

The effects of acrylamide and vitamin E administration during pregnancy on adults' ovarian tissue: An experimental study

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Abstract

Aim: The aim of the present study is to investigate the effects on ovarian tissues of adult female rats, which were the offspring of rats administered acrylamide (AA) and vitamin E during pregnancy.

Material and Methods: Thirty rats were divided into 5 groups of 6 pregnant rats, as the Control, Corn Oil, Vitamin E, AA, Vitamin E + AA groups. The births were monitored on the 21st day to select the female rats. The selected female rats were decapitated at the end of the 8th week and their ovarian tissues were removed under anesthesia malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant capacity (TAS), total oxidant capacity (TOS), oxidative stress index (OSI), superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO) levels were examined.

Results: Administration of AA during pregnancy caused an increase in MDA, TOS, OSI and NO levels and a decrease in GSH, SOD, CAT and TAS levels in the ovarian tissues of the rats when compared to the control group. It was determined that vitamin E administration caused an increase in GSH, SOD, CAT and TAS levels in ovarian tissues, compared to all other groups ($P < 0.05$).

Conclusion: Exposure to food-induced AA toxicity increases each day and the parallel increase in infertility suggests that it could be related to AA toxicity. Although vitamin E is capable to exert a protective effect against AA toxicity through increasing the antioxidant capacity of ovarian tissue, there is certain necessity for further studies.

Keywords: Pregnancy; acrylamide; vitamin E; oxidative stress; infertility; ovarian tissue.

INTRODUCTION

Acrylamide (AA) is widely used in industry, especially in printing, textile and research laboratories (1,2). It is a toxic substance that has a high chemical activity that can be synthesized chemically and does not exist naturally. AA occurs spontaneously in the carbohydrate and protein-rich foods once these foods reach temperatures above 120 °C, (3). The actual risk for humans is the constant exposure to food-induced AA toxicity. It was determined that AA was neurotoxic, genotoxic and carcinogenic and highly toxic for the animal reproductive systems, furthermore, it was classified as neurotoxic and 2A class carcinogenic for humans (4,5). The daily dose of AA intake was reported as 0.2-0.8 µg/kg/day in adults. Such intake varies based on factors such as lifestyle, type of nutrition and diet and age

and gender (6,7). Due to easy solubility in water, AA can easily pass from placenta to fetus. Given that fetuses are in the developmental stages during intrauterine period the occurrence of a life-long, AA-induced permanent damage is possible (8). Researchers that investigated the effects of AA on reproductive toxicity in the reproductive tissues reported that it decreased reduced glutathione levels, increased malondialdehyde levels and led to DNA damage (9). It was indicated that such effects of AA shifted oxidant/antioxidant balance towards oxidants and caused oxidative stress, hence occurred infertility (10,11).

Vitamin E is a powerful antioxidant that can easily pass through the placenta (12). It can exhibit properties that are capable of protecting the cells and tissues against oxidative stress-induced damage through increasing the

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antioxidant capacity against free radicals (13).

It is a fact that infertility continues to increase every day. The present study, for the first time, investigates the effects of acrylamide and vitamin E on ovarian tissues of adult rats (8 weeks old), which were the offspring of rats administered Acrylamide (AA) and vitamin E during pregnancy.

MATERIALS and METHODS

Animals

Before starting the present study, ethics approval was obtained from the experimental animal ethics committee of the Faculty of Medicine at Inonu University (2017/A-11). Thirty young female Sprague Dawley rats, which weighed 250 ± 25 grams and were produced at the Experimental Animal Production and Research Center of the Faculty of Medicine at Inonu University (INUTF-DEHUM), were used in the present study. One male and two female rats were taken into special cages at 5 PM. The rats were kept in the cages until 8 AM the next morning. At the end of this period, the male rats were separated from the female rats. Vaginal smears were taken from the female rats, examined under a microscope, and the rats with sperm in their smear were accepted as half-day pregnant. The rats, without positively confirmed pregnancies through the smear test, were excluded from the study. The pregnant rats were kept at $21 \pm 2^\circ\text{C}$ rooms at the INUTF-DEHUM for 20 days (pregnancy period) in a ventilated and 12 hours light and 12 hours dark environment. The rats were fed *ad-libitum* during the experimental period.

Study Design

Before starting the experiment, 30 pregnant Sprague Dawley rats with positive vaginal smear were randomly selected for five groups, each group containing 6 rats, and the administrations were carried out between the 0th and 20th days of the pregnancy.

Control group: The rats were fed *ad-libitum* during pregnancy.

Corn oil group: 1 mL corn oil was administered during pregnancy.

Acrylamide group: 10 mg/kg/day acrylamide (Sigma A8887, St Louis, Missouri, USA) was administered during pregnancy (14).

Vitamin E group: 100 mg/kg/day α -Tocopherol (Sigma T3251) was administered during pregnancy (15).

Acrylamide + Vitamin E group: 10 mg/kg/day acrylamide + 100 mg/kg/day Vitamin E were administered during pregnancy.

Administrations: 1 mL of oral gavage was given at the same time for 20 days.

After the births on the 21st day, the fetuses were separated as male and female rats. No procedures were performed until female fetuses reached adulthood. They were

allowed to be fed by their mothers and later were fed *ad-libitum*. Over tissues, retrieved from 8 weeks old female rats, were used in biochemical analyzes.

Preparation of the Tissues for Biochemical Analysis

Over tissues kept in the freezer (-80°C) were taken out and weighed on the analysis day. Phosphate buffer was added to obtain a 10% homogenate and it was homogenized in ice for 12 minutes at 12000 rpm (IKA, Germany). The supernatant were obtained through centrifuging the tissue homogenates at 5000 rpm and $+4^\circ\text{C}$ for 30 minutes.

Measurement of Malondialdehyde (MDA) Levels

The MDA analysis was conducted based on the method by Uchiyama and Mihara (16). The MDA concentration was determined through the measurement of the supernatant, extracted from the n-butanol phase of the pink colored product occurring as a result of the MDA in the supernatant reacted with thiobarbituric acid at 95°C , by a spectrophotometer at 535 and 520 nm. The results are presented as nmol/g wet tissue.

Measurement of Reduced Glutathione (GSH) Levels

The GSH analysis was conducted based on Elman's method (17). The GSH in the analysis tube reacted with, 5'-dithiobis 2-nitrobenzoic acid, obtained a yellow-greenish color and in order to determine the GSH concentration, light intensity of the obtained color was measured by a spectrophotometer at a wavelength of 410 nm. The results are presented as nmol/g wet tissue.

Measurement of Superoxide Dismutase (SOD) Levels

SOD activity was determined by the total reduction in nitroblue tetrazolium by xanthine and xanthine oxidase induced superoxide anion production (18). One unit of SOD activity was measured by the quantity of protein that inhibits NBT reduction by 50 %, and the results are reported as units per milligram protein. SOD activity is presented as U/g protein.

Measurement of Catalase (CAT) Levels

Aebi's method was adopted for the measurement of CAT activity (19). This method determines the rate constant k (dimension: s^{-1} , k) H_2O_2 (10 mM of initial concentration) based on the absorbance at 240 nm. CAT activity is presented as U/g protein.

Measurement of Total Oxidant Status (TOS) Levels

In order to determine the TOS levels, ELISA was adjusted to 25°C , as indicated in the kit procedure, 500 μL of reactive 1 (measurement buffer) and 75 μL of serum were mixed, and the absorbance was measured at 530 nm. 25 μL reactive 2 (pro-chromogen solution) was added to the mixture and after an incubation of 10 minutes, TOS levels were determined due to absorbance measurement at 530 nm (20). TOS activity is presented as $\mu\text{mol H}_2\text{O}_2$ Equiv/L.

Measurement of Total Antioxidant Status (TAS) Levels

In order to determine the TAS levels, ELISA was adjusted

to 25°C, as indicated in the kit procedure, 500 mL of reactive 1 (measurement buffer) and 75 mL of serum were mixed, and the absorbance was measured at 660 nm. 75 mL reactive 2 (colored ABTS solution) was added to the mixture and after an incubation of 10 minutes, TAS levels were determined due to absorbance measurement at 660 nm (21). TAS activity is presented as mmol Trolox Eq/L.

Oxidative stress index (OSI) Levels

The ratio of TOS to TAS was accepted as OSI (21). The OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (micromole H₂O₂ Eq/L) / TAS (millimole Trolox Eq/L) X 10.

Measurement of Nitric Oxide (NO) Levels

The instability of Nitric oxide (NO) in physiological solutions facilitates the determination of the NO content through its rapid transformation to nitrite (NO₂⁻) and dinitrate (NO₃⁻). NO detection kit was used, based on the guidelines provided by the producer, to identify the serum levels of NO₂⁻ /NO₃⁻. Griess reagent was used to measure the total nitrite, which appears as a result of nitrate conversion induced by aspergillus nitrite reductase. Absorbance was determined by a spectrophotometer at 540 nm (22). The results are presented as µmol/L.

RESULTS

The examination of the ovarian tissues of young adult rats did not present any difference between the control and corn oil groups. It was observed that the AA administered group

exhibited significant increase in MDA, TOS, OSI and NO levels, while the GSH, TAS, SOD and CAT levels decreased, when compared to all other groups. In the Vitamin E administered group, a statistically significant increase in GSH, SOD, CAT and TAS levels was observed when compared to the control group. It was also established that MDA, TOS, OSI and NO levels decreased in the AA + Vitamin E group, and the decreased SOD, CAT, GSH and TAS levels increased when compared to AA group (Tables 1 and 2).

DISCUSSION

In the present study, which was investigated by us for the first time, we examined the ovarian tissues of female rats that reached adulthood at the end of the post-natal period and that were the offspring of rats, which were orally administered AA and Vitamin E during pregnancy (0-20 days) and determined that AA negatively affected the oxidant-antioxidant parameters and AA-induced oxidative stress could be eliminated via Vitamin E administration.

Certain levels of free oxygen radicals (FORs) appear in entire eukaryotic cells due to the regular physiologic metabolism. Superoxide anion radical (O₂⁻), hydroxyl radical (·OH), hydrogen peroxide (H₂O₂), peroxy radicals (LOO·) and the singlet oxygen (¹O₂) are the main free radicals. Even though physiological conditions cause a constant formation of free radicals in the body, inhibitive antioxidant mechanisms detoxify such harmful radicals. Such antioxidant mechanisms are composed of several

Table 1: Biochemical some parameters of all groups

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)	CAT (U/g protein)	NO (µmol/L)
C	763 ± 18 ^a	1424 ± 130 ^a	91 ± 6 ^a	4.1 ± 0.5 ^a	137 (115-145) ^a
CO	756 ± 18 ^a	1461 ± 103 ^a	95 ± 9 ^a	4.1 ± 0.5 ^a	133 (121-141) ^a
Vit E	631 ± 23 ^b	2054 ± 71 ^b	200 ± 23 ^b	6.7 ± 0.3 ^b	116 (163-181) ^b
AA	1026 ± 61 ^c	1258 ± 102 ^c	53 ± 5 ^c	1.9 ± 0.3 ^c	174 (108-135) ^c
AA + Vit E	762 ± 59 ^a	1234 ± 86 ^d	63 ± 3 ^d	2.6 ± 0.3 ^d	140 (121-146) ^a

C; Control, CO; Corn Oil, Vit E; Vitamine E, AA; Acrylamide, AA + Vit E; Acrylamide + Vitamine E

MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; Data are expressed Mean ± SD (MDA, GSH, SOD, CAT) and Median (min - max) (NO) of eight animals. gwt; gram wet tissue. Groups with different letters in columns are significantly different from each other (p < 0.05).

Table 2: Oxidative stress index parameters of all groups

Groups	TAS (mmol/L)	TOS (µmol/L)	OSI (A U)
C	1.8 ± 0.3 ^a	4.6 (4-5.7) ^a	25 ± 5 ^a
CO	1.8 ± 0.3 ^a	4.7 (3.3-5.5) ^a	26 ± 4 ^a
Vit E	4.4 ± 0.5 ^b	1.6 (1-5) ^b	3.6 ± 2 ^b
AA	0.5 ± 0.2 ^c	10.1 (8-11.6) ^c	202 ± 8 ^c
AA + Vit E	2.8 ± 0.5 ^d	5.7 (4.3-7.5) ^d	20 ± 5 ^d

C; Control, CO; Corn Oil, Vit E; Vitamine E, AA; Acrylamide, AA + Vit E; Acrylamide + Vitamine E

TAS, total antioxidant status; TOS, total oxidant status, OSI, Oxidative stress index. Data are expressed Mean ± SD (TAS, OSI) and Median (min - max) (TOS) of eight animals. gwt; gram wet tissue. Groups with different letters in columns are significantly different from each other (p < 0.05).

organic and inorganic molecules (selenium, melatonin and GSH), catalase, glutathione-S-transferase, glutathione peroxidase, antioxidant enzymes (superoxide dismutase [SOD]) and vitamins (A, E, and C). Normal physiological conditions suggest a balance between the antioxidant mechanisms and free radicals and through such balance harmful effects of the free radicals are removed. Once the balance shifts towards the oxidants, such condition results in oxidative stress and the related oxidative tissue damage.

Research indicated that an AA administration of 14 mg/kg/bw between the 6th and the 21st day (birth) resulted with an increased lipid peroxidation and AA caused connective tissue deformation, atrophic tubule and multinuclear giant cell in testis tissues of the offspring adult male rats (23). Erdemli et al. administered AA, with a dose of 10 mg/kg/bw/day and Vitamin E with a dose of 100 mg/kg/bw/day to rats from the day 1 to the day 20 of pregnancy and grouped rats as male and female in the postpartum period. Male rats were fed with breast milk and later were not interfered with their ad-libitum feeding for 8 weeks and no surgical intervention was performed on the rats. At the end of the 8th week, the effects on the testis tissues of male rats were examined and it was determined that acrylamide (AA) negatively affected the serum hormone levels (Total Testosterone, Progesterone, FSH, LH, Estradiol), oxidant-antioxidant parameters in the tissues (MDA, GSH, NO, SOD, CAT, TAS, TOS) ($p < 0.05$) and the histological findings (the Johnson's score, seminiferous tubule diameter, histopathological images), furthermore, Vitamin E administration resulted with an increase in the total testosterone, progesterone, FSH, LH, GSH, TAS, NO, SOD, CAT levels ($p < 0.05$) and an improvement in histopathological findings (24). In a study that administered AA to male adult rats for 8 weeks in doses of 5, 10 and 15 mg/kg/bw researchers established that AA administration decreased testosterone and TAS levels in testis tissues, increased MDA levels and caused spermatogonia degeneration, in a dose-dependent manner (25). In their ovarian torsion model, Sarac et al. administered Vitamin E to rats as a preservative. They measured NUCB2 / nesfatin-1, ghrelin, atropine, and irisin levels in serum and reported that Vitamin E could exhibit protective properties in ischemia-reperfusion injury (26). Wang et al. administered 500 mg/kg bis (2-ethylhexyl) phthalate (DHEP) and 200 mg/kg Vitamin E as a preservative between the 12.5th day of pregnancy to the 3rd postnatal day. On the 3rd postnatal day, male rats were selected and examined for changes in their testis tissues. Researchers observed testicular cell apoptosis, decreased PPARs expression and histological damage due to DHEP administration and reported that such negative effects were eliminated due to Vitamin E administration (27). Xavier et al. examined the relationship between hypothyroidism and infertility. In order to establish a hypothyroidism model, they administered 10 mg/kg of propylthiouracil (PTU) and Vitamin E as a preservative in a dose of 75 mg/kg for 56 days. They established that

PTU deteriorated sperm amount, viability and structure in the testis tissues, increased MDA amount, and decreased catalase and super oxide dismutase levels when compared to control group. The researchers stated that vitamin E administration improved negative effects of PTU, hence increased the levels closer to control group levels (28).

The results of the present study indicated similarities, based on oxidant-antioxidant parameters and oxidative stress index, with the findings of other researchers. It was determined that AA caused oxidative stress damage in female ovaries and it was presented that Vitamin E had strong antioxidant properties that could suppress AA-induced oxidative stress.

CONCLUSION

Recent increase in infertility suggests that it could be due to food-induced effects. Exposure to toxic substances such as AA continues to increase each day. AA toxicity during development can cause permanent damage throughout life. It is evident that powerful antioxidants such as Vitamin E are necessary on daily basis, in order to prevent or alleviate the effects of exposure to AA toxicity. Further studies are essential to explain the effects of AA on reproductive toxicity.

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