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Curcumin Ameliorates Acrylamide Induced Ovarian Toxicity in Albino Female Rats: A Biochemical and Histological Study

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Abstract

Acrylamide (AA), an industrial compound, causes reproductive toxicity in female rats due to oxidative stress. In the present study, we investigated the protective effects of Curcumin (Cur) against acrylamide-induced ovarian toxicity in female rats. The animals utilized in this study were divided into 4 groups: GI- Control group received a daily oral administration of 0.5ml DMSO (1%) saline (9%), GII- Acrylamide (AA) group received acrylamide (20 mg/kg b.w.) for 21days, GIII- Acrylamide + Curcumin (AA + Cur) group treated with Curcumin (100 mg/kg b.w.) after acrylamide intoxication for 21 days, GIV- Curcumin (Cur) group that received only Curcumin (100 mg/kg b.w.). In our study, we observed acrylamide intoxication to be associated with a significant ($P<0.05$) increase in the serum levels of estradiol, luteinizing hormone (LH), testosterone (T), and tumor marker CA125 and CEA. The mean values of serum progesterone (P4), follicle-stimulating hormone (FSH), and total antioxidant capacity (TAC) significantly decreased in the AA- treated group as compared to the control. Histological investigations revealed that AA treatment results in an apparent regression of ovarian follicles, cyst formation, and corpus luteum degeneration. Treatment with curcumin restored altered ovarian histology and serological indices concomitantly to normal

Keywords: Reproductive; female toxicity; Acrylamide; Curcumin

1. Introduction

Exposure to different xenobiotics leads to ovarian toxicity, resulting in alterations at physiological, cellular, and molecular levels, which ultimately leads to disruptions in follicle growth, follicle recruitment, sex steroid hormone synthesis, and oocyte quality, among others[1-2]. Over the past few decades, increasing instances of the impairment of the female reproductive functions in humans have raised major concerns about food products and chemicals present in our environment [3]. Acrylamide is a vinyl monomer (**Figure1**), which exists in the form of a white crystalline powder [4]. Acrylamide can be easily detected in the starchy foods products that have been treated at high temperatures (120°C). Humans are exposed to acrylamide in daily life via consumption of processed food, drinking water, or industrial and laboratory use. Therefore, the normal population is exposed to acrylamide through their dietary intake and other sources [5-8]. Acrylamide is neurotoxic to experimental animals and humans [9-10], Various studies on different experimental animal models have shown that acrylamide exposure results in reproductive toxicity [11-13], hepatic toxicity [14-15], skeletal muscle weakness, ataxia, and hind-limb foot splay [16], and development of cancer [17]. Hence, the potential harm of acrylamide on human health should not be underestimated. Acrylamide exposure results in oxidative stress at both cellular and tissue level. Additionally, cytochrome P450 2E1

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(CYP2E1) leads to oxidative biotransformation of acrylamide into a more potent metabolite, glycidamide, which in turn is more is more reactive toward proteins, including hemoglobin and DNA, in comparison with acrylamide [18]. Recent studies have reported the promising therapeutic potential of alternative medicines (Herbs) for the treatment of diseases or amelioration of adverse effects of chemotherapy and other toxicants [19] . Among medicinal herbs, *Curcuma longa* is an important herbaceous plant species that yields curcumin, which in turn has been used as a natural antioxidant [20].

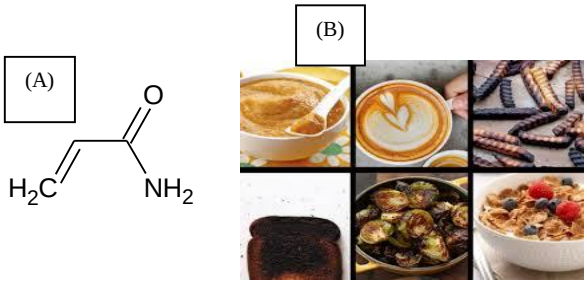

Figure 1: (A) Chemical structure of acrylamide (B) Acrylamide source in food products

Curcumin (diferuloylmethane) (Cur), a famous flavonoid, with the chemical formula of 1,7-bis(4-hydroxy-3 methoxyphenyl)-1,6-hepta-diene-3,5-dione (**Figure 2**), is extracted from the ground rhizome of the Curcuma species, and it has been used for centuries for culinary and food coloring purposes, and as a key ingredient of many medicinal preparations [21-22]. Curcumin has been scientifically demonstrated to function as an antioxidant [23], anti-inflammatory [24-25], anti-bacterial substance [26] along with anticarcinogenic activities [27-28]. Several studies that have reported curcumin used as protective reagent such as the hepatoprotective and nephroprotective [29-30], and myocardial infarction a protective role [31], in various animal models [32] and human studies [33]. Also, many studies have reported that curcumin directly reduces proliferation and increases apoptosis in ovarian cancer cells [33-36] , protects against the negative effects of ovarian insufficiency [37-38] and oxidative stress on ovarian function [39]. Some studies have also demonstrated that curcumin promotes ovarian growth, follicullogenesis and steroidogenesis [37]. Recently, it has been reported that curcumin was used as a supplement in the treatment of COVID-19 patients, and possible mode of action was reported [40].

Considering the toxic effects of acrylamide and properties of curcumin and that no study has ever evaluated the effects of curcumin on the acrylamide-induced ovarian toxicity, the current study was performed to assess the ameliorating effect of curcumin on the acrylamide-induced ovarian toxicity via the study of some reproductive parameters, histological alteration, and possibility ovarian cancer induction.

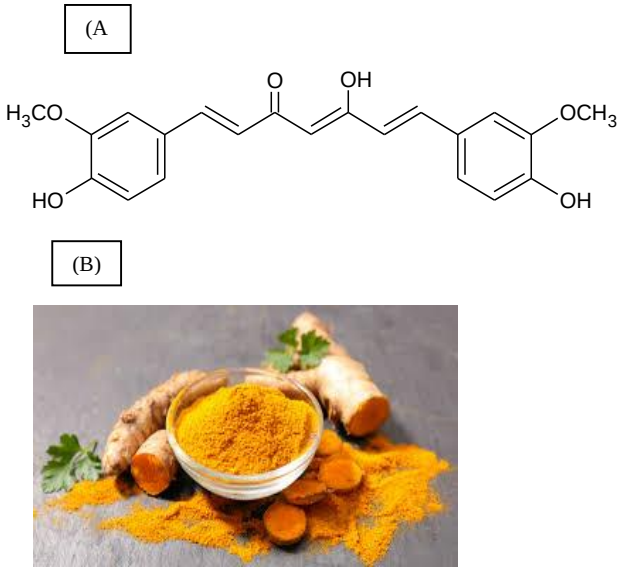

Figure 2: (A) Chemical structure of Curcumin (B) Curcumin (*Curcuma longa*)

2. Material and Methods

2.1. Chemicals

Acrylamide, ≥ 98.0 % (GC), CAS: 79-06-1, MW: 71.08 g / mol, mp: 81-87 ºC and P Code: 101601204. Curcumin from curcuma longa (Turmeric), powder ≥ 65.0 % , CAS: 458-37-7, $C_{20}H_{21}O_6$, MW: 368.38 g/mol, and P Code: 1002542750. Acrylamide and curcumin were obtained from SIGMA-ALDRICH Chemical Co**.**

2.2. Animals

The experiment was upon forty healthy female albino rats, (120-150 gm). Animals were provided by an animal house in Sohag University, faculty of Science. Rats were kept in the experimental room two weeks before starting the experiment for acclimateization. They were kept in metal cages under hygienic conditions, Animals were fed on a commercial pellet diet and kept under a normal light/dark cycle. This work was carried out following the guidelines of Sohag University for animal use and approved by Ethics and Animal Care Committee (approval number: Sohag-2-2-2021-03).

2.3. Experimental design

Rats were kept in the experimental room two weeks before starting the experiment for acclimatization.

They were kept in metal cages under hygienic conditions with free access to standard rat diet .The animals were sorted into 4 groups, (N = 10), GI: control group (received a daily oral administration of 0.5ml DMSO (5%) saline(9%); GII: (AA) group (received a daily oral administration with freshly prepared of AA , 20 mg/kg body weight) for 21days which is less than lethal dose the LD_{50} of acrylamide in rats (150 mg /kg/ BW) [41]; GIII:AA+Curcumin group (recei-ved a daily oral administration of Cur, 100 mg/kg body weight) after acrylamide intoxication for 21 day and GIV: Curcumin group (received a daily oral administration of Cur only, 100 mg/kg body weight) for 21 consecutive days [42].

2.4.Sample collection

Animals were first deeply ether anesthetized and blood samples were taken from the heart. Subsequently, serum was separated from the blood samples in plain tubes and centrifuged at 5000 *rpm* for 10 min, and the samples were stored at -20˚C till further analysis. After blood collection, rats were euthanized, the abdomen was opened, and the whole ovaries were dissected. The ovaries were then washed with isotonic saline, and fixed in 10% neutral formalin for further histopathological investigations.

2.5. Biochemical study

2.5.1. Hormonal assay

ELISA procedure was used for the quantitative determination of serum female reproductive hormones (Progesterone (P4) [43] (PG362S), Estradiol (E2) [44] (ES150S-100), follicle-stimulating hormone (FSH) [45] (FS232F), luteinizing hormone (LH) [46] (LH231F), Testosterone [47] (TE187S) Kits were purchased from CALBIOTECH, an Egyptian company.

2.5.2. Ovarian tumor markers assay

Enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of ovarian tumor markers (CA125) [48] (CA239T), and carci-noembryonic antigen (CEA)[49] (CE236T) using specialized CALBIOTECH kits obtained from Calbiotch, an Egyptian company.

2.5.3. Assay of total antioxidant capacity TAC

To measure total antioxidant capacity, the sample was mixed with a predetermined amount of exogenously delivered hydrogen peroxide to determine the total antioxidant in serum quantitatively using a colorimetric approach utilizing a kit obtained from Biodiagnostic, an Egyptian firm (H_2O_2). The antioxidants in the sample help to remove some of the H_2O_2 that is present. An enzymatic reaction involving the conversion of 3,5, dichloro-2-hydroxy-benzenesulphonate into a colored product was used to quantify the remaining H_2O_2 using calorimetry**:** TAC (TA 2513) kit obtained from bio diagnostic company, Egypt [50].

2.6. Histopathology study

Preparation of ovary Section and Histopathological Examination

The ovaries were collected and fixed in a 10% formalin solution. Subsequently, the samples were then routinely processed and 4-5 mm thickness sections were prepared. The tissue sections thus obtained were mounted on a glass slide, depara-ffinized and the sections were then stained with Hematoxylin,and Eosin stain (Thermo Fisher Scientific, USA) by following standard procedure. The sections were then examined and observed under a light microscope at 100X and 400X magnifications (Leica, Germany)[51].

2.7. Statistical Analyses

Graph Pad Prism 5 software (San Diego, CA. USA) was used to perform statistical analyses. One way analysis of variables (ANOVA) was used in the analyses as posted by Newman-keuls test. The results are presented as mean ± SD and the level of significance between groups is denoted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ [52].

3. Results

3.1. Biochemical study

3.1.1. Ovarian hormones

3.1.1.1. Progesterone (P4) (ng/mL)

In comparison to the control group GI, a highly significant drop ($P<0.001$) in the progesterone levels was observed in response to acrylamide treatment given for 21 days as shown in **Table 1**. The mean values of serum P4 in the GIII (AA + Cur) group were found to increase significantly ($P<0.001$) when compared to a control (GI) and non-treated group (GII). Curcumin-treated rats showed a highly significant ($P<0.001$) increase in the progesterone levels as compared to control (GI) rats.

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

In our experiments, the levels of estradiol in the serum were found to be significantly higher ($P<0.001$) after acrylamide treatment (GII) compared to the control group (GI). A highly significant

difference (P<0.001) in the mean values of E2 was observed in the GIII (AA + Cur) and GIV (Curcumin alone) groups compared to the control group (GI). However, when comparing GIII to GII (AA), the level of E2 was found to be significantly lower as indicated in **Table 1**.

3.1.1.3. Testosterone (T) (ng/mL)

The data presented in **Table 1** show that the mean values of serum testosterone (T) (ng/mL) in the GII group were significantly higher (P<0.001) in comparison with the control group (GI), and that the mean values of serum testosterone in the GIII (AA+ Cur)) group increased significantly (P<0.001) when compared to the control group (GI), and there was also a significant decrease compared to the non-treated GII group (AA). In addition, there was no significant difference (P > 0.05) in the mean values of testosterone in the Cur group (GIV) as compared to the control group (GI).

3.1.2. Pituitary hormones

3.1.2.1. FSH (mIU/mL)

As shown in the data presented in **Table 1**, the mean values of serum FSH (mIU/mL) level were found to significantly decrease (P<0.001) in AA group GII (AA) as compared to the control GI. The FSH level was found to significantly increase (P<0.01) in the group GIII (AA+Cur) compared to GII (AA) and also, there was a significant decrease in FSH level compared to the control GI. However, there was a non-significant change (P > 0.05) in the mean values of FSH in GIV (Cur) group as compared to the control GI.

3.1.2.2. LH (mIU/mL)

The data in **Table1** shows that the mean values of serum LH (mIU/mL) showed a highly significant increase (P<0.001) in the acrylamide group (GII) as compared with the control group. The LH level was significantly decreased (P<0.001) in GIII compared to GII (AA). Also, there was a significant increase (P<0.001) in the mean values of serum LH in GIII (AA + Cur) observed as compared to the control group (GI). On the other hand, there was a non-significant change (P > 0.05) in the mean values of T in Cur group GIV as compared with control GI.

3.1.3. Total antioxidant Capacity TAC level.

TAC concentration (mmol/L) of the serum in both the treated and control group are shown in **Table 2**, which revealed that the TAC concentration showed a highly significant decrease (P<0.001) in the GII (AA) compared to the control GI. TAC levels in GIII (AA+Cur) group showed a non-significant difference compared to the control GI and a highly significant increase when compared to the non-treated group GII. In the GIV (Cur alone) group, TAC levels were found to be significantly increased when compared to the control GI.

3.1.3. Ovarian tumor markers

3.1.3.1. CA125 (U/L) level

The oral administration of acrylamide for three weeks led to a highly significant increase (P<0.001) in the mean values of serum CA125 (U/L) in the AA group (GII) as compared to the control (GI). The level of CA125 in the GIII (AA+Cur) group was significantly low in comparison to the non-treated GII (AA). Also, the level of CA125 in the GIII group was still high in comparison with the control GI group. On the other hand, there was a non-significant change (P<0.01) in the mean values of CA125 in Curcumin group (G1V) as compared to the control as illustrated in **Table 3**.

3.1.3.2. CEA (ng/mL) level

The data is presented in **Table 3**, clearly shows highly significant increase in CEA (ng/mL) in the mean values (P<0.001) in the GII (AA) group. The level of CEA in GIII (AA+Cur) group was significantly lower as compared to the non- treated GII group (AA). The level of CEA in the GIII group was still high in comparison with the control GI group. Oral administration of Curcumin (G1V) for 21 days revealed that the change in the mean values of CEA were not significant (P<0.01) when compared with the control.

3.2.Histological study

To support our biochemical results, we performed a histological study to provide further evidence regarding the protective role of curcumin. Control ovary (**Figure 6 -GI**) showed the normal texture of ovarian follicles with intact ova, normal corpus luteum and normal interstitial cells; no alterations in normal ovarian histological features were observed in the group that received curcumin (Cur) alone (**Figure 6-GII**). On the other hand, administration of acrylamide (AA) (**Figure 6-GIII**) for 21days resulted in the formation of the atretic follicle, the cyst transformation with an attenuated layer of granulosa cells. Marked degeneration of corpus luteum cells (shrunken cytoplasm and dark nuclei) were also observed. In the AA+Cur group (**Figure 6-GIV**), ovarian tissue showed potential protection with the

preservation of growing follicles. Residual cysts are still present with a prominence of interstitial cells (white arrow).

5. Discussion

The ovary is a complex, heterogeneous organ essential for the reproductive and overall health of females. The ovary releases female sex hormones such as estrogen and progesterone, as well as the female gamete, ova (also known as "eggs"). The follicle and corpus luteum (CLs) are the two temporary endocrine glands found in the ovary. An oocyte is found in each mature follicle, which in turn is covered by multiple layers of hormone-secreting cells (granulosa cells). The two most common hormones secreted by these cells are estradiol and testosterone [53]. A follicle ovulates when it reaches its full maturity, and the oocyte is then discharged into the oviduct. As a result, surviving cells undergo physical and chemical modification, resulting in the formation of corpus lutea (CLs). The principal job of CL is to secrete progesterone, which helps regulate pregnancy maintenance. Subsequently, if the egg is not fertilized and pregnancy does not occur, the corpus lutea regresses, and follicle maturation and ovulation start again [53]. In the hypothalamic–pituitary–gonadal axis, the hormones released by follicles and CLs work in complex feedback loops with gonadotropins and hypothalamic hormones [54]. The ovary's structural diversity and unique biochemical processes make it a potential target for chemical toxicants such as pharmaceutical drugs, occupational chemicals, and environmental pollutants, all of which can cause ovarian toxicity. Acrylamide (AA) is a dietary contaminant that can be found in a wide variety of commonly consumed foods, making human exposure to this toxicant unavoidable [55].

The study of acrylamide in female rats has been focused on its effect on ovarian function, cancer markers, total antioxidant, histological, and the possible effective role of curcumin as a treatment

Data collected in the present study show that P4 levels were significantly lower in the experimental group GII (AA), and the estradiol E2 (ng/ml) and testosterone T levels (ng/ml) were significantly enhanced ($P<0.001$) post acrylamide treatment as compared to the control group (GI). These results are similar to that reported by Janssen *et al*., (2004) in the polycystic ovarian syndrome which is characterized by low progesterone, normal to high estradiol, and high testosterone levels [56].

Also, the present results are in partial agreement with Mannaa et al., where it was reported that acrylamide induced a significant decrease in the levels of estrogen and progesterone in rats [57]. The lower levels of progesterone and higher levels of estradiol in the AA-treated group could imply that acrylamide directly or indirectly affects ovarian follicles by reducing pituitary gland release of FSH, which is responsible for increasing the follicle growth and regulating androstenedione to estradiol conversion [58]. The decline in the value of progesterone indicated a decrease in the number or function of corpus lutea. In the acrylamide treated group (GII), an increase in the testosterone levels can be explained by the inhibitory activity of acrylamide inhibitor activity on aromatase cytochrome P-450, which is an enzyme necessary that is essential to convert androgens into estradiol. Estradiol is secreted by the ovarian follicles [59]. In contradiction with the present result, Nagata et al, 2013 reported that in non-pregnant women, a lower level of estradiol was associated with higher acrylamide intake via food sources.

The significant decrease in Follicle-Stimulating Hormone (FSH) (mIU/L) levels reported in the current study after exposure to acrylamide can be explained because of the increase of estradiol levels due to feedback inhibition of FSH release by the anterior pituitary [60-61]. A significant increase in the luteinizing hormone LH level was observed as compared to the control group (GI). The observed increase in the LH (mIU/L) levels over the normal physiological limits could be because of the low progesterone levels as observed in the present study.

In contradiction with the present results, Nagata et al. (2014) observed that an increase in FSH level is associated with high acrylamide intake, and they reported hypothesized that it could be a result of negative feedback triggered by low levels of estrogen [60]. Curcumin is a well- known antioxidant that can mitigate or prevent the harmful action of environ-mental contaminants. Curcumin positively impacts many physiological processes, including the female reproductive system (ovarian follicular development, puberty, reproductive aging) [41,62-63]. Curcumin affects the female reproductive system by modulating the release of pituitary, and ovarian hormones, as well as growth factors and cytokines[64].

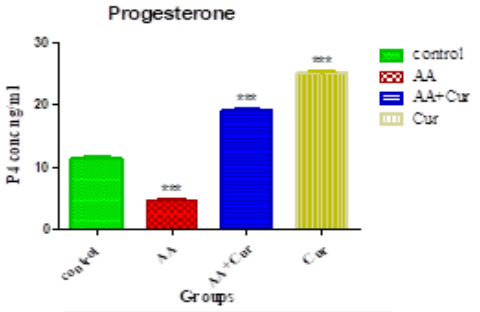

Figure 3a: Progesterone levels in the sera of different groups

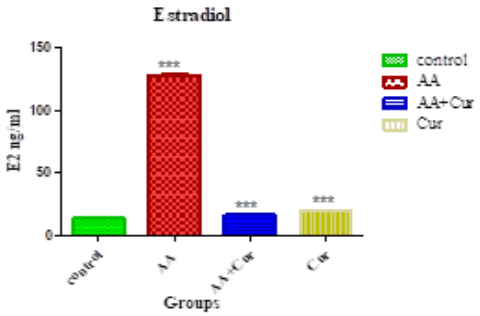

Figure 3b: Estradiol levels in the sera of different groups

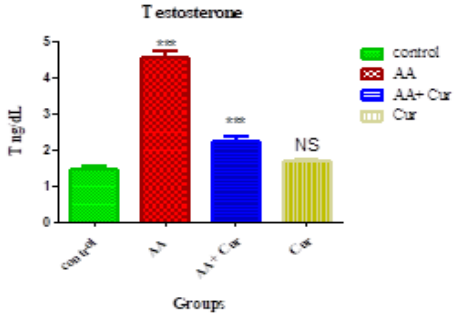

Figure 3c: Testosterone levels in the sera of different groups

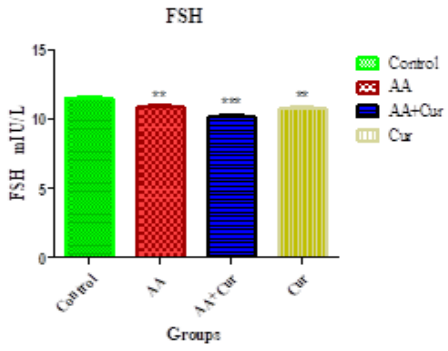

Figure 3d: FSH levels in the sera of different groups

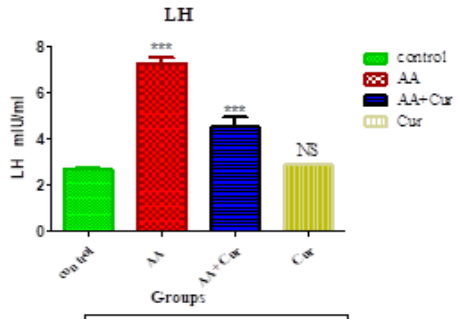

Figure 3e: LH levels in the sera of different groups

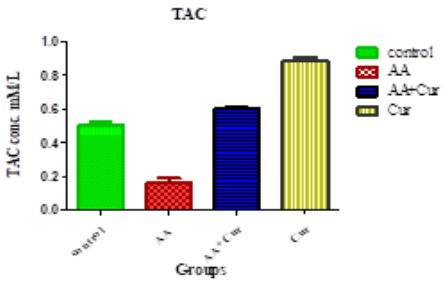

Figure 4: TAC levels in the sera of different groups

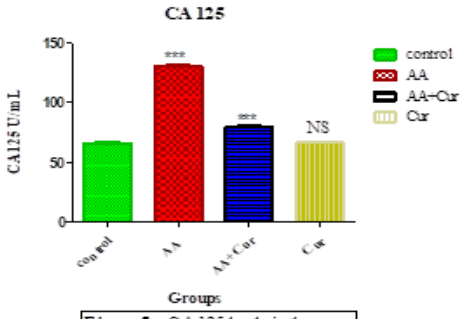

Figure 5a: CA 125 levels in the sera of different groups

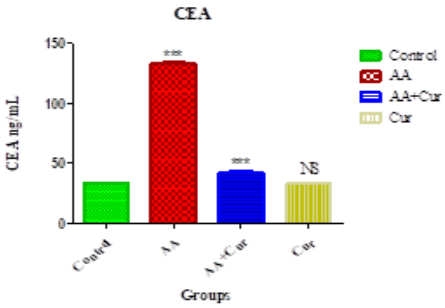

Figure 5b: CEA levels in the sera of different groups

Table 1: Effect of acrylamide (20 mg/kg b.w.) and curcumin (100 mg/kg b.w.) on female reproductive hormones.

Parameter	GI	GII	GIII	GIV
P4 (ng/mL)	11.49±0.74	4.7951± 0.36^{a***}	19.12±0.56 ^{a***,b***}	25.16 ±0.64 ^{a***}
E2 (ng/mL)	13.61± 0.21	128±2.7 ^{a***}	16.29 ±0.83 ^{a***,b**}	19.24± 0.27 ^{a***}
T (ng/mL)	1.46±0.34	4.57±0.57 ^{a***}	2.23±0.50 ^{a,b***}	1.67±0.14 ^{a,NS}
FSH (mU/mL)	11.48± 0.47	10.37±0.03^{a***}	10.76±0.26 ^{a***,b**}	11.30±0.14^{a,NS}
LH (mU/mL)	2.6±0.22	7.25±0.78 ^{a***}	4.30±0.51 ^{a***,b***}	2.84±0.12^{a,NS}

P4: Progesterone, E2: Estradiol, T: Testosterone, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone Data are expressed as mean ± SD, Significant change in comparison between groups: *p<0.05;**p<0.01; ***p<0.001, N.S Nonsignificant (P > 0.05)

[a] Significant change in comparison between GI & GII; GI& GIII; GI& GIV

[b] Significant change in comparison between GII& G^{III}

Where rats received orally GI: control kept on a balanced diet and 0.5 mL 1% DMSO saline(9%), ; GII: Acrylamide group received acrylamide in drinking water (20 mg/kg b.w.) for 21 days. GIII:Treated with curcumin (100 mg/kg b.w.) after acrylamide intoxication for 21 days; GIV; curcumin only as control

Table 2: Effect of acrylamide (20 mg/kg b.w) and curcumin (100 mg/kg b.w) on total antioxidant capacity.

Parameter	GI	GII	GIII	GIV
TAC mM/L	0.50±0.05	0.16±0.08^{a***}	0.54± 0.07$^{a***,b\ NS}$	0.68±0.04^{a***}

TAC: Total antioxidant capacity

Significant differences in comparison between groups are represented as mean ± SD. *p<0.05;**p<0.01; ***p<0.001, N.S Nonsignificant (P> 0.05), [a] Significant change in comparison between GI & GII; GI& GIII; GI& GIV, [b] Significant change in comparison between GII& GIII

Table 3: Effect of acrylamide (20 mg/kg b.w) and curcumin (100 mg/kg b.w) on tumor markers.

Parameter	GI	GII	GIII	GIV
CA125 U/mL	66.10±2.3	130.90±2.4***	77.02±1.89^{***,b***}	66.95± 2.15^{a***}
CEA ng/mL	33.38±1.8	133.60±2.4***	43.16±0.39 $^{a***\ b***}$	31.26±0.17$^{a\ NS}$

CA 125: Cancer antigen 125, CEA: Carcinoembryonic antigen.

Significant differences in comparison between groups are represented as mean± SD. *p<0.05;**p<0.01; ***p<0.001, N.S Non significant (P > 0.05) [a] Significant change in comparison between GI & GII; GI& GIII; GI& GIV. [b] Significant change in comparison between GII& GIII

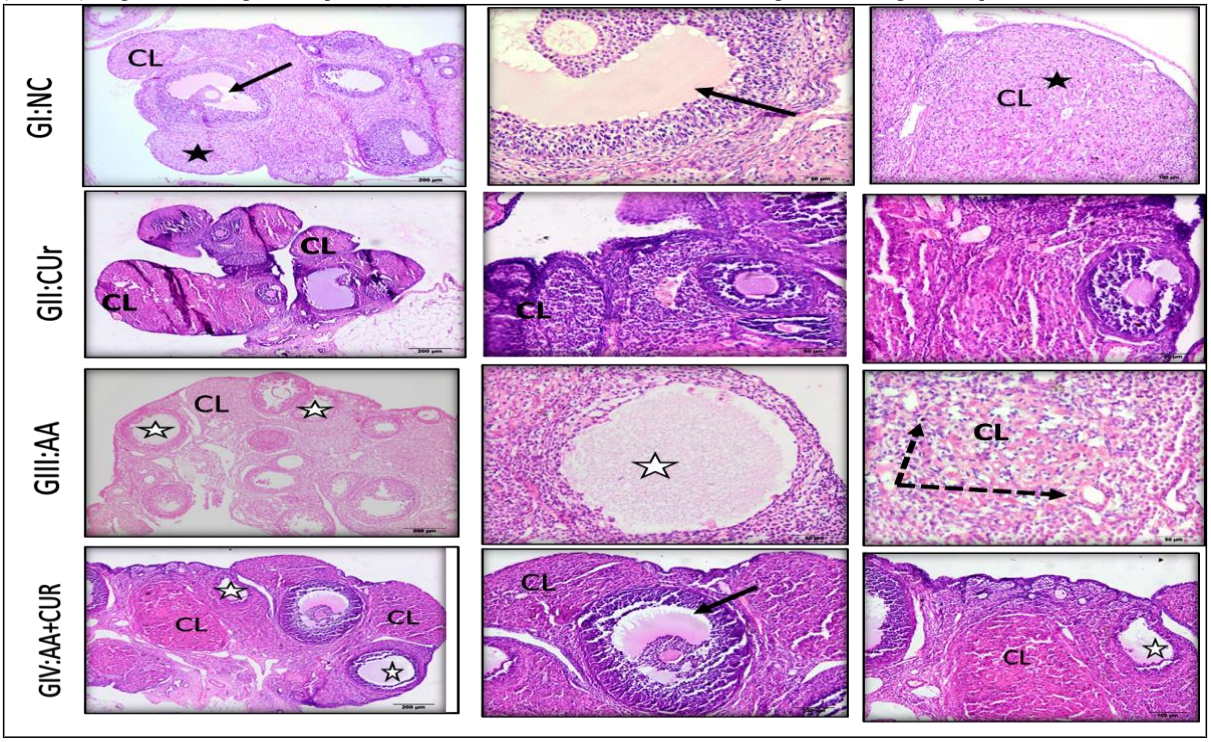

Figure 6: Photomicrography of Paraffin sections of rat ovary stained by H&E show: GI: NC- Normal control with normal follicles (arrows) and corpora lutea (CL). GII: Cur treated. No marked alteration in the normal structure of the ovary. GIII: AA acrylamide group: showed degenerative cystic changes of mature follicles (white stars) and few corpora. GIV): AA+CUR treated group: showed degenerative cystic changes of mature follicles (white stars) and few corpora. GIV): AA+CUR treated group showing potential preservation of ovarian structure with intact growing follicles (arrows) and many corpora (CL)

The improvement in the release of all sex hormones (P4, E2, FSH, LH and testosterone) as a result of curcumin administration corroborated with the results presented by Mohammadi et al (2017), who they reported increased levels of FSH and decreased LH and testosterone levels post curcumin treatment in a PCOS model, and these results are in agreement with the findings of our study [65]. These effects may probably be due to the improvement in ovulation and corpus luteum development as a result of curcumin treatment [65]. These results clearly indicate that curcumin has the potential to ameliorate the reproductive endocrine function and induce follicular maturation. Studies performed by Azami SH, *et. al* and Melekoglu R, *et.al*. have also demonstrated the ability of curcumin to decrease the concentrations of gonadotropins FSH (follicle-stimulating hormone) and LH (luteinizing hormone) [39, 66].

Sirotkin et.al, (2018) reported that rabbits who were fed curcumin showed an increased progesterone production and also a simultaneous reduction in testosterone production by ovaries . Additionally, curcumin treatment also resulted in an altered response of ovarian cells to the luteinizing hormone in rabbits [67]. Not surprisingly, similar observations were also observed in the in vitro experiments performed in the porcine granulosa cells, where curcumin treatment was shown to stimulate progesterone and testosterone release upon curcumin treatment [68]. In conclusion, curcumin treatment enhances the release of gonadotropins by pituitary cells, the release of hormones by ovarian cells, and the response of ovarian cells to gonadotropins.

Analysis of TAC concentration showed a highly significant decrease in TAC concentration post acrylamide treatment. A low level of total antioxidants in serum in GII (AA) might be attributed to oxidative stress. Oral administration of curcumin after acrylamide intoxication for 21 days showed a highly significant increase in TAC concentration as compared to the non- treated G II (AA) group. Curcumin may function by activating the detoxification enzymes, which in turn detoxify reactive oxygen species (ROS) produced in the cells after the toxicants have been administered [69]. Acrylamide has been established as a potent is a carcinogen in the animal studies, and acrylamide has also been classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen [70]. Glycidamide, an epoxide metabolite generated by cytochrome P4502E1 (CYP2E1) activity on acrylamide, and acrylamide are clastogenic, and glycidamide is also known to form DNA adducts. Acrylamide has been reported to react with glutathione, thereby influencing the redox status of cells and also altering the gene transcription profile of the impacted cells, or it may hinder the DNA repair process or hormonal balance; all these changes constitute non-genotoxic pathways that are impacted by acrylamide treatment [12]. In the present study, a 20 mg/kg/day acrylamide dose was administered to the female rats and the carcinogenicity of acrylamide and the interaction between acrylamide and hormonal disorders in the female rats were studied in detail. Elevated levels of CEA, which is a well-known cell surface glycoprotein, have been reported in patients with different cancers, and the stage and extent of the tumor directly correlate with the elevated plasma levels of CEA [71]. The analyses of ovarian tumor marker CA125 and CEA showed that the mean value of the two parameters in GII (AA) significantly increased than the control group, but in GIII (AA+Cur) it was highly significantly decreased than GII, Also in GIV was non-significant than the control group. These findings demonstrated that acrylamide induces oxidative DNA damage, which could contribute to its carcinogenic potential [72], and these results were consistent with acrylamide (AA) being a gene mutagen in rats *via* the metabolism of glycidamide.

In this study, an evaluation of the protective effect of curcumin against ovarian damage and histological changes induced by acrylamide in rats was done. Female rats who received acrylamide for 21 days showed the formation of many cystic cells. The ovarian cortex exhibited the presence of an atretic follicle, and the corpus luteum showed degenerated cells and massive degeneration of follicles. Similar results have been recorded by Wei Q., et al., Amin, K., et al., and Duan X., et al., reported that rats exposed to oral acrylamide showed a noticeable reduction in the number of follicles and the atrophy of the ovary and atretic follicles [41,73-74]. However, these results do not agree with Rawi et al., who reported that acrylamide does not affect the ovary significantly with mature follicles and corpus luteum in ovarian sections after 28-day treatment with acrylamide [75]. Histological studies supported our findings and validated that low serum levels of FSH and increased estradiol levels in the acrylamide treated group treated resulted in reduced folliculogenesis. Treatment with curcumin reduces ovarian structural deterioration and follicular maturation alterations. The increased folliculogenesis in the curcumin-treated group also avoided decreased ovarian steroidogenesis and impacted the hypothalamic-hypophysial-ovarian axis, presumably by reducing oxidative stress and increasing antioxidant levels [47]. The current work reveals that curcumin's antioxidant activities protect against the development of acrylamide-induced ovarian toxicity.

5.Conclusion

In conclusion, our results showed that curcumin protecte the ovary against the development ovarian toxicity induced by AA exposure in female rats via its antioxidant properties. Therefore, it is a safe and an effective component in the diet, it could be applied as a potential strategy for the intervention of AA toxicity.

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Conflict of interest

The authors declare that they have no competing interests.

References

[1] Wang, X., Jiang, S.W. , Wang, L., Sun, Y., Xu, F., He, H., Wang, S., Zhang, Z., Pan, X., Interfering effects of bisphenol A on in vitro growth of preantral follicles and maturation of oocytes, (2018) *Clin. Chim. Acta* 485: 119-125.

[2] Zhou,C., Wang,W., Peretz,J., Flaws, J.A., Bisphenol A exposure inhibits germ cell nest breakdown by reducing apoptosis in cultured neonatal mouse ovaries. (2015) *Reprod. Toxicol* 57:87-99.

[3] Colborn, T., Vom Saal, F. S., Soto, A. M., Developmental effects of endocrine disrupting chemicals in wildlife and humans. (1993) *Environ Health Perspect* 101: 378-84.

[4] Parzefall, W., Minireview on the toxicity of dietary acrylamide. (2008) *Food Chem Toxicol* 46(4):1360-4.

[5] Claus A, Carle R, Schieber A. Acrylamide in cereal products. (2008) A review J Cereal Sci 47:118-33.

[6]Shalaby, R., Anwar, D., Hassan, R. (2022). Acrylamide Formation In Cake Baked In Different Utensils And Novel Intervention Strategies. *Egyptian Journal of Chemistry*, (), doi: 10.21608/ ejchem. 2022. 126624.5611

[7] El-sayed, O., Mahmoud, A., El-nikeeti, M., Sallam, Y. (2022). Preventive Action of Acrylamide Formation as A Chemical Hazard During Deep Fat Frying of Potato Strips. *Egyptian Journal of Chemistry*, (), -. doi: 10.21608/ejchem. 2022. 111897. 5086

[8] Abdel-Haleem, A. M. (2021). The Relationship Between Varieties and Acrylamide Formation In Roasted Barley. *Egyptian Journal of Chemistry*, 64(9), 1-2.

[9] Hung, C. Y., Chang, C.H., Lin, T. J., Yi, H. H., Tsai, N. Z., Chen, Y. R., Chen, Y.T. (2022) AQP4 Attenuated TRAF6/NFκB Activation in Acrylamide-Induced Neurotoxicity. *Molecules* 27(3):1066.

[10] Seale, S.M., Feng, Q., Agarwal, A.K., El-Alfy, A.T., Neurobehavioral and transcriptional effects of acrylamide in juvenile rats. (2012) *Pharmacol Biochem Behav* 101(1):77-84.

[11] Favor, J., Shelby M.D., Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure. (2005) *Mutat Res* 580: 21–30.

[12] Ma, Y., Shi, J., Zheng, M., Liu, J., Tian, S., He, X., Zhang, D., Li, G., Zhu, J. (2011) Toxicological effects of acrylamide on the reproductive system of weaning male rats. *Toxicol Ind Health* 27(7): 617-627.

[13] Zhao, C. Y., Hu, L. L., Xing, C. H., Lu, X., Sun, S. C., Wei, Y. X., Ren, Y. P. (2022)Acrylamide Exposure destroys the distribution and functions of organelles in mouse oocytes. *Front Cell Dev Biol* 10:834964.

[14] Elsawi, N. M. , Aldajani, W. A. Mounir, A. A., M., Ali, A. M. Gamal .S.A, Ali, S.S. Abd Ellah, S. A. (2019) Copper (I) Nicotinate complex Abrogates Acrylamide Induced Hepatotoxicity in Male Rats: Biochemical and Histological Studies *J Food Nutr Sci* 6(1):21-31.

[15] Er, R., Aydın, B., Şekeroğlu, V., Atlı, Ş. Z. (2020) Protective effect of Argan oil on mitochondrial function and oxidative stress against acrylamide-induced liver and kidney injury in rats. *Biomarkers*. 5(6):458-67.

[16] Zhao M, Zhang, B., Deng, L. (2022) the mechanism of acrylamide-induced neurotoxicity: current status and future perspectives. *Front Nutr* 9:859189.

[17] Maier, A., Kohrman-Vincent, M., Hertzberg, R., Allen, B., Haber, L.T. Dourson, M. Critical review of dose-response options for F344 rat mammary tumors for acrylamide - Additional insights based on mode of action. (2012) *Food Chem. Toxicol* 50:1763-75.

[18] Baum, M., Loeppky, R.N., Thielen, S., Eisen, G. (2008) Genotoxicity of glycidamide in comparison to 3-N-nitroso oxazolidin-2-one. *J Agric Food Chem* 56:5989-93.

[19] Hosseini, A., Hosseinzadeh, H. (2018) Antidotal or protective effects of Curcuma longa (turmeric) and its active ingredient, curcumin, against natural and chemical toxicities: A review. *Biomed Pharmacother* 99: 411-421.

[20] Sharifi-Rad, J., El Rayess, Y., Abi Rizk, A., Sadaka, C., Zgheib, R., Zam, W. (2020) Curcumin Health Applications and Safety. *Front Pharmacol* 11(01021):1-23.

[21] Hewlings SJ, Kalman DS. (2017) Curcumin: a review of its'effects on human health. *Foods* 6(10):92.

[22] Sahu, P.K. (2016) Design, structure activity relationship, cytotoxicity and evaluation of

antioxidant activity of curcumin derivatives/analogues. *Eur J Med Chem* 121: 510-16.

[23] Augustyniak, A., Bartosz, G., Cipak, A., Duburs, G., Horáková, L., Luczaj, W., Majekova, M., Odysseos, A. D., Rackova, L., Skrzydlewska, E. (2010) Natural and synthetic antioxidants: an updated overview. *Free Radic Res* 44: 1216-62.

[24] Ueki, M., Ueno, M., Morishita, J., Maekawa, N. (2013) Curcumin ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in mice. *J Biosci Bioeng* 115:547-51.

[25] Geng, X., Hong, Q., Wang, W., Zheng, W., Li, O., Cai, G., Chen, X., Wu, D. (2017) Biological membrane-packed mesenchymal stem cells treat acute kidney disease by ameliorating mitochondrial-related apoptosis. *Sci Rep* 7: 41136.

[26] Mun, S. H., Joung, D. K., Kim, Y. S., Kang, O. H., Kim, S. B., Seo, Y. S., Kim, Y. C., Lee, D. S., Shin, D. W., Kweon, K. T., *et al*. (2013) Synergistic antibacterial effect of curcumin against methicillin-resistant Staphylococcus aureus. *Phytomedicine* 20:714-18.

[27]Ahmed, S., Mohamed, W., El Hamouly, S., maziad, N., abd elmonam, M. (2022). In Vitro Release of Curcumin as an Anticancer Drug from Gelatin Nanoparticles. *Egyptian Journal of Chemistry*, (),-.doi: 10.21608/ ejchem.2022 129597. 5738

[28] Barcelos, K.A., Mendonça, C.R., Noll, M., Botelho, A.F., Francischini, C.R.D., Silva, M. A. M. (2022) Antitumor Properties of Curcumin in Breast Cancer Based on Preclinical Studies: A Systematic Review. *Cancers* 14: 2165.

[29] L ee, Y. S., Oh, S. M.; Li, Q. Q., Kim, K. W., Yoon, D., Lee, M. H., Kwon, D. Y., Kang, O. H., Lee, D.Y. (2022) Validation of a Quantification Method for Curcumin Derivatives and Their Hepatoprotective Effects on Nonalcoholic Fatty Liver Disease. *Curr. Issues Mol Biol* 44:409-32.

[30] Venkatesan, N., Punithavathi, D., Arumugam, V. (2000) Curcumin prevents adriamycin nephrotoxicity in rats. *Br J Pharmacol* 129 (2): 231–4.

[31] Qureshi, S., Shah, A. H., Ageel, A. M. (1992) Toxicity studies on Alpinia galanga and Curcuma longa. *Planta Med* 58 (2):124-7

[32] Lao, C. D., Ruffin, M. T., Normolle, D.,Heath, D. D., Murray, S. I.; Bailey, J. M., Boggs, M. E., Crowell, J, Rock, C. L., Brenner, D. E. (2006) Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med* 17: 6-10.

[33] Shehzad, A., Wahid F., Lee, Y. S., Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. (2010) Archiv Der Pharmazie 343 (9):489-99.

[34] Terlikowska, K. M, Witkowska, A. M., Zujko, M.E., Dobrzycka, B., Terlikowski, S. J. (2014) Potential application of curcumin and its analogues in the treatment strategy of patients with primary epithelial ovarian cancer. *Int J Mol Sci* 15(12): 21703-22

[35] Vallianou, N.G., Evangelopoulos, A., Schizas, N., Kazazis, C. (2015) Potential anticancer properties and mechanisms of action of curcumin. *Anticancer Res* 35(2):645-51.

[36] Seo, J.A., Kim, B., Dhanasekaran, D.N., Tsang, B.K., Song, Y.S. (2016) Curcumin induces apoptosis by inhibiting sarco/endoplasmic reticulum Ca2+ATPase activity in ovarian cancer cells. *Cancer Lett* 371: 30-7.

[37] Aktas, C., Kanter, M., Kocak, Z. (2012) Antiapoptotic and proliferative activity of curcumin on ovarian follicles in mice exposed to whole body ionizing radiation. *Toxicol Ind Health* 28:852-63.

[38] Qin, X., Cao, M., Lai, F., Yang, F., Ge, W., Zhang, X., Cheng, S., Sun, X., Qin, G.,Shen, W., Li, L. (2015) Oxidative stress induced by zearalenone in porcine granulosa cells and its rescue by curcumin in vitro. *PLOS ONE* 10(6):e0127551.

[39] Azami, S.H., Nazarian, H., Abdollahifar, M.A., Eini, F., Farsani, M.A., Novin, M.G. (2020) The antioxidant curcumin postpones ovarian aging in young and middle-aged mice. *Reprod Fertil Dev* 32: 292-303

[40] Heidari, Z., Mohammadi, M., Sahebkar, A., (2021) possible mechanisms and specia clinical considerations of curcumin supplemenatation in patients with COVID-19. Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health, Advances in Experimental Medicine and Biology 1308, Springer Nature Switzerland AG.

[41] Wei, Q., Li, J., Li, X., Zhanga, L., Shi, F. (2014) Reproductive toxicity in acrylamide-treated female mice, *Reprod. Toxicol*., 46: 121-8.

[42] Ashrafzadeh, A.A, Jahromi, H. K., Kamfiruzi, S., Davami, M. H. (2016) Investigation of Curcumin Effects on ovaries and the hormones of LH and Progestron in Wistar rats Treated with Cadmium Chloride. *Int J Pharm Res Allied Sci*5(1):146-53.

[43] Pedersen, S.B., Kristensen, K., Richelsen, B. (2003) Anti-glucocorticoid effects of progesterone in vivo on rat adipose tissue metabolism; *Steroids* 68(6): 543-50.

[44] Tietz, N.W. (1995) Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Co., Philadelphia, 216-17.

[45] Soldin, O.P., Hoffman, E. G., Waring, A., Soldin, S. J. (2005) Pediatric refrance intervals for FSH, LH, Estradiol, T3, Cortisol and growth hormone on the DPC Immunolite 1000. *Clinica Chemica Acta* 355: 205-10

[46] Ulloa-Aguirre, A., Timossi, C. (1998) Structure-function relationship of follicle-stimulating hormone and its receptor. *Hum Reprod Update* 4(3): 260-83.

[47] Heinonen, P.K. (1991) Androgen production by epithelial ovarian tumours in post-menopausal women. *Maturitas* 13: 117-122

[48] Alexandre, J., Brown, C., Coeffic, D. (2012) CA-125 can be part of the tumor evaluation criteria in ovarian cancer trials: experience of the GCIG CALYPSO trial. *Br J Cancer* 106(4): 633-63.

[49] Zhang, T., Cheng, X.J., Zhang, B. (2016) CA199, Clinical value of combined detection serum CA125 and CEA for ovarian cancer diagnosis. *Chin. Med.* 40:973-75.

[50] Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., Cosic, V. (2001) Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 54: 356-61.

[51] Bancroft, J. D., Gamble, M., (2002) Theory and Practice of Histological Techniques, 1796, Churchill Livingstone, Edinburgh, London, 5th edition.

[52] H.J. Motulsky, Prism 5 Statistics Guide, 2007, GraphPad Software. Inc., San Diego CA**,** www.graphpad.com**.**

[53] Guraya, S.S. (1977) Recent advances in the morphology, histochemistry, and biochemistry of the developing mammalian ovary. *Int Rev Cytol* 51: 49-131.

[54] Kaprara, A., Huhtaniemi, I.T. (2018) Thehypothalamus- pituitary-gonad axis: tales of mice and men. Metabolism. *Clin Exp Med* 86: 3-17.

[55] Mourikes, V. E., Flaws, J. A. (2021) Effects of chemical mixtures on the ovary. Reproduction 162: F91–F100.

[56] Janssen, O. E., mehlmauer, N., Hahn, S., Offiner, A.H., Gartner, R.. (2004) High prevalence of autoimmune thyroiditis in patient with polycystic ovary syndrome. *Eur J Endocrinol* 150(3):363-9.

[57] Mannaa, F., Abdel-Wahhab, M.A., Ahmed, H.H., Park, M.H. (2006) Protective role of Panax ginseng extract standardized with ginsenoside Rg3 against acrylamide induced neurotoxicity in rats. *J Appl Toxicol* 26: 198-206.

[58] Channing, C.P., Schaerf, F.W., Anderson, L.D., Tsafriri,A. (1980) Ovarian follicular and luteal physiology. *Int Rev Physiol* 22:117-201.

[59] Carr, B.R., Disorders of the ovary and female reproductive tract. (1992) In Williams Textbook of Endocrinology, 8th edn, Wilson JD, Foster DW (eds).W. B. Saunders: Philadelphia. 733-798.

[60] Nagata, C., Konishi, K., Tamura, T., Wada, K., Tsuji1, M., Hayashi, M., Takeda, N., Yasuda, K. (2014) Associations of Acrylamide Intake with Circulating Levels of Sex Hormones and Prolactin in Premenopausal Japanese Women. *Cancer Epidemiol Biomark Prev* 24(1):249-54.

[61] Maeda, K.I., Ohkura, S., Tsukamura, H. (2000) Physiology of reproduction.. In: Krinke GJ, editor. The Laboratory Rat. London, UK: Academic Press, 145-74.

[62] Murphy, C. J., Tang, H., Van Kirk, E.A., Shen, Y., Murdoch, W.J. (2012) Reproductive effects of a pegylated curcumin. *Reprod Toxicol* 34:120-24.

[63] Tiwari-Pandey R, Ram Sairam, M. (2009) Modulation of ovarian structure and abdominal obesity in curcumin and flutamide-treated aging FSH-R haploinsufficient mice. *Reprod Sci* 16: 539-50.

[64] Sirotkin AV. (2021) The Influence of Turmeric and Curcumin on Female Reproductive Processes, Reviews. *Planta Med* 88: 1-6.

[65] Mohammadi, S., Kayedpoor, P., Karimzadeh-B. (2017) The effect of Curcumin on TNF-α, IL-6 and CRP expression in a model of polycystic ovary syndrome as an inflammation state. *J Reprod Infertil* 18(4):352-60

[66] Melekoglu, R., Ciftci, O., Eraslan, S., Cetin, A., Basak, N. (2018) Beneficial effects of curcumin and capsaicin on cyclophosphamide-induced premature ovarian failure in a rat model. *J Ovarian Res* 11:33.

[67] Sirotkin, A.V., Kadasi, A., Stochmalova, A., Balazi, A., Földesiová, M., Makovicky, P., Chrenek, P., Harrath, A. H. (2018) Effect of turmeric on the viability, ovarian folliculogenesis, fecundity, ovarian hormones and response to luteinizing hormone of rabbits. *Animal* 12: 1242-49.

[68] Kádasi, A., Maruniaková, N., Štochmaľová, A., Bauer, M., Grossmann, R., Harrath, A. H., Kolesárová, A., Sirotkin, A.V. (2017) Direct effect of curcumin on porcine ovarian cell functions. *Anim Reprod Sci* 182:77-83.

[69] Al-Rubaei, Z. M., Mohammad, T. U., Ali, L. K. (2014) Effects of Local Curcumin on Oxidative Stress and Total Antioxidant Capacity in vivo Study. *PJBS* 17: 1237-41.

[70] Mei, N., McDaniel, L.P., Dobrovolsky, V. N., Guo, X., Shaddock, J.G., Mittelstaedt, R. A., et al. (2010) The genotoxicity of acrylamide and glycidamide in big blue rats. *Toxicol Sci* 115: 412-21.

[71] Yamao, T., Kai, S., Kazami, A., Koizumi, K., Handa, T., Takemoto, N., et al. (1999) Tumor markers CEA, CA19-9 and CA125 in

monitoring of response to systemic chemotherapy in patients with advanced gastric cancer. *Jpn J Clin Oncol* 29: 550-55.

[72] Bergmark, E., Calleman, C. J., Costa, L. G. (1991) Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol Appl Pharmacol* 111: 352-63.

[73] Amin, K., Mahmood, Sh. F., Rahman, H. S., Othman, H. H. (2016) The Pathophysiological Effects of Acrylamide in Albino Wister Rats. *Int J Med Res Health Sci* 5(7):42-48.

[74] Duan, X., Wang, Q.C., Chen, K.L., Zhu, C.C., Liu, J. and Sun, S.C. (2015) Acrylamide toxic effects on mouse oocyte quality and fertility in vivo. *Sci Rep* 5: 11562.

[75] Rawi, S.M., Marie M.A.S., Fahmy, S.R., EL-Abied, S.A. (2012) Hazardous effects of acrylamide on immature male and female rats. *Afr. J. Pharmacy Pharmacol* 6 (18): 1367-86