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Acrylamide-Induced Ovarian Toxicity in Female Wistar Albino Rats

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Abstract: **Background**: Acrylamide was well known to induce reproductive toxicity with an impact on reproductive functions on both male and female. The current investigation was designed as a trial to assess any changes in ovarian hormones and histology brought on by the administration of acrylamide for three weeks. **Methods**: Animals were split into two groups (N = 10) and fed a healthy diet. and DMSO (1%) saline (9%), Acrylamide (AA) group received acrylamide in saline (9%), (20 mg/kg b. w.) for 21days. Alteration in the level of ovarian hormones were assayed and correlated with histological changes in ovarian tissue. **Results**: Estradiol levels in the serum were considerably increased after acrylamide administration, but progesterone levels were significantly decreased. Histological study revealed that AA causes cyst formation, corpus luteum d generation which pointed to ovarian follicular regression. **In conclusion**: Results showed that AA modulated hormonal levels in female rats that related to alteration in ovarian structure

Keywords: Acrylamide, Oxidative stress, Ovarian Toxicity, Histological study.

1 Introduction

Acrylamide, also referred to as 2-propenamide (AA, C_3H_5NO), is a crystalline compound that is white in colour, water soluble and has a relative molecular mass of 71.08 kDa (Figure 1, a) [1]. Since AA has been deemed a potential human carcinogen by the International Agency for Research on Cancer (IARC), its presence in heat-processed foods is a global health concern [2]. Foods containing raw ingredients that are high in carbohydrate and lower in proteins tend to produce the most acrylamide. [3,4]. When high-carbohydrate foods are processed at temperatures above 120 ^{o}C, it is produced. The largest levels of AA are thought to be found in fried, deep-fried, or baked products such cake, bread, French fries, and chips (Figure 1, b) [5]. Additionally, AA has been used to create polyacrylamide polymer, which is still commonly used as a coagulant in water treatment, a grout for dams and other underground architectural projects, and as electrophoresis gels [6–9]. Studies conducted in vivo and in vitro have shown that AA has harmful effects on neurotoxicity [10], hepatotoxicity [11], genotoxicity [12], cancer [13-14] and the reproductive system [15]. So, the risks of AA to human health must thus not be disregarded. The female reproductive system is primarily regulated by estrogen, progesterone, which have an important role in controlling female reproductive cycles [16]. These steroids' concentrations fluctuate throughout the ovarian cycle and during various reproductive stages, and they are responsive to variations in energy and social stressors, and environmental toxins [17]. These hormones play a role in one or more stages of development and function of the female reproductive system [16]. Numerous studies have shown that environmental xenobiotic and chemical compounds affect the levels and operations of female reproductive hormones [18-19].

The goal of the current study was to evaluate the potential toxicity of acrylamide on adult female albino rats'

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ovarian structures and hormonal levels.

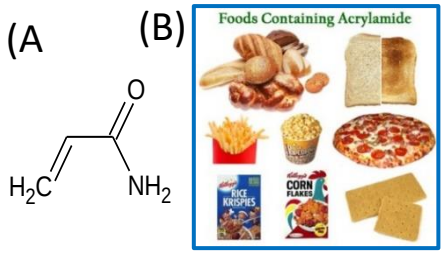

Figure 1: (A) Chemical structure of acrylamide (B) Acrylamide source in food products.

2 Material and Methods

2.1. Chemicals

Acrylamide, ≥ 98.0 %, MW: 71.08 g / mol, mp: 81-87 °C and P Code: 101601204 were obtained from SIGMA-ALDRICH Chemical Co.

2.2. Animals and study design

A total of twenty healthy female albino rats were used in the experiment (120-150 gm). The Medical Animal House at the Sohag, Faculty of Science is home to animals that were purchased from the Sohag University, Faculty of Science. Two weeks prior to the experiment's start, rats were allowed to acclimate in the testing room. They had unrestricted access to the typical rat diet while being housed in metal cages under hygienic environments. The Medical Research Ethics Committee of the Faculty of Medicine, Sohag University, registered the study under the number Sohag-2-2-2021-03, considering the handling and use of laboratory animals. (N = 10) The animals were divided into 2 groups: GI: control group (received daily oral dosing of 0.5 ml DMSO (1%) and saline (9%); GII: (AA) group (For 21 days, the freshly made AA (20 mg/kg body weight) group got daily oral administration, which is less than the lethal dose). Rats exposed to acrylamide had an LD50 of 150 mg/kg/BW [20].

2.3. Sample collection

First, ether was used to completely anaesthetize animals before cardiac blood samples were obtained. Following centrifugation at 5000 rpm for 10 min to separate the serum from the blood, the samples were kept at -20°C for additional analysis. Rats were put down after blood was collected, and then their abdomens were opened and their whole ovaries were dissected. The ovaries were cleaned with isotonic saline and then preserved in 10% neutral formalin for additional histological research.

2.4. Biochemical study

Progesterone (P4) [21] and estradiol (E2) [22] serum levels were quantitatively determined using the ELISA method. Kits were bought from the Egyptian business CALBIOTECH.

2.5. Histopathology study

Preparation of ovarian Sections and Histopathological Examination

The ovaries were removed and preserved in a formalin solution with a 10% concentration. The samples were then routinely treated, and sections with a thickness of 4-5 mm were obtained. The tissue sections were deparaffinized, mounted on a glass slide, and stained with hematoxylin and eosin (Thermo Fisher Scientific, USA) The slices were then examined at and observed using a light microscope (Leica, Germany) at 100X and 400X magnifications [23].

2.6. Statistical Analysis

The statistical analysis was carried out using Graph Pad Prism 5 software (San Diego, CA, USA). In the analysis, a T test analysis of two variables was used. The results are presented as mean ± SD and the difference in significance between groups is indicated by the notation ***$p < 0.001$ [24].

3 Results

3.1. Biochemical study

3.1.1. Progesterone (P4) (ng/mL)

The progesterone levels decreased significantly ($P<0.001$) in compared with the control group (GI) after receiving acrylamide treatment for 21 days (Table 1, Figure 2).

3.1.2. Estradiol Level (E2) (ng/mL)

As shown in Table 1 and Figure 3, the levels of estradiol in the serum were discovered to be substantially greater ($P<0.001$) following acrylamide treatment (GII) compared to the control group (GI).

Table 1: Female reproductive hormones and the impact of acrylamide (20 mg/kg b.w.).

Parameter	P4 (ng/mL)	E2 (ng/mL)
Control	11.59±0.28	13.61± 0.07
AA	4.79± 0.38***	128±0.88 ***

P4: Progesterone, E2: Estradiol

Where rats received orally GI: control kept on balanced diet and 0.5 mL of DMSO (1%) Saline (9 %); Acrylamide (AA) group received acrylamide in 0.5 mL of DMSO (1%) saline (9%), (20 mg/kg b. w.) for 21days.

3.2. Histopathology study

The purpose of the current histological investigation was to provide explanation for the biochemical results. Ovaries of control group (Figure 4-A) showed normal texture of ovarian follicles with intact ova, normal corpus luteum and normal interstitial cells; On the other hand, administration of acrylamide (AA) (Figure 4-B) for 21

days resulted in the formation of atretic follicles, cyst transformation with attenuated layer of granulosa cells. Marked degeneration of corpus luteum cells (shrunken cytoplasm and dark nuclei) was also observed.

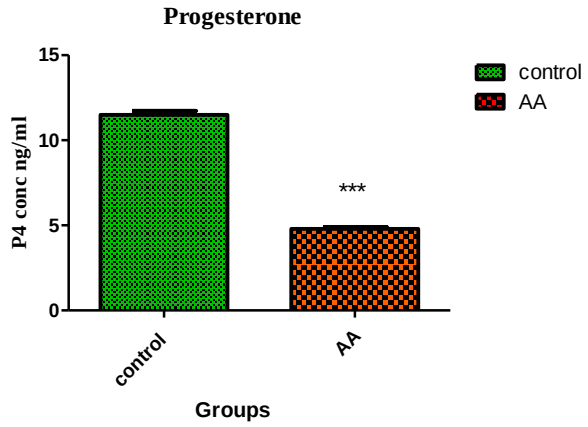

Figure 2: Progesterone levels in the sera of different groups

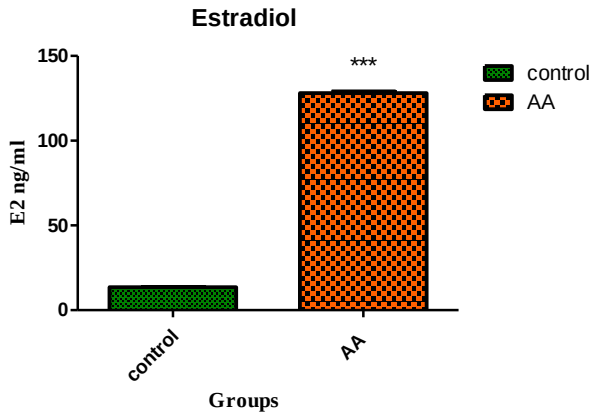

Figure 3: Estradiol levels in the sera of different groups

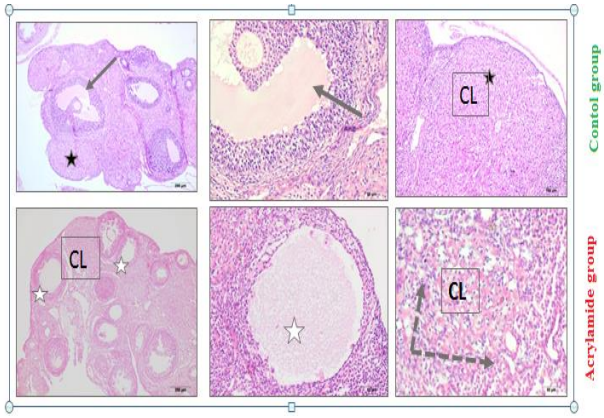

Figure 4: Paraffin sections of female rat ovary stained by H&E to show: Control group showed normal follicles (arrows) and corpora lutea (CL) (black stars). Acrylamide group: showed degenerative cystic changes of mature follicles (white stars) and few corpora with vacuolated degenerated cells (dotted arrow).

4 Discussion

The present study was focused in re-evaluation of acrylamide effects in female rats regarding ovarian hormones levels (P4 & E2) in correlation to ovarian histological structure .

Data revealed that progesterone levels in the (AA) group were considerably lower than those in the control group, which is consistent with Wei, et al. [20] who reported that progesterone was decreased by AA in female mice in a dose-dependent manner. The authors explained this decline as being caused by AA reducing the quantity of corpora lutea. Based on the histology observation of deteriorated corpora lutea in the (AA) group, a comparable explanation for the current results may be offered. Another study reported that in female rat, steroid hormone release was affected by AA administration and the authors linked this disfunction to the apoptotic changes on granulosa cells and absence of corporal lutea [25]. When compared to the control group, the mean value of estradiol E2 (ng/ml) increased in a highly significant manner (P 0.001) after (AA) administration. This result was disagreed with what was reported by Wei, et al., who found a decrease in E2 in contrast with the increase in progesterone level and explained this decrease by degeneration of follicular granulosa cells which was similar finding to what was found in ovarian histological examination in the present study [20].

Cystic changes observed in in the present study pointed to a status like polycystic ovarian syndrome (PCOS) changes. Janssen et al., reported that low progesterone, normal to high estradiol, and high testosterone are all symptoms of PCOS [26]. In the current investigation, it was discovered that progesterone was significantly drop and estradiol was significantly elevated, and this may suggest that acrylamide directly or indirectly impacts ovarian follicles by decreasing pituitary gland release of the hormone FSH, which promotes follicle growth and controls androstenedione to estradiol conversion [27]. Additionally, these outcomes partially correspond to those of Mannaa et al. who discovered that acrylamide significantly decreased estrogen and progesterone levels in rat females [28].

The toxic effect of AA-induced ovarian damage was established in this investigation by histological analysis of ovarian tissue. Wei Q., et al., [20], Amin, K., et al., [29], and Duan X., et al., [30] observed that oral acrylamide exposure to rats resulted in an obvious decrease in ovarian follicle number, as well as ovarian atrophy and the a number of atretic follicles. The presence of mature follicles and corpus luteum in ovarian sections after a 28-day AA treatment by Rawi et al. contradicts previous findings,

which show that AA does not significantly alter the ovary [31].

5 Conclusion

Acrylamide exposure disrupted the steroidogenic pathway (altered P4 & E2 levels). Histological investigation also demonstrated that AA exposure caused follicular maturation problems and ovarian structural deterioration. Avoiding uncontrolled combustion of food rich in acrylamide especially in females is recommended to avoid such toxicity.

Abbreviations

AA: acrylamide; TA: P4: Progesterone, E2: Estradiol, CL: corpus lutum; PCOS: polycystic ovarian syndrome

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Declarations

Ethics approval and consent to participate The study was designed and conducted according to ethical norms approved approved by Sohag University Animal Research Ethics Committee (Sohag, Egypt).

Consent for publication

Not application.

Competing interests

The author declare they have no competing interest.

Availability of data and materials

The corresponding author will provide the raw data used in this work upon reasonable request.

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