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## Genotoxic evaluation for the estrogenic mycotoxin zearalenone

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**Abstract** — Genotoxic effects of the mycotoxin Zearalenone (ZEN) were evaluated on albino mice. The investigation was assessed using 4 criteria: chromosome aberrations in bone marrow and spermatocytes of adult male mice; chromosome analysis and teratological effects of mice embryos. Zearalenone was administered to both adult males and pregnant females with 2 doses level ( $5 \mu\text{g}\cdot\text{kg}^{-1}$  and  $10 \mu\text{g}\cdot\text{kg}^{-1}$  ZEN). Zearalenone was found to reduce the mitotic activity in treated males and the embryos proving that it is a cytotoxic substance. In treated males and females, it induced some chromosome abnormalities with no significant increase over the control at the doses investigated, except for some few figures. Similar results were observed for the teratological study. The results in general could consider zearalenone as a toxic mycotoxin for both adult animals and embryos. It is highly recommended that a great attention should be paid towards the toxicity of zearalenone to mono-gastric animals and human, especially it contaminate corn that is widely used in human and animal feeding.

**genotoxicity / zearalenone / mice / embryo / chromosome / spermatocyte**

### 1. INTRODUCTION

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced mainly by several species of *Fusarium* (*graminearum*,

*crookwellemsse*, *culmorum* and *semitectum*) [23]. It is mainly infecting maize but also can infect some other crops such as barley, oats, wheat and sorghum as well as other cereals [12, 27]. Stob et al. [26] were the

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first to isolate it from infected corn, while Urry et al. [31] identified this compound as zearalenone.

In Egypt, Abd El-Hamid [2] reported that 56.3% of several Egyptian foods and feeds are contaminated with ZEN. Abd Alla [1] detected ZEN in 30% of corn samples with an average concentration of 22.3 ppb., the author detected it also in 8.9% from rice samples with an average 15.5 ppb. Aziz et al. [3] detected ZEN in wheat at levels ranging from 103 to 287  $\mu\text{g}\cdot\text{kg}^{-1}$ . It is also detected in corn by El-Maghraby et al. [6], with a ratio of 22.6 to 80.4  $\mu\text{g}\cdot\text{kg}^{-1}$ .

Studies in many species have shown that zearalenone and its metabolites have anabolic and estrogenic activities since it mimics follicle-stimulating hormone (FSH) [7, 17]. There are some reproductive problems associated with the zearalenone were reported in cows and sows [19, 21].

Zearalenone is produced commercially as an intermediate in the preparation of zearanol ( $\alpha$ -Zearalenol), which is a substance used as a growth promotor hormone in beef cattle, feedlot lambs and suckling pigs [11, 32].

Conflicting reports about the genotoxicity of the Zearalenone were published. Zearalenone was found to be negative in the *Salmonella typhimurium* assay [13, 34]. It was also negative in a eukaryotic cell mutation assay with *Saccharomyces cerevisiae* [16]. In contrast, zearalenone and its estrogenic metabolites showed a positive DNA damaging effect in recombination tests with *Bacillus subtilis* [30]. It induces sister chromatid exchange, chromosomal aberration and polyploidy in Chinese hamster ovary (CHO) cells cultured in vitro [28].

The objective of this research was to evaluate the genotoxicity and teratogenicity effects of the zearalenone in both male and pregnant female mice as a model for monogastric organism, since this toxin is commercially used as an anabolic agent in farm animals.

## 2. MATERIALS AND METHODS

### 2.1. Zearalenone

Zearalenone was purchased in a pure crystalline form (Sigma chemical Co., St.-Louis, USA), and dissolved in propylene glycol.

### 2.2. Animals

Sixty adult male and female Swiss albino mice (20–25 g) obtained from the animal house of the National Research Center. Animals were maintained on feed and water ad libitum.

### 2.3. Slide preparation from male bone marrow and spermatocytes

Fifteen adult male mice were used for this part of study. They were classified into 3 groups (5 animals each). The first and second groups were injected intraperitoneally (i.p.) with 5  $\mu\text{g}\cdot\text{kg}^{-1}$  and 10  $\mu\text{g}\cdot\text{kg}^{-1}$  from the Zearalenone respectively, while the third group received only propylene glycol and acts as a control group. Injected doses were chosen after the recommendations of Ito and Ohtsubo [14].

Mitotic and meiotic chromosomes were obtained from the same animal. Animals were injected (i.p.) 2.5 h before killing with 0.2 mL colchicine (0.05%) per 100 gm body weight. Bone marrow chromosomes were prepared according to the method of Yosida and Amano [35], while meiotic chromosome preparation from testicles were prepared following the technique described by Brewen and Preston [4].

### 2.4. Preparations of chromosomes and skeletons from embryos

Adult virgin female and male mice were paired overnight (1 male per 2 females). The day on which the vaginal plug was

detected was designated as day zero of gestation. Pregnant females were divided into three groups (10 animals each). On day 7 of pregnancy, the first and the second groups were intraperitoneally injected with  $5 \mu\text{g}\cdot\text{kg}^{-1}$  and  $10 \mu\text{g}\cdot\text{kg}^{-1}$  ZEN respectively, while the third group received only propylene glycol. In all groups, 5 females were killed at day 13 of pregnancy, the chromosomes preparations were obtained from the produced whole embryos using the methodology described by Romagnano et al. [25]. The other 5 females in each group were killed at day 17 of pregnancy and the embryos were morphologically examined, counted, weighed and the number of resorption sites for live and dead fetuses was recorded. At the same time, the life embryos were used for skeletal preparation following the methodology described by Weesner [33]. For all cytogenetic work 50 well spread metaphases were examined, chromosome abnormalities were recorded.

### 2.5. Mitotic index

Mitotic index is referred also as mitotic activity, which is counted as the number of divided cells in 2000 cells per animal.

### 2.6. Statistical analysis

The results were statistically analyzed using Chi-Square for chromosomal abnormalities and T-test for mitotic index and teratological data.

## 3. RESULTS

### 3.1. Effects on adult males

A significant reduction ( $P < 0.01$ ) for the mitotic activity was observed for the tested doses after ZEN treatment. This means that zearalenone has cytotoxic effects.

The observed structural abnormalities in bone marrow cells in control and treated

males are presented in Table I. Types of abnormalities were chromatid gaps (Fig. 1b), chromatid breaks (Fig. 1c), centric fusion (Fig. 1d), centromeric attenuations (Fig. 1e) and end to end associations (Fig. 1f). Chromatid gaps were the most frequent type of abnormality observed. It was increased significantly over the control ( $P < 0.05$  and  $0.01$ ) for doses  $5 \mu\text{g}\cdot\text{kg}^{-1}$  and  $10 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively. Similar result was observed for centric fusion with less percentage. Other abnormalities were also increased after the treatment but it didn't reach to the significant level. No trend for any abnormalities was found to be associated with special chromosome.

Table II represents the observed types of abnormalities in the spermatocytes. Only 2 types of abnormalities were observed (X-Y univalents and break). Although these abnormalities were increased after the treatment, only break was significantly increased over the control at  $P$  level of 0.01 for the dose  $10 \mu\text{g}\cdot\text{kg}^{-1}$ .

### 3.2. Effect on embryo somatic chromosomes

Similar to the effect on male bone marrow, significant reduction at  $P$  level of 0.01 for the mitotic activity was observed for the two doses tested after ZEN treatment.

As well as, similar types of chromosome abnormalities to the male mice bone marrow were observed in mice embryos after the mothers were treated with zearalenone. Data of the examination presented in Table III indicated that both chromatid gaps and chromatid breaks were increased significantly over than the control at  $P$  level of 0.01 for the 2 doses tested. In contrast, ends to end associations were increased significantly over than the control at  $P$  level of 0.05 for the 2 doses tested. Centric fusion showed a significant increase over the control at  $P$  level of 0.01 for the high dose only ( $10 \mu\text{g}\cdot\text{kg}^{-1}$ ). Similar to the male also, no trend for any abnormalities was found to be associated with special chromosome.

**Table I.** Effect of zearalenone treatment on male mice bone marrow cells.

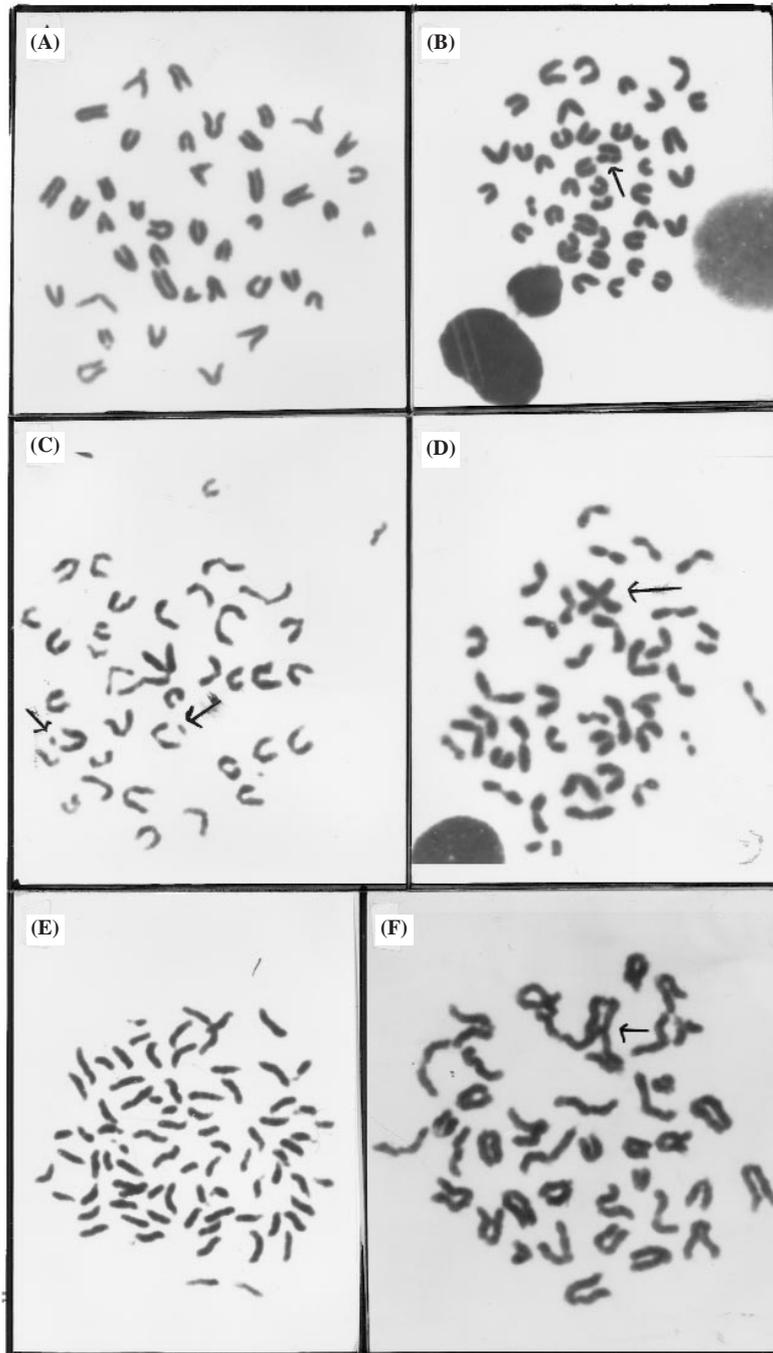
Treatment	Structural aberrations					Total structural aberrations	Mitotic activity
	Chromatid gap	Chromatid break	Centric fusion	Centromeric attenuation	End to end associations		
Propylene glycol (control)	1.4 ± 0.548	0.8 ± 0.447	0.0 ± 0.0	1.6 ± 0.548	0.8 ± 0.837	4.6 ± 1.140	105.2 ± 3.673
5 µg ZEN per kg	* 3.600 ± 0.894	1 ± 0.707 *	* 0.6 ± 0.894	3.2 ± 0.837	1.2 ± 1.304	** 9.8 ± 4.087	** 71.6 ± 3.130
10 µg ZEN per kg	** 5.4 ± 1.673	1 ± 1.732	** 2 ± 1.225	2 ± 0.701	1.8 ± 0.447	** 12.2 ± 2.490	** 82.4 ± 10.807

Data presented as mean ± SD.

No. of examined metaphases were 250 per each group.

\* Significantly different ( $P < 0.05$ ).

\*\* Significantly different ( $P < 0.01$ ).



**Figure 1.** Normal and different bone marrow metaphases with chromosome: (A) normal metaphase, (B) chromatid gap, (C) chromatid break, (D) centric fusion, (E) centromeric attenuation, (F) end to end association.

**Table II.** Effect of zearalenone on mice spermatocytes.

Treatment	Structural abnormalities		
	X-Y univalents	Break	Total
Toxin solvent (control)	2 ± 0.707	1 ± 1	1 ± 0.707
5 µg ZEN per kg	1.2 ± 0.837	1.8 ± 1.924	3 ± 2
10 µg ZEN per kg	1.6 ± 1.140	** 4.4 ± 2.074	** 6 ± 1.225

Data presented as mean ± SD.

No. of examined spermatocytes were 250 per each group.

\*\* Significantly different ( $P < 0.01$ ).

**Table III.** Effect of zearalenone on mice embryos.

Treatment	Structural aberrations							Total structural abnormalities	Mitotic activity
	Gap		Break		Centric fusion	Centromeric attenuation	End to end associations		
	Chromatid	Chromosome	Chromatid	Chromosome					
Propylene glycol (control)	1.6 ± 0.957	0.04 ± 0.2	1.12 ± 1.053	0.0 ± 0.0	0.68 ± 0.690	6.28 ± 1.173	0.0 ± 0.0	9.720 ± 1.838	73.12 ± 15.292
5 µg ZEN per kg	** 5.72 ± 1.4	0.2 ± 0.408	** 5.72 ± 2.151	0.04 ± 0.2	0.44 ± 0.821	6.32 ± 2.940	* 0.16 ± 0.374	** 18.48 ± 3.765	** 40.88 ± 10.967
10 µg ZEN per kg	** 4.92 ± 1.66	0.04 ± 0.2	** 4.56 ± 2.468	0.04 ± 0.2	** 2.760 ± 2.83	7.16 ± 1.795	* 0.16 ± 0.473	** 19.64 ± 3.86	** 54.4 ± 14.776

Data presented as mean ± SD.

No. of examined metaphases were 1 250 per each group.

\* Significantly different ( $P < 0.05$ ).

\*\* Significantly different ( $P < 0.01$ ).

### 3.3. Teratological effects

Results of the embryo teratological examinations on days 13 and 17 of gestation are presented in Tables IVa and IVb. Other parameters such as total number of implantation sites and living, resorbed (Fig. 2a), dead embryos (Fig. 2c) were recorded. Embryo weight and the number of embryos with haematoma (Fig. 2e) were also included. In general, no significant differences from the control were noticed for most of these parameters, except for embryo weight which decreased significantly ( $P < 0.05$ ) and the haematoma embryos which was significantly difference over the control for the dose  $10 \mu\text{g}\cdot\text{kg}^{-1}$  at day 17, as shown in Table IV. The number of resorbed embryos was significantly increased over the control at  $P < 0.01$  for the dose  $10 \mu\text{g}\cdot\text{kg}^{-1}$  at days 13, 17. No skeleton abnormalities were observed in the treated embryos.

## 4. DISCUSSION

The inhibition of mitotic activity in both male and embryo bone marrow cells, reported in this study indicates that zearalenone has a cytotoxic effect on these cells. Some previous reports [5, 10] mentioned that the increase in cytotoxic effect was usually accompanied by a decrease in number of cells with chromosomal aberrations. As a result of severe cytotoxic effect, heavily damaged cells will be eliminated from the cell population, leading to decrease in number of cells with aberrations. Although some of the trichothecenes fungi, which is the family of the zearalenone producing fungi, have some effects on the cell growth through inhibition of protein and DNA synthesis and subsequently affecting the cell cycle and division [29], there is no available data concerning zearalenone from this aspect.

Concerning the chromosomal abnormalities observed at male germ cells, break was the most frequent abnormality observed, it was highly significant at ( $P < 0.01$ ) for the

high dose only. Cells, which have break, will be died and the total number of resulted sperm may be reduced. Our observation concerning the abnormalities in germ cells does not agree with that of Pylkkanen et al. [24], who studied the effect of zearalenone on the mouse germ cells using sperm head abnormality and their results were negative.

In general, our observation concerning the scored percentages of abnormalities at the germ cells which were lower than that of the somatic cells, could be explained by the idea, that there is some sort of natural protection for the testicles against chemicals and toxins.

Observations of some chromosomal abnormalities at the control group may be due to the propylene glycol, which is recently reported that it is a mild toxic substance [18, 20]. From another side, a similar observation was noticed during genotoxic evaluation of the mycotoxin diacetoxyscirpenol on mice using the same solvent [10].

In general, there were very conflicting reports about the genotoxicity of zearalenone. Zearalenone showed a positive DNA damaging and adducts effect, sister chromatid exchange and chromosomal aberrations in CHO cells in vitro [23, 28, 30]. Our results agree with these previous reports and disagree with those informing that ZEN has not genotoxic effect [13, 16, 22, 34].

These conflicting reports concerning the genotoxicity of zearalenone could be attributed to many reasons such as the sensitivity of the treated animal itself, from this point of view, sows were reported to be very sensitive to zearalenone treatment than other farm animals [8, 17]. Also, the structure of the gastric system since it is found in ruminants that zearalenone is transformed by rumen microflora into ten less toxic another compounds, unfortunately there is no available such data concerning the metabolic pathway of the mono-gastric animals such as pigs and rodents [23]. The way, time and the administrated dose of zearalenone may be also reasons for such discrepancy.

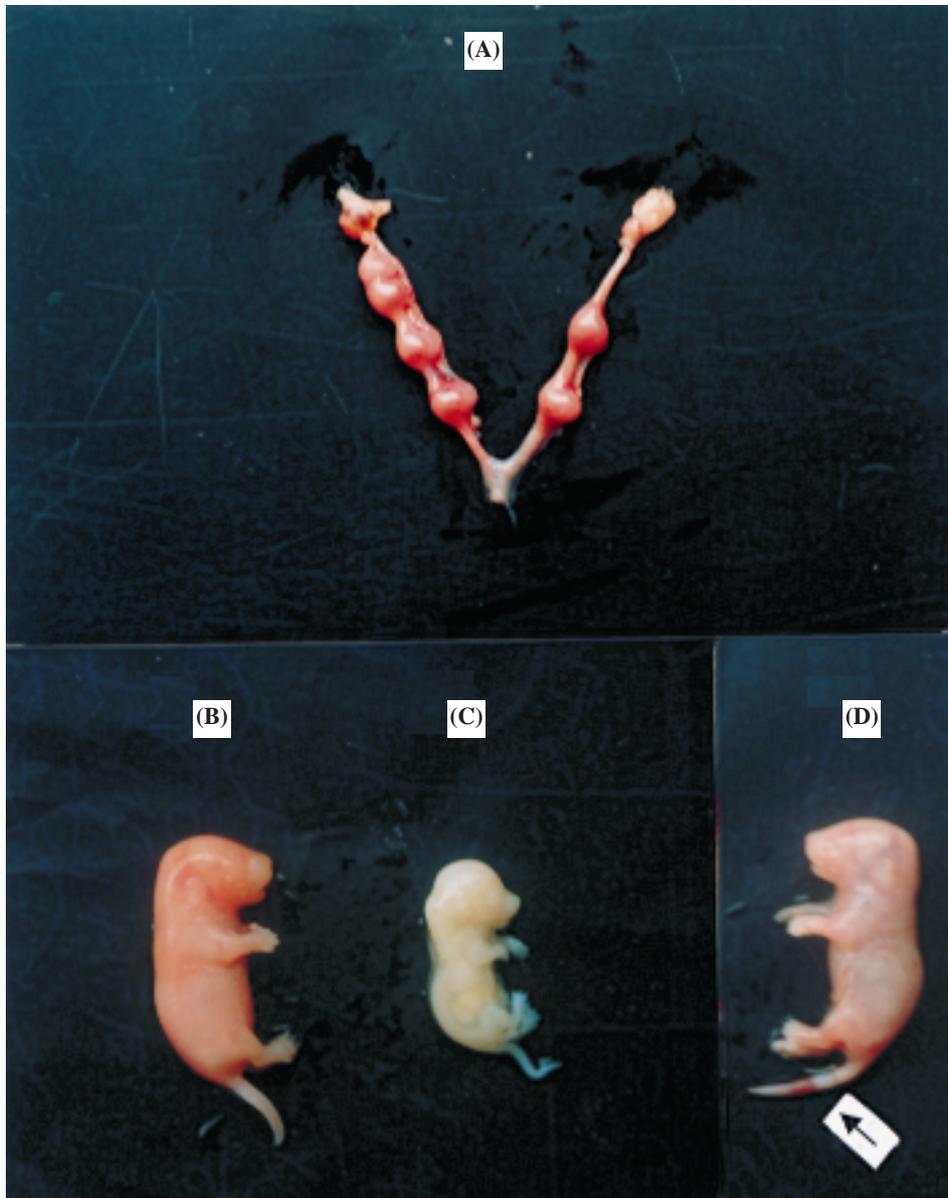
**Table IV.** Teratogenic effects on mice embryos on day 13 (a) and day 17 (b) of gestation after zearalenone treatment.

Treatment	No. of implanted embryos	No. of live embryos	No. of resorbed embryos	No. of dead embryos	No. of haematoma embryos	Embryos body weight
<b>(a)</b>						
Control	8.6 ± 1.342	8.2 ± 1.095	0.4 ± 0.894	0.0 ± 0.0	0.0 ± 0.0	0.299 ± 0.030
5 µg ZEN per kg	8.2 ± 1.304	7.0 ± 1.870	1.2 ± 1.095	0.0 ± 0.0	0.0 ± 0.0	* 0.231 ± 0.053
10 µg ZEN per kg	7.4 ± 2.302	5.2 ± 3.564	** 2.2 ± 3.347	0.0 ± 0.0	0.0 ± 0.0	* 0.176 ± 0.101
<b>(b)</b>						
Control	6.8 ± 1.643	6.8 ± 1.643	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.894	1.097 ± 0.124
5 µg ZEN per kg	7.2 ± 1.643	6.2 ± 2.588	0.8 ± 1.304	0.2 ± 0.447	1.8 ± 1.304	* 0.721 ± 0.428
10 µg ZEN per kg	7.0 ± 2.121	4.8 ± 3.114	** 2.2 ± 3.347	0.0 ± 0.0	* 2.0 ± 1.581	* 0.708 ± 443

Data presented as mean ± SD.

No. of pregnant females were 5 per each group.

\* Significantly different ( $P < 0.05$ ) from control.



**Figure 2.** Embryo abnormalities after ZEN treatment: (A) uterus with resorbed and dead embryos, (B) day-17 normal embryo, (C) day-17 dead embryo, (D) embryo with an haematoma in the tail\*.

\* This figure is available in colour at [www.edpsciences.org](http://www.edpsciences.org)

The number of resorbed embryos was significantly increased over the control at  $P < 0.01$  for the dose  $10 \mu\text{g}\cdot\text{kg}^{-1}$  at days 17, 19. This is mean that ZEN caused an early embryonic death, chromosome abnormalities in the embryo may be one of the factors leading to early embryonic death [9].

Concerning teratogenic effects on the embryos, embryo weight were significantly decreased, this result agrees with that reported by Kiessling [15], who reported that zearalenone treatment reduced body weight when given to rats during their growing stage. From another side since our study was dealing with embryos and there were very little and insignificant abnormalities scored, their may be some protection role of the placental barrier protects the embryo from foreign chemicals and toxins.

It is highly recommended that a great attention should be paid towards the toxicity of zearalenone to mono-gastric animals and human, especially it contaminate corn that is widely used in human and animal feeding.

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