

Toxicity Assessment of Zearalenone in Ovarian Female Rats

Nagwa M. EL-Sawi¹, Ohoud M. Marie², Soad Shaker Ali³, Amany M. Hamed¹

¹ Chemistry Department, Biochemistry lab, Faculty of Science, Sohag University, Egypt.

² Chemistry Department, Faculty of Science, Suez Canal University, Egypt.

³ Histology, faculty of Medicine, Assuit University, Egypt..

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Abstract: Background: Zearalenone (ZEA) is particularly toxic to the reproductive system, resulting in uterine enlargement, alterations to the reproductive tract, decreased fertility, and changes in the levels of ovarian hormones in laboratory animals. The purpose of this study was to investigate the toxicity effect of zearalenone on ovarian biochemical, and histological changes. **Methods:** Animals were divided into two groups (N= 12); Control and ZEA group: orally intoxicated by a dose of 2.7mg/kg. b.w. The biochemical and histological changes were reported for two groups and statistically analyzed. **Results:** Estrogenic mycotoxin increases the level of CA-125, Caspase-3 and decreases the level of Progesterone. Histologically there is deformity and degeneration of ovarian follicles and corpus luteal with increased inter-follicular fibrosis. **Conclusion:** ZEA caused toxicological effects including abnormal hormone levels and biomarkers in the female rats.

Keywords: Mycotoxins, Zearalenone, ovarian fibros.

1 Introduction

ZEA, also known as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-b-resorcylic acid l-lactone (Figure.1), is a non-steroidal mycotoxin with estrogen-like activity that is a member of the mycoestrogen group of compounds with a stable structure. It is produced by *Fusarium* species [1]. In addition to immunological toxicity, carcinogenicity, mutagenicity, and genotoxicity, studies on ZEA have demonstrated that it also causes hemato-toxicity [2]. Inhibiting ovarian activity (an agonist) is one way that ZEA and its metabolites can bind to the oestrogen receptors (ERs) and affect both animal and human reproductive behaviour [3]. Additionally, the edematous uterus, ovarian cysts, increased follicular maturation, and lower fertilisation rate may be caused by the steroidal qualities of ZEA [4]. The present work was designed to assess the effect of Zearalenone toxicity on hormonal levels and ovarian

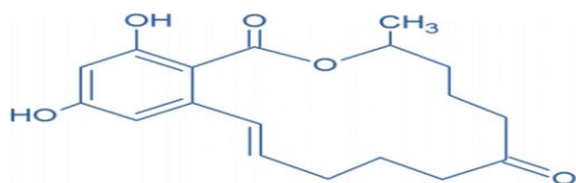


Figure 1: Chemical structure of zearalenone.

2 Material and Methods

Chemicals

Zearalenone (CAS No. 17924-92-4) was supplied by Sigma Aldrich, Egypt.

Animals and study design

Twenty-four adult female Wistar rats (age 10-12 weeks, 180-200 g) were provided by the animal house of Sohag University, Sohag, Egypt. All were nourished under standard laboratory conditions, along with free access to water ad libitum and a balanced pellet diet. Animals were adapted to the laboratory conditions prior to the experiment and maintained under standard housing environments. All ethical guiding principles were followed according to "Ethics and Animal Care Committee of Suez Canal University, Ismailia, Egypt".

The animals were divided randomly into two groups, one control and one treated with ZEA (12 Rats/group), as follow:

Control: Rats were given orally 2.7 mg/kg b.w. of 1% DMSO saline, two doses (Twice a week) for two weeks.

ZEA group: Rats were given orally 2.7 mg/kg b.w. of ZEA, two doses (Twice a week) for two weeks. (Chronic dose), according to EL-Sawi et al., [5].

Sample collection

At the end of each specified period, Rats were scarified and dissected, also blood samples from two groups were collected from the heart into plain tubes and centrifuged at 4000 rpm for 10 min to separate the serum, then divided into several aliquots and stored at -20°C until analysis was performed. Moreover, the whole ovaries of two groups were collected for histological studies.

Biochemical study (Hormonal and Biomarker assay)

Enzyme immunoassay test kit for the quantitative determination of ovarian cancer antigen Concentration in Serum was purchased from (BIOS Company, South San Francisco, CA 94080, USA). Enzyme immunoassay for the quantitative determination of Progesterone in serum was purchased from (PerkinElmer Company, Hayward, CA 94545). ELISA kit applies to the in vitro quantitative determination of Rat Caspase-3 concentrations in serum was purchased from (Elabscience Biotechnology Company, Catalog Number: E-EL-R0160, USA).

Histopathological Study

Ovaries from different rat groups were collected, cut along their axial directions, each into 2 halves, and immediately fixed in 10% neutral buffered formalin. The tissue specimens were prepared and stained with hematoxylin & eosin stain. all procedures were done according to Suvarna *et al.*, [6].

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Science (S.P.S.S. version 26). Results were expressed as the mean \pm SE (standard error). Statistical analysis was done using analysis of t-tests to compare means, the values in each group have characterized by a normal distribution and identical variance. While for the non-parametric data, the equality of groups' means was additionally checked by the Mann-Whitney test. The level of significance was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

3 Results

3.1. Biochemical study

3.1.1. Ovarian hormones and biomarkers

3.1.1.1. Progesterone Concentration

Table (1), Figure (2) indicated that there was a highly significant decrease ($***P < 0.001$) in the mean value of Progesterone Concentration in ZEA group compared with the control.

3.1.1.2. CA-125 concentration

As shown in Table (1), Figure (3) there is a highly significant increase ($***P < 0.001$) of CA-125 concentration (U/mL) in the mean value of ZEA group compared to control.

3.1.1.3. Caspase-3 concentration

Table (1), Figure (4) indicates that the Caspase3

concentration (ng/ml) in the serum showed a highly significant increase ($***P < 0.001$) in the mean value in ZEA group when compared with the control group 2-

Table 1: Effect of zearalenone (2.7 mg/kg b.w.) on Progesterone and Ovarian biomarkers of female rats.

Parameters /Groups	Progesterone (ng/ml) ^{\$}	CA-125(U/mL) ^{\$}	Caspase-3 (ng/ml) [@]
Control group	29.79 \pm 2.07	21.34 \pm 1.0	0.83 \pm 0.11
ZEA group	7.81 \pm 0.68 ^{***}	35.10 \pm 2.51 ^{***}	9.06 \pm 1.09 ^{***}

Values are represented by mean of 12 Rat \pm S.E. \$Parametric data (normal distribution of data) are tested by T-Test for comparison between two groups. @Non- Parametric data are tested by Mann-Whitney test. *** p < 0.001 vs. Control

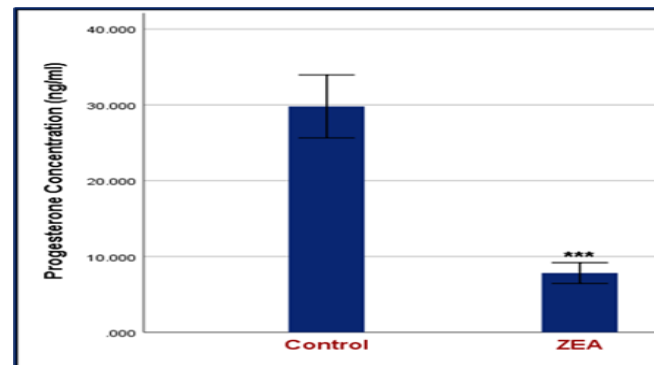


Figure 2: Effect of zearalenone (2.7 mg/kg b.w.) on Progesterone concentration in studied groups. Error bars indicate the standard error of the mean.

3.2. Histopathological study

A histological study in the present work was done to give evidence for biochemical results. Examination of paraffin sections of control and treated groups of rat ovaries stained with hematoxylin & eosin and photographed at different powers; Lowx100 and High x200 at ovarian follicles (OF) and corpora lutea (CL) regions (Figure 5) which observed that administration of ZEA exerted a toxic effect on ovarian tissue of rat represented in the form of cystic changes of ovarian follicles, degeneration and vacuolation of corpora lutea cells and increased interalveolar fibrosis compared to normal features of a control group.

4 Discussion

The current research has focused on ovarian toxicity because ZEA has been shown to cause severe reproductive disorders in animals and hyperestrogenic syndromes in humans as it has an estrogenic effect [7, 8].

The concentration of progesterone was observed to be significantly lower in the ZEA group than in the control group in the current investigation. The ovaries secrete a hormone called progesterone. Progesterone levels fluctuating might cause irregular menstrual cycles and menopausal symptoms [9]. We expected that a

considerably larger decline in progesterone in the ZEA group at the end of the trial indicates that zearalenone's cytotoxicity effects can also result in abnormal periods and trouble becoming pregnant in female rats. The body cannot create the ideal environment for the egg and growing foetus without this hormone. Low progesterone levels may raise the chance of abortion if a woman becomes pregnant [10]. The pathogenesis behind infertility in the setting of low levels of progesterone is due to the loss of these necessary endometrial changes, resulting in an impaired ability of the endometrium to allow for proper implantation of a fertilized egg [11].

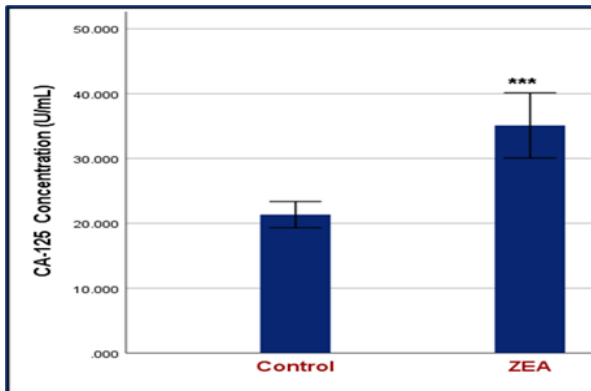


Figure 3: Effect of zearalenone (2.7 mg/kg b.w.) on CA-125 concentration in studied groups. Error bars indicate the standard error of the mean.

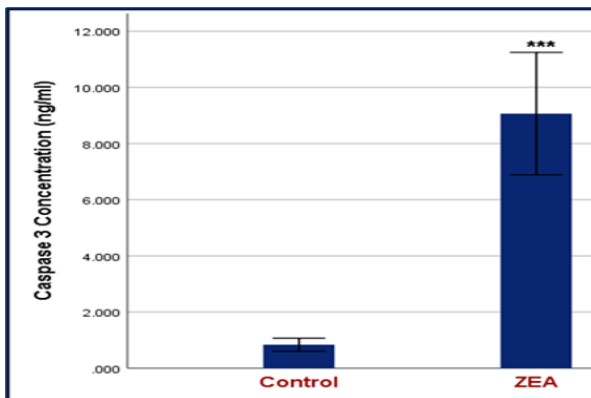


Figure 4: Effect of zearalenone (2.7 mg/kg b.w.) on Caspase-3 concentration in studied groups. Error bars indicate the standard error of the mean.

Rats fed ZEA for two weeks in the current study displayed a significantly significant rise in CA-125 level compared to control group. Several types of cells, especially ovarian cancer cells, produce the protein known as CA-125 [12]. Due to its ability to reveal details about the biological nature of the disease, it is frequently referred to as a biomarker or tumour marker (ovarian cancer). The most used test to help diagnose and monitor ovarian cancer is measuring CA-125 [13]. The CA-125 blood levels can be increased in ovarian cancer and other cancers including the fallopian tube, pancreatic, breast, colorectal, lung, and stomach [14, 15]. In agreement with Minervini et al., [2],

Who reported that a pulse dose of ZEA causes toxic effects on the reproductive tract; Our results suggested that elevation of CA-125 induced by ZEA may indicate a risk of ovarian and fallopian tube cancer.

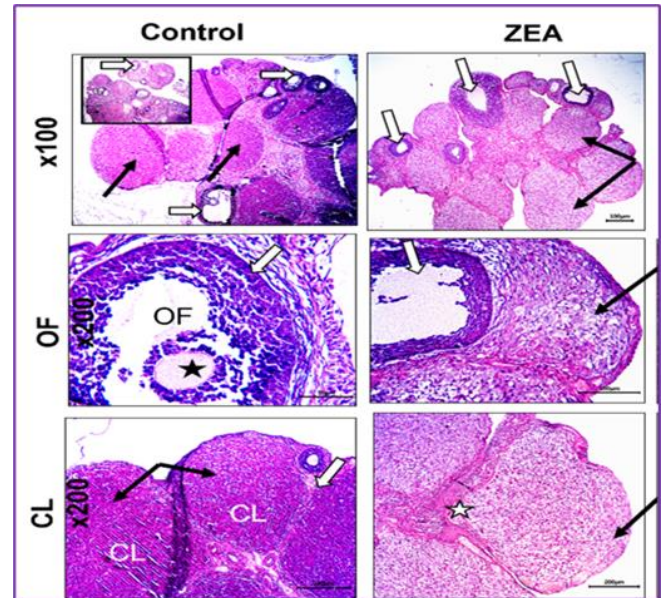


Figure 5: Paraffin sections of female rat ovary stained by H&E to show: **Control group:** Showed different ovarian follicles (white arrows, OF) and corpora lutea (Black arrows, CL) with intact ova (star). **ZEA group:** showed an absence of ovarian follicles with intact ova. The rest showed cystic changes (white arrows). Corpora showed degenerative changes marked by loss of cellular features and cellular vacuolation (black arrows) with increased inter-corpora fibrosis (white star).

In our research, we found that the level of caspase-3 activity increased significantly in the ZEA group compared to the control group. caspase-3 is a major executioner caspase that is cleaved and activated by both caspase-8 and caspase-9 initiator caspases [16]. Several cellular proteins are broken down by active caspase-3, which is also in charge of the morphological modifications and DNA fragmentation in cells. during apoptosis [17]. The apoptotic signalling pathway's convergence point, caspase-3, is a component of many different apoptotic signalling routes. The apoptotic cascade was started once caspase-3 was activated [18]. One of the hallmarks of cancer cells is a deficiency in caspase-3 production or activation [19]. Similarly, Liu et al., [20] reported that the protein levels of caspase-3 in tumor tissues were significantly higher compared to those in adjacent normal tissues. Our findings suggested that marked activation of caspase-3 induced by ZEA caused inflammation and fibrosis of the ovary.

In the present study, the results of the ovarian histopathological examination on H&E-stained slides of the ZEA group showed cystic changes in ovarian follicles, degeneration, and vacuolation of corpora lutea cells, and increased interalveolar fibrosis compared to normal features of the control group.

5 Conclusion

The experiment concludes that mycotoxin ZEA causes an increase in the CA-125, and caspase-3 expression, decreases progesterone levels, and thus causes cystic changes in ovarian follicles, degeneration, vacuolation of corpora lutea cells, and increased interalveolar fibrosis in the rat ovaries.

Abbreviations

ZEA: Zearalenone, CA: Cancer Antigen, H&E stain: Hematoxylin & Eosin stain, OF: Ovarian Follicles, CL: Corpora Lutea

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Consent for publication

The manuscript is original. It has not been published previously by any of the author and even not under the consideration in any other journal at the time of submission.

Declaration of Competing interest

No potential conflict of interest was reported by the authors.

Ethics approval

All procedures for using experimental animals were checked and permitted by the "Ethics and Animal Care Committee of Suez Canal University, Ismailia, Egypt" which is fully accredited by the Committee for Purpose of Control and Supervision on Experimental Animals.

Consent to participate

Not applicable.

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