AA-rich foods (3,4). AA is rapidly metabolized in the body, and its metabolites are normally excreted by urine (1). In humans and rodents, AA is oxidized by CYP450 2E1 and converts into glycidamide (GA) which is an epoxide derivative. Studies show that both *in vivo* and *in vitro*, GA has genotoxicity features (5). Glutathione S-transferase (GST) is a metabolic enzyme that plays an important role in reducing oxidative stress and eliminating free radicals. The conjugation of AA or its metabolite, i.e. GA with GST enzyme, disrupts cellular function and causes cell death. Studies have also indicated that the destructive effects of AA or GA are by influencing sulfhydryl groups of proteins (6,7). Due to AA poisoning, the level of glutathione decreases and the body's defense system against free radicals weakens (8). Free radicals combine with cell membrane and unsaturated fatty acids, producing radical lipids with oxygen molecule and as a result, phospholipids in the endoplasmic reticulum decompose and release enzymes, which ultimately lead to cell death (9). Some studies represent that AA has destructive effects on the reproductive system of both male and female (10-13). AA-treated rats have low-weight ovaries and low-quality or undeveloped oocytes due to increased oxidative stress and this can lead to a significant reduction in the fertilization rate or low-quality embryos and consequently subfertility or infertility (12).

N-acetylcystein (NAC) is a kind of acetylated L-amino acid, used as an antidote for acetaminophen poisoning, as well as for treating various disorders associated with oxidative stress (14). Studies have shown that during oxidative stress that decreases glutathione levels, NAC can act as an antioxidant by increasing glutathione and naturally neutralizes the effects of intracellular damage of free radicals by repairing oxidative damage or collecting oxygen reactive species (15). In previous studies, severe side effects associated with NAC consumption have not been reported so that its consumption at high doses for one year was uncomplicated (16). Therefore, in the current study, the protective effect of NAC on ovarian tissue and serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone in AA-treated adult rats were studied.

MATERIALS AND METHODS

Animals

In the present experimental study, 42 adult female Wistar rats weighing 220±20 g and 8 weeks old were provided from the animals' house at the Islamic Azad University of Kazerun. All animals were maintained on a 12 h/12 h light-dark cycle at 22±2℃ and 70% relative humidity in polycarbonate cages and they had free access to sufficient water and food ad libitum. Before any experimental manipulation, the animals were kept together for two weeks to adapt to the laboratory conditions. The current study was approved by the Ethics Committee of the Islamic Azad University of Kazerun, Iran, regarding to work with laboratory animal care (Ethical No: IR.Kiau 16330525651006).

Experimental Design

Animals were randomly divided into 7 groups of 6 control, placebo, AA, NAC, AA+NAC10, AA+NAC20 and AA+NAC40 groups. The control group did not receive any treatment. Because the medications' solvent in this study was distilled water, animals in the placebo group received distilled water intraperitoneally. The animals in AA group received 50 mg/kg AA (Merck, Germany) by oral gavage. In NAC group, the animals received 40 mg/kg NAC (Merck, Germany) intraperitoneally. The rats in AA+NAC10, AA+NAC20 and AA+NAC40 groups received 10, 20 and 40 mg/kg NAC intraperitoneally, respectively at 9 o'clock in the morning and then received 50 mg/kg AA by oral gavage at 5 o'clock in the afternoon. All groups were treated over a period of 28 days. The basis for selecting the dose of AA and NAC was based on the previous studies (10,17). Regarding the above mentioned protocol, after receiving the last dose of AA, the animals were anesthetized using ether and blood sampling was carried out directly from the heart. Then, for histological evaluations, the left and right ovaries of all rats were removed from the abdominal cavity.

Before starting the treatment, vaginal smear was prepared to ensure that all rats were at one stage of the estrus cycle. For this purpose, 100 µg of estradiol valerate (Aboreyhan, Iran), dissolved in 0.2 ml of olive oil, was injected intramuscularly using an insulin syringe. After 42 hours, 50 µg of progesterone (Aboreyhan, Iran) was injected intramuscularly. Six hours after the injection of progesterone, the vaginal smear was prepared and examined by light microscope. The previous method was used to determine the stages of estrous cycle. In this method, each stage of the estrus cycle is detected based on the ratio between three types of cell population (epithelial cells, keratinized cells and leukocytes) observed in vaginal smear (18). Microscopic observations showed that all the rats were synchronized in the estrous phase.

Hormonal Analysis

Using a 5ml syringe, blood sampling was done directly from the heart. After agglutination process in the laboratory, the blood samples were centrifuged for 5 minutes at 3000 rpm to discrete the serum. The serums were stored at -20℃ prior to FSH, LH, estradiol, and progesterone measurement. The serum

levels of FSH, LH, estradiol and progesterone were measured using radioimmunoassay (RIA) kits (FSH and LH: BT Lab, China; Estradiol and Progesterone: IBL, Germany) according to the manufacturer's instructions.

Based on the RIA kits, 125 µl of the standard solution was decanted into NSB tube and then 25 µl of the serum was added. 500 µl of the labeled solution was added and 100 µl of antiserum was added at the last step and incubated for 60 minutes at 37°C. Then, the amount of 1000 µl of the secondary antibody was added and incubated for 15 minutes at 20 to 35°C. At the final stage, the mixture was centrifuged at 2000 rpm for 20 minutes, and after separating the supernatant, the levels of hormones were counted using a gamma counter (Kentron, Switzerland).

Histopathologic Analysis of Ovarian Tissue

After blood sampling, the left and right ovaries of all animals were removed and once the surrounding adipose tissue was removed, they were fixed in a 10% buffered formalin solution. After standard histological processing, the samples were blocked in paraffin and then, using a microtome (Aisan, China), midsagittal and longitudinal serial sections of 5 µm thickness were prepared and stained with hematoxylin-eosin (Merck, Germany). Under the light microscope (Nikon, Tokyo, Japan), the number of primary follicles, secondary follicles, graafian follicles, atretic follicles and corpus luteum were counted in each section. Counting the follicles and corpus luteum were performed in a spiral way from a point at the cortex in clockwise direction toward the medulla.

Statistical Analysis

Data were analyzed using SPSS version 20 software (SPSS Inc., Chicago, IL, USA). The normal distribution of the resulting data was confirmed by non-parametric Kolmogorov-Smirnov test. Then, all data were analyzed applying one way ANOVA by bonferroni test at a significance level of $p < 0.05$. The data were described as mean ± standard error (SE) in the graphs . GraphPad Prism version 6 (GraphPad Prism, Inc., San Diego, CA, USA) was used to represent the graphs.

RESULTS

Hormonal Findings

Based on the hormonal findings, there was no significant difference in the serum levels of FSH, LH, estradiol and progesterone among the control, placebo, NAC and AA+NAC40 groups ($p > 0.05$) (Figures 1A-1D). The serum Levels of FSH, estradiol and progesterone in the AA and AA+NAC10 groups were decreased significantly compared to the control, placebo, NAC and AA+NAC40 groups ($p < 0.05$) (Figures 1A, 1C and 1D), but the level of LH increased significantly in both groups compared to the control, placebo, NAC and AA+NAC40 groups ($p < 0.05$) (Figure 1B). The serum levels of FSH, estradiol and progesterone in AA+NAC10, AA+NAC20 and AA+NAC40 groups were increased in a dose-dependent manner compared to the AA group, and were significantly different in AA+NAC20 and AA+NAC40 groups. Conversely, the level of LH was decreased compared to AA group in a dose-dependent manner, and was significantly different in AA+NAC40 group ($p < 0.05$) (Figure 1B).
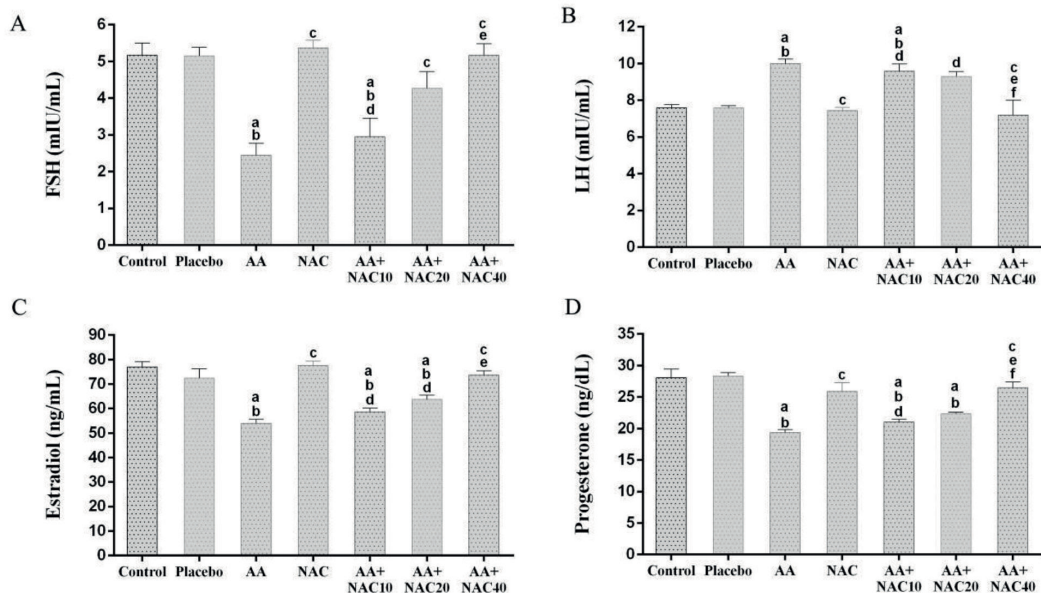

Figure 1. A-D. Comparison of mean and standard error of the serum levels of FSH, LH, estradiol and progesterone in control, placebo, AA-treated rats in AA group, NAC-treated rats in NAC group and AA+NAC-treated rats in AA+NAC10, AA+NAC20 and AA+NAC40 groups. a: compared to control group; b: compared to placebo group; c: compared to AA group; d: compared to NAC group; e: compared to AA+NAC10 group; f: compared to AA+NAC20 group (a, b, c, d, e and f= p<0.05).

Histopathologic Findings

Figure 2 compares the mean and the standard error of the number of primary follicles, secondary follicles, graafian follicles and corpus luteum. There was no significant difference in the number of primary follicles, secondary follicles, graafian follicles and corpus luteum among the control, placebo, NAC, AA+NAC20 and AA+NAC40 groups (p>0.05) (Figures 2A-2D). Also, there was no significant difference in the number of graafian follicles among the control, placebo, NAC and AA+NAC10 groups (p>0.05) (Figure 2C). The number of primary follicles, secondary follicles and corpus luteum in the AA group was significantly decreased compared to the control, placebo, NAC, AA+NAC20 and AA+NAC40 groups (p<0.05) (Figures 2A, 2B and 2D). Furthermore , the number of graafian follicles in the AA group was significantly decreased compared to the control, placebo, NAC, AA+NAC10, AA+NAC20 and AA+NAC40 groups (p<0.05) (Figure 2C). The number of primary follicles, secondary follicles and corpus luteum increased in a dose-dependent manner in the AA+NAC10, AA+NAC20 and AA+NAC40 groups in comparison to the AA group, and this increase was significant in the AA+NAC20 and AA+NAC40 groups (p<0.05) (Figures 2A, 2B and 2D). Also, the number of graafian follicles in the AA+NAC10, AA+NAC20 and AA+NAC40 groups was significantly increased in comparison with the AA group (p<0.05) (Figure 2C).

Figure 3 compares the mean and standard error of the number of atretic follicles. There was no significant difference in the number of atretic follicles among the control, placebo, NAC, AA+NAC20 and AA+NAC40 groups (p>0.05), but the number of atretic follicles increased significantly in AA and AA+NAC10 groups compared to the control, placebo, NAC, AA+NAC20 and AA+NAC40 groups (p<0.05).
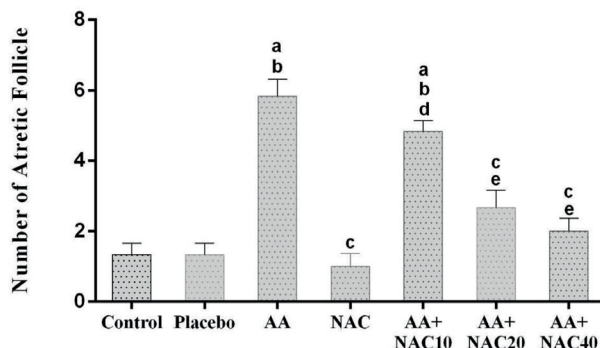

Figure 3. Comparison of mean and standard error of atretic follicles in control, placebo, AA-treated rats in AA group, NAC-treated rats in NAC group and AA+NAC-treated rats in AA+NAC10, AA+NAC20 and AA+NAC40 groups. a: compared to control group; b: compared to placebo group; c: compared to AA group; d: compared to NAC group; e: compared to AA+NAC10 group (a, b, c, d and e= p<0.05).
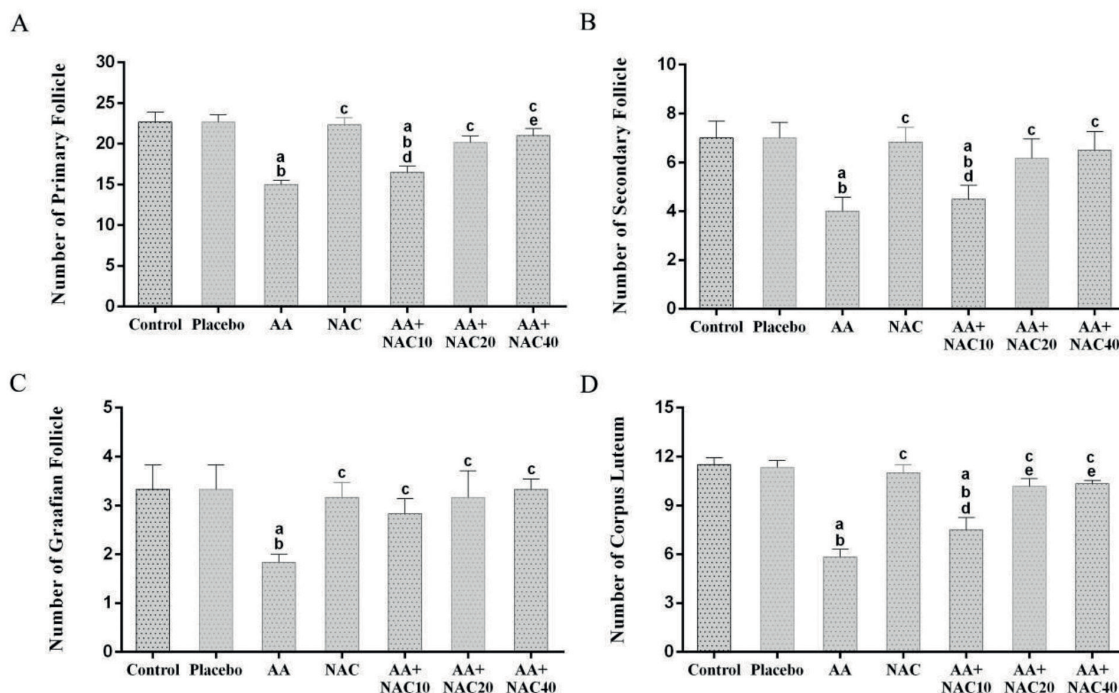

Figure 2. A-D. Comparison of mean and standard error of the number of primary follicles, secondary follicles, graafian follicles and corpus luteum in control, placebo, AA-treated rats in AA group, NAC-treated rats in NAC group and AA+NAC-treated rats in AA+NAC10, AA+NAC20 and AA+NAC40 groups. a: compared to control group; b: compared to placebo group; c: compared to AA group; d: compared to NAC group; e: compared to AA+NAC10 group (a, b, c, d and e= p<0.05).
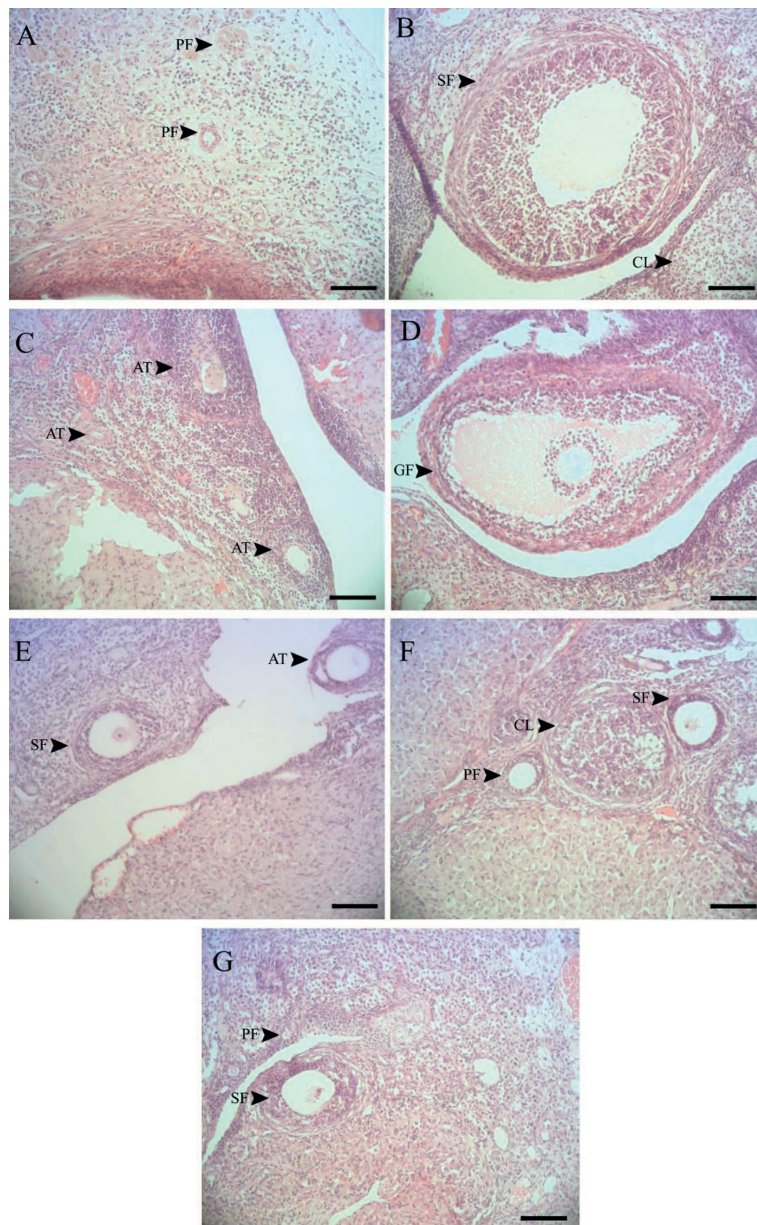
Figure 4. A-G. Photomicrograph of ovarian tissue in rats treated with AA, NAC and AA+NAC in different groups. In control (A) and placebo (B) groups, types of ovarian follicles and normal folliculogenesis are observed. In the AA group (C) the reduction of ovarian follicles and elevation of atretic follicles can be observed. In the NAC group (D) graafian follicles along with a thick layer of granulosa are observed. In the AA+NAC10 group (E) a large number of atretic follicles are observed. In the AA+NAC20 (F) and AA+NAC40 (G) groups different ovarian follicles are seen. Folliculogenesis has been improved. PF: Primary follicle; SF: Secondary follicle; GF: Graafian follicle; CL: Corpus luteum; AT: Atretic follicle. H & E staining. The photos are with 10X magnification. Black bars: 50 μm.

Figure 4 represents the morphology of ovarian tissue in different groups. The histological sections of the ovaries in the control, placebo, NAC, AA+NAC20 and AA+NAC40 groups show normal folliculogenesis with all types of follicles, including graafian follicles with a thick layer of granulosa cells and corpus luteum (Figures 4A, 4B, 4D, 4F and 4G). In AA and AA+NAC10 groups, atretic follicles with a very thin granulosa layer, which is characterized by cystic follicles and few corpus luteum indicating ovulation decrease, were observed (Figures 4C and 4E).

DISCUSSION

In the present study, the effect of NAC administration on the changes of ovarian tissue and the serum levels of FSH, LH, estradiol and progesterone was evaluated in AA-treated rats. Our results showed that the number of primary follicles, secondary follicles, graafian follicles and corpus luteum decreased significantly while the number of atretic follicles increased significantly by administering 50 mg/kg AA in AA group

compared to the control, placebo and NAC groups. Although toxic effects of AA on male reproductive system have been investigated in several studies, few quantitative studies have been done on the female reproductive system. It was suggested that administering different amounts of AA (20 and 40 mg/kg) in rats decreases the number of primordial follicles and corpus luteum, while the increase in the number of primary and antral follicles was observed in a dose-dependent manner (10).

The results of this study as well as our study suggest that AA can affect follicular growth, development and atresia in different stages and prevent the formation of corpus luteum as a normal ovarian function index. It was indicated that the low dose-administration of AA in rats, infertility can occur due to the reduction of mature follicles and cystic changes in the ovary (11). In this study, the administration of AA decreased FSH, estradiol and progesterone levels and increased LH level. It has been indicated that AA can decrease the FSH level in a dose-dependent manner, and also affect the proliferation and survival of granulose cells, therefore, it seems that the reduction in estradiol level may be due to decreased level of FSH and the degradation of granulosa cells function (10,19). The main function of the corpus luteum is secreting progesterone, so reduced level of progesterone can be due to the reduction of the corpus luteum following AA administration (19). An elevated level of LH with a reduced level of estradiol and progesterone suggest that the pituitary-gonad axis can provide a suitable negative feedback after reducing estradiol and progesterone levels as a result of AA administration.

NAC is a low molecular weight thiol derived from cysteine amino acid and due to antioxidant, anti-aging and anti-inflammatory activities, it has an important role in reducing oxidative stress, eliminating ROS and improving nitric oxide activity (20,21). Some studies confirm the protective and positive effects of NAC on various tissues of the body, including the liver, kidney, intestine and ovary (22-25). In patients with polycystic ovarian syndrome, NAC can decrease and increase insulin level and peripheral insulin sensitivity, respectively, nevertheless, co-administer ing of NAC and assisted reproductive technology medications does not significantly increase the number of ovarian follicles in these patients (26). It was demonstrated that the NAC administration in AA-treated rats can decrease the level of Malondialdehyde and increase glutathione level and GST activity in the liver and intestine (27). The results of this study showed that administering NAC at a dose of 40 mg/ kg in the NAC group does not influence the level of FSH, LH, estradiol and progesterone and also the number of ovarian follicles and corpus luteum compared to control and placebo groups. In confirmation of our results, it has been shown that in NAC-treated pregnant rats, the progesterone level and the number of corpus luteum did not change in comparison with the control group (28). What's more , It was revealed that the administration of 100 mg/kg of NAC in male rats did not affect the serum levels of FSH, LH and testosterone compared to the control group (13). It seems that administering NAC alone in this study does not affect the level of the pituitary-gonadal

axis hormones as well as the number of ovarian follicles and the corpus luteum. However, it has been shown that proper treatment with NAC delayed the rate of apoptosis and death of healthy follicles during the aging process of the ovary and since oocytes and follicles can be exposed to oxidative stress in the body, so the ovary can be a proper place for NAC activity (29).

Based on our observations in the current study, administer ing NAC at 20 and 40 mg/kg in AA-treated rats was significantly increased the number of primary follicles, secondary follicles, graafian follicles and corpus luteum, but the number of atretic follicles was significantly increased compared to AA groups; while there was no significant difference between control, placebo and NAC groups. Also, our study represented that the levels of FSH, LH, estradiol and progesterone after NAC administration at 40 mg/kg in AA-treated rats did not differ significantly with control, placebo and NAC groups. Follicular atresia is a natural process dependent on the developmental stage and apoptosis plays an important role in this process. AA has been shown to increase follicular atresia in primordial and primary follicles. It has been suggested that AA can induce caspase enzymes in oocytes and causes proteolysis of vimentin intermediate filament, which results in apoptosis promotion (30). Nitric oxide plays an important role in modulating oxidative stress, and its excessive increase can be cytotoxic by reacting with reactive oxygen and nitrogen species that impairs mitochondrial function (28). It has also been indicated that nitric oxide (NO) plays an important role in folliculogenesis, follicular atresia, oocyte development, ovulation, luteolysis, and steroidogenesis. NO is synthesized by nitric oxide synthase (NOS) and AA can exert its toxic effects through NOS pathway on the female reproductive system (10). Nevertheless, it appears that NAC can modulate the toxic effects of AA on the ovary by affecting the NOS activity (21,28).

CONCLUSION

Our study shows that AA has toxic effects on ovaries and could impair the folliculogenesis as well as the level of pituitary-gonadal axis hormones in the rats. The administration of NAC in healthy female rats did not influence the folliculogenesis and pituitary-gonadal hormones levels. Administering NAC at different doses in AA-treated rats doesn't have a similar effect on follicular development and atresia as well as pituitary-ovarian axis hormones. The lowest effect of NAC was observed at 10 mg/ kg, while 20 and 40 mg/kg NAC had the highest effect on the folliculogenesis and levels of pituitary-ovarian axis hormones in AA-treated rats, respectively. Therefore, administering NAC in AA-treated rats could improve folliculogenesis and serum levels of pituitary-ovarian axis hormones in a dose-dependent manner. Therefore, NAC supplementation may be beneficial when there is a risk of AA female reprotoxicity.

References

1. Rahangadale S, Jangir BL, Patil M, Verma T, Bhandarkar A, Sonkusale P, et al. Evaluation of protective effect of vitamin E on acrylamide induced testicular toxicity in wister rats. Toxicol Int 2012; 19(2): 158-61.

2. Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. Int J Cancer 2008; 122(9): 2094-100.

3. Yener Y and Kalipci E. The carcinogenic effects of acrylamide formed during cooking of some foods. Academic J Cancer Res 2009; 2(1): 25-32.

4. Lingnert H, Grivas S, Jagerstad M, Skog K, Tornqvist M, Aman P, et al. Acrylamide in food: mechanisms of formation and in influencing factors during heating of foods. Scand J Nutr 2002; 46(4): 159-72.

5. Klaunig JE. Acrylamide carcinogenicity. J Agric Food Chem 2008; 56(15): 5984-8.

6. Barber DS, Hunt JR, Ehrich MF, Lehning EJ, Lopachin RM. Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. Neurotoxicology 2001; 22(3): 341-53.

7. Tyl RW and Friedman MA. Effects of acrylamide on rodent reproductive performance. Reprod Toxicol 2003; 17(1): 1-13.

8. Collí-Dulá RC, Friedman MA, Hansen B, Denslow ND. Transcriptomics analysis and hormonal changes of male and female neonatal rats treated chronically with a low dose of acrylamide in their drinking water. Toxicol Rep 2016; 19(3): 414-26.

9. Zarei M and Shivanandappa T. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of Decalepis hamiltonii in mice. Food Chem Toxicol 2013; 57: 179-84.

10. Wei Q, Li J, Li X, Zhang L, Shi F. Reproductive toxicity in acrylamide-treated female mice. Reprod Toxicol 2014; 46: 121-8.

11. ALKarim S, ElAssouli1 S, Ali S, Ayuob N, ElAssouli Z. Effects of low dose acrylamide on the rat reproductive organs structure, fertility and gene integrity. Asian Pac J Reprod 2015; 4(3): 179–87.

12. DuanX, Qiao- Wang C, Chen KL, Zhu CC, Liu J, Suna SC. Acrylamide toxic effects on mouse oocyte quality and fertility *in vivo*. Sci Rep 2015; 5: 11562.

13. Kunle-Alabi OT, Akindele OO, Odoh MI, Oghenetega BO, Raji Y. Comparative effects of coconut water and N-Acetyl cysteine on the hypothalamo-pituitary-gonadal axis of male rats. Songklanakarin J Sci Technol 2017; 39(6): 759-64.

14. Ercal N, Treeratphan P, Lutz P, Hammond TC, Matthews RH. N-acetylcysteine protects chinese hamster ovary (CHO) cells from lead-induced oxidative stress. Toxicology 1996; 108(1-2): 57- 64.

15. Xue C, Liu W, Wu J, Yang X, Xu H. Chemoprotective effect of N-acetylcysteine (NAC) on cellular oxidative damages and apoptosis induced by nano titanium dioxide under UVA irradiation. Toxicol in Vitro 2011(1); 25: 110-6.

16. Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med. 2005; 353(21): 2229-42.

17. Chiew AL, Isbister GK, Duffull SB, Buckley NA. Evidence for the changing regimens of acetylcysteine. Br J Clin Pharmacol 2016; 81(3): 471–81.

18. Marcondes FK, Bianchi FJ, Tanno AP. Determination of estrous cycle phases of rats: some helpful considerations. Braz J Biol 2002; 62(4A): 609-14.

19. Camacho L, Latendresse JR, Muskhelishvili L, Patton R, Bowyer JF, Thomas M, et al. Effects of acrylamide exposure on serum hormones,gene expression, cell proliferation, and histopathology in male reproductive tissues of Fischer 344 rats. Toxicol Lett 2012; 211(2): 135–43.

20. Pan X, Wu X, Yan D, Peng C, Rao C, Yan H. 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. Toxicol Lett 2018; 288: 55-64.

21. Kojsová S, Jendeková L, Zicha J, Kunes J, Andriantsitohaina R, Pechánová O. The effect of different antioxidants on nitric oxide production in hypertensive rats. Physiol Res 2006; 55(Suppl): S3-16.

22. Galhardo MA, Júnior CQ, Riboli Navarro PG, Morello RJ, De Jesus Simões M, De Souza Montero EF. Liver and lung late alterations following hepatic reperfusion associated to ischemic preconditioning or N-acetylcysteine. Microsurgery 2007; 27(4): 295-9.

23. Usta U, Inan M, Erbas H, Aydogdu N, Puyan OF, Altaner S. Tissue damage in rat ovaries subjected to torsion and detorsion: effects of L-carnitine and N-acetyl cysteine. Pediatr Surg Int 2008; 24: 567-73.

24. Danilovic A, Lucon AM, Srougi M, Shimizu MHM, Lanhez LE, Nahas WC, et al. Protective effect of N-acetylcysteine on early outcomes of deceased renal transplantation. Transplant Proc 2011; 43: 1443-9.

25. Millea PJ. N-Acetylcysteine: Multiple clinical applications. Am Fam Physician 2009; 80: 265-9.

26. Mokhtari V, Afsharian P, Shahhoseini M, Kalantar SM, Moini A. A review on various uses of n-acetyl cysteine. Cell J 2017; 19(1): 11-17.

27. Altinoz E, Turkoz Y, Vardi N. The protective effect of N-acetylcysteine against acrylamide toxicity in liver and small and large intestine tissues. Bratisl Lek Listy 2015; 116(4): 252-8.

28. Helal MA. The effects of N-acetyl-L-cysteine on the female reproductive performance and nephrotoxicity in rats. Ren Fail 2016; 38(2): 311-20.

29. Liu J, Liu M, Ye X, Liu K, Huang J, Wang L, et al. Delay in oocyte aging in mice by the antioxidant N-acetyl-L-cysteine (NAC). Hum Reprod 2012; 27(5): 1411-20.

30. Hułas-Stasiak M, Dobrowolski P, Tomaszewska E, Kostro K. Maternal acrylamide treatment reduces ovarian follicle number in newborn guinea pig offspring. Reprod Toxicol. 2013; 42: 125-31.