



Possible role for glutathione-S-transferase in the oligozoospermia elicited by acute zearalenone administration in Swiss albino mice

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ABSTRACT

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by several species of *Fusarium*, commonly found in the soil in temperate and warm countries and is a frequent contaminant of cereal crops worldwide. Accordingly, it has been implicated in several mycotoxicosis in farm animals and in humans, but the underlying mechanisms remain largely unknown. Therefore, the current study was aimed to investigate the effect of an acute dose of ZEA (40 mg/kg, p.o.) on reproductive and hematological parameters, as well as on markers of oxidative stress in liver, kidney and testes in mice. Adult Swiss albino male mice were exposed to a single oral administration of ZEA, and 48 h thereafter behavioral and biochemical tests were performed. No differences in locomotor or exploratory activity were observed in the open-field test. On the other hand, ZEA increased the number of leukocytes, segmented neutrophils, sticks, eosinophils, monocytes and decreased platelets and lymphocytes number. Moreover, ZEA drastically reduced the number and motility of live spermatozoa.

Additionally, while levels of thiobarbituric acid reactive substances (TBARS), non-protein thiols (NPSH) and ascorbic acid in liver, kidney and testes were not altered by ZEA administration, superoxide dismutase activity increased in all tissues evaluated, catalase activity increased in the kidney, and glutathione-S-transferase activity decreased in kidney and testes. In summary, we showed that ZEA have acute toxic effects mainly in reproductive system of adult male Swiss albino mice and its effect probably is related to a reduced activity of GST and increased in SOD activity in testes.

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Abbreviations: CAT, catalase; CDNB, 1-chloro-2,4 dinitrobenzene; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); ERs, estrogens receptors; ER α , estrogen receptors α ; ER β , estrogen receptors β ; GSH, reduced glutathione; GST, glutathione S-transferase; GSTM1, glutathione S-transferase M1; GSTM3, glutathione S-transferase M3; GSTM5, glutathione S-transferase M5; LD50, lethal dose; MDA, malondialdehyde; NPSH, non protein thiols; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; ZEA, zearalenone; α -ZEA, α -zearalanol; α -ZOL, α -zearalenol; β -ZEA, β -zearalanol; β -ZOL, β -zearalenol.

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1. Introduction

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by the fungi *Fusarium culmorum* and *Fusarium graminearum* (Langseth et al., 1998; Marasas et al., 1984), which are commonly found in the soil in temperate and warm countries and are frequent contaminants of cereal crops worldwide (Zinedine et al., 2007). ZEA is rapidly absorbed following oral intake and, during subsequent metabolism mainly in the liver and intestine, it is transformed into α - and β -zearalenol (α - and β -ZOL), α -and β -zearalanol (α - and β -ZEA) and zearalanone (ZEA), all of which are subsequently conjugated to glucuronic acid (Gromadzka et al., 2008). A variety of other tissues, including the kidney, testis, prostate, hypothalamus and ovary, also contain the major enzymes (3 α - and 3 β -hydroxysteroid dehydrogenase) able to metabolize mycotoxins (Olsen et al., 1981). ZEA is genotoxic and responsible of a potent reproductive toxicity in humans and animals (Abbes et al., 2007; Salah-Abbes et al., 2009a; Tomaszewski et al., 1998). ZEA has been shown to be immunotoxic (Abbes et al., 2006; Ben Salah-Abbes et al., 2008), hepatonephrotoxic (Salah-Abbes et al., 2009b) and apoptotic (Abid-Essefi et al., 2003).

Such toxic effects of ZEA and its metabolites have been ascribed primarily to its chemical structure that resembles that of naturally occurring estrogens (Gromadzka et al., 2008), but the exact underlying mechanisms remain largely unknown. In this context, oxidative stress has been considered to play an important role in the toxic effects after mycotoxins exposure. In fact, oxidative damage has been described in rats fed with diets containing high levels of ZEA (Becci et al., 1982). Moreover, it has been demonstrated that ZEA and its metabolites induces lipid oxidation and increases the production of malondialdehyde in several cell lines (Hassen et al., 2007; Kouadio et al., 2005; Othmen et al., 2008). Furthermore, antioxidants such as vitamins A, E and C reduced the formation of DNA adducts induced by this mycotoxin in renal cells (Szkudelska et al., 2002).

The reproductive system is a major target of ZEA toxicity (Minervini and Dell'Aquila, 2008; Tiemann and Danicke, 2007). ZEA has strong estrogenic effects and it is mainly distributed in reproductive organs, particularly uterus and ovaries. ZEA and its metabolites have been shown to bind competitively to estrogen receptors (ER α and ER β) in a number of *in vitro* or *in vivo* systems and to activate transcription of estrogen responsive genes (Mehmood et al., 2000; Turcotte et al., 2005). So, it is frequently implicated in hyperestrogenism and other reproductive disorders in laboratory and farm animals (Green et al., 1990; Kuiper-Goodman et al., 1987; Lopez et al., 1988; Minervini and Dell'Aquila, 2008). In humans, ZEA was associated to precocious pubertal changes, endometrial adenocarcinoma and hyperplasia in women (Tomaszewski et al., 1998). Moreover, ZEA was found to be hepatotoxic, to disturb haematological parameters, and it was associated to several alterations of immunological parameters in humans and rodents (Abid-Essefi et al., 2004; Hassen et al., 2007). In experimental chronic studies, ZEA caused alterations in the reproductive tract of laboratory animals (mice, rats, and

pigs) and farm animals. It decreased fertility, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and changed serum levels of progesterone and estradiol (EFSA, 2004). Moreover, it has been demonstrated that while small amounts of ROS have been shown to be required for several functions of spermatozoa, their excessive levels can negatively impact the quality of spermatozoa and impair their overall fertilizing capacity (Tvrda et al., 2011). Regarding male fertility, increased levels of ROS have been correlated with decreased sperm motility (Eskenazi et al., 2003), increased sperm DNA damage (Armstrong et al., 1999), sperm cellular membrane lipid peroxidation (Aitken, 1995). Nevertheless, to the best of our knowledge, there are no studies investigating the acute effects of ZEA on male reproductive system and fertility and the possible association of oxidative stress. Therefore, this study aims to evaluate the effects of a single acute dose of ZEA on reproductive and hematological parameters, as well as on markers of oxidative stress in liver, kidney and testes of mice.

2. Materials and methods

2.1. Animals and reagents

Twenty male Swiss albino mice (25–30 g in weight and 90 days old) from our own breeding colony were used. Animals were housed in groups of 5 in Plexiglas cages (41 cm \times 34 cm \times 16 cm) with the floor covered with sawdust. They were kept in a room with light-dark cycle of 12 h with the lights on between 7:00 and 19:00 h and temperature controlled (20–25 °C) and received water and food *ad libitum*.

The animals were maintained and used in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources (process #071/2011) of the Federal University of Santa Maria, Brazil.

Zearalenone (ZEA), Thiobarbituric acid (TBA), trichloroacetic acid (TCA), reduced glutathione (GSH), 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4 dinitrobenzene (CDNB), Epinephrine bitartrate salt, glycine, tris, were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were obtained from standard commercial suppliers. The stain used for the blood smear was the quick panoptic (Laborclin Produtos para Laboratório Ltda, Pinhais, PR, Brazil). ZEA was prepared in olive oil, immediately before administration.

2.2. Experimental design and sampling

Mice were weighed and randomly divided in two groups which received one administration of ZEA (40 mg/kg – 8% of LD50) or olive oil by gavage (10 ml/kg). Forty eight hours after ZEA or vehicle administration the animals received a dose of pentobarbital (180 mg/kg, i.p.), and blood was collected by cardiac puncture into tubes containing heparin (1 UI/ μ l). The liver, kidneys and testes were removed, weighed and homogenized in Tris-HCl 50 mM, pH 7.4 for the determination of enzymatic and non-enzymatic indicators of oxidative stress. The epididymis were weighed and used for determining the number and motility of spermatozoa.

2.3. Open-field test

The open field task is a simple assessment used to determine general activity levels, gross locomotor activity and exploration habits in rodents. Two days (48 h) after the treatment with ZEA or vehicle, mice were submitted to the open field test. Mice were placed in a wooden box (20 × 30 cm) with the floor divided in twenty-one identical squares, and the number of squares crossed with all paws, the number of rearings and the time of cleaning were counted during 10 min.

2.4. Body weight and vital organs relative weight

In order to evaluate any possible toxic action of acute ZEA administration, the body and vital organs relative weight were determined. Mice were weighted before, and two days (48 h) after the treatment with ZEA and some vital and reproductive organs (lungs, liver, spleen, kidneys, testes and epididymis) were weighted relatively to the body weight.

2.5. Blood cells

Total leucocyte count was performed using 25 µl of blood and 500 µl of solution Turkey in a Neubauer chamber with the aid of optical microscope with a 40× objective (Nikon Eclipse 50i). Subsequently, we applied the technique of blood smears for differential counts of neutrophils (segmented and sticks), eosinophils, lymphocytes and monocytes with 5 µl blood. After performing the same, the slides were stained (panotico fast) and viewed under a microscope according to the method described by (Failace et al., 2009).

2.6. Number and motility of spermatozoa

Assessment of spermatozoa count and motility was performed according to Freund and Carol (1964). The two cauda epididymides from each mouse were homogenized in 2 mL of warmed (37 °C) saline solution (0.9% NaCl). Briefly, 10 µL of the diluted spermatozoa suspension was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 min. The cells settled during this time were counted with the help of light microscope at 200× magnification (Nikon Eclipse 50i).

2.7. Thiobarbituric acid reactive substances (TBARS) determination

Lipid peroxidation was estimated by measuring TBARS and expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa et al. (1979). In this method, MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. TBARS content was estimated in a medium containing the supernatant fraction of liver, kidneys or testes, 0.05 ml of 8.1% SDS, 0.2 ml of acetic acid buffer (2.5 M, pH 3.4), and 0.38 ml of 0.81% thiobarbituric acid (TBA). The mixture was finally made up to 1 ml with type I ultrapure water and heated at 95 °C for 90 min in a water bath using

a glass ball as a condenser. After cooling to room temperature, absorbance was measured in the supernatant at 532 nm. Results were calculated as nmol MDA/mg of protein.

2.8. Non protein thiols (NPSH) determination

NPSH levels of liver, kidney and testes samples were determined according to the method proposed by Ellman (1959) with some modifications. Samples were precipitated with TCA (10%) and subsequently centrifuged at 3000 g for 10 min. After the centrifugation, the supernatant fraction (60 µl) was added to a reaction medium containing potassium phosphate buffer (1 M, pH 7.4) and DTNB (10 mM). NPSH levels were measured spectrophotometrically at 412 nm. Results were calculated in relation to a standard curve constructed with cysteine and corrected by the protein content. Results were calculated as nmol NPSH/mg of protein.

2.9. Ascorbic acid determination

Hepatic, renal and testicular ascorbic acid determination was performed as described by Jacques-Silva et al. (2001). Protein was precipitated in 10 V of a cold 5% trichloroacetic acid solution. An aliquot of sample (300 µL), in a final volume of 575 µL of the solution, was incubated with TCA 13.3%, and a color reagent containing dinitrophenyl hydrazine, thiourea and CuSO₄, at 37 °C for 3 h, then 500 µL H₂SO₄ 65% (v/v) was added to the medium. The reaction product was determined spectrophotometrically at 520 nm as µg ascorbic acid/mg of protein.

2.10. Enzyme assays

2.10.1. Catalase (CAT) activity

CAT activity was determined by following the decomposition of 30 mM hydrogen peroxide in 50 mM potassium phosphate buffer (pH 7.0) at 240 nm for 120 s in a thermostatized (37 °C) spectrophotometer, according to the method proposed by Aebi (1984). CAT specific activity was expressed as first-order rate constant k, per mg of protein. Appropriate controls for non-enzymatic decomposition of hydrogen peroxide were included in the assays.

2.10.2. Superoxide dismutase (SOD) activity

SOD activity was determined in liver, kidney and testes, according to the method described by Misra and Fridovich (1972). This method is based on the ability of SOD in inhibiting autoxidation of adrenaline to adrenochrome. Briefly, the supernatant fraction (20–60 µl) was added to a medium containing glycine buffer (50 mM; pH 10.5) and adrenaline (1 mM). The kinetic analysis of SOD was started after adrenaline addition, and the color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 30 °C, and results were expressed as Units (U)/mg of protein.

2.10.3. Glutathione S-transferase (GST) activity

GST activity was assayed spectrophotometrically at 340 nm by the method of Habig et al. (1974). The reaction

mixture contained an aliquot of supernatant of liver, kidney or testes, 0.1 M potassium phosphate buffer (pH 7.4), 100 mM GSH and 100 mM CDNB, which was used as substrate. The enzymatic activity was expressed as nmol CDNB/min/mg of protein.

2.11. Protein determination

Protein content was measured colorimetrically by the method of Bradford (1976), and bovine serum albumin (1 mg/ml) was used as standard.

2.12. Statistical analysis

Graphpad prism 5 software was used for statistical analysis and for plotting graphs. Statistical analysis was carried out by the Student's *t* test, and *P* < 0.05 was considered significant. All data are reported as mean and S.E.M.

3. Results

In order to investigate whether ZEA affects motor and exploratory behavior in mice, animals were visually observed in open field paradigm. No significant differences in locomotor or exploratory activity (crossing, rearing and time of cleaning) were observed in ZEA-treated mice when compared with control group in open field test (data not shown).

The effect of ZEA on percent of body weight gain did not differ among groups (data not shown). The effect of acute administration of ZEA on isolated weight of vital and reproductive organs was also evaluated. Mice organs (kidneys, liver, lungs, spleen, testes and epididymis) were visually observed *ex vivo* for any signs of damage and weighed relatively to the body weight. No significant differences were observed when compared to control group, with exception of significantly increase in liver weight (Table 1).

Fig. 1 shows the effect of ZEA on number of blood cells. Hematotoxic effect of ZEA was evident after 48 h of exposition to a single dose of mycotoxin. ZEA significantly increased the number of leukocytes (Fig. 1A), segmented neutrophils (Fig. 1B), sticks (Fig. 1C), eosinophils (Fig. 1D) and monocytes (Fig. 1E). On the other hand, ZEA decreased lymphocytes (Fig. 1F) and platelets number (Fig. 1G).

Table 1

Effect of ZEA (40 mg/kg, p.o.) 48 h after a single oral administration on organs relative weight. Data are mean ± S.E.M. for *n* = 10 animals in each group.

Tissue	Relative weight (g/%)	
	Olive oil	ZEA
Kidney	1.502 ± 0.054	1.581 ± 0.040
Liver	5.230 ± 0.172	5.832 ^a ± 0.171
Lung	0.561 ± 0.027	0.535 ± 0.020
Spleen	0.137 ± 0.004	0.128 ± 0.006
Testes	0.619 ± 0.040	0.602 ± 0.049
Epididymis	0.119 ± 0.007	0.111 ± 0.009

^a Indicates a significant difference (*P* < 0.05) in relative liver weight (*t*(18) = 2.478, *P* = 0.0234) compared with olive oil group.

In addition to the hematological effects of ZEA, we evaluated the number and motility of spermatozoa after ZEA administration, since there are only a few evidences that the male reproductive system is affected by acute ZEA treatment. Interestingly, ZEA significantly reduced the number of spermatozoa (Fig. 2A) and its motility (Fig. 2B).

In order to evaluate the role of oxidative stress on the effects induced by acute administration of ZEA, we measured several enzymatic and non-enzymatic indicators of oxidative stress in liver, kidneys and testes. Statistical analyses revealed that levels of non-enzymatic markers for oxidative stress, TBARS, NPSH and ascorbic acid were not altered by ZEA administration (data not shown).

On the other hand, activities of enzymatic markers for oxidative stress were altered by ZEA treatment. In fact, catalase activity increased in kidneys (Fig. 3), while SOD activity increased in the liver, kidney and testes (Fig. 4). However, ZEA decreased GST activity in the kidney and testes (Fig. 5).

4. Discussion

ZEA is a fusariotoxin produced mainly by *Fusarium* species that grow on foodstuffs at high incidence in several countries (Eriksen and Alexander, 1998). Since ZEA is a potent toxin and may cause a risk to animal and human health, it is important to investigate the acute harmful effects of this mycotoxin.

In the present study we showed that acute ZEA administration caused deleterious hematologic effects (Fig. 1) and drastically reduced the number and motility of live spermatozoa (Fig. 2) in male Swiss albino mice. The role of oxidative stress in the toxic effects of ZEA was also investigated. Interestingly, this mycotoxin decreased GST activity in the testes and kidney (Fig. 5B–C), increased SOD activity in the liver, kidney and testes (Fig. 4A–C), and increased CAT activity in the kidney (Fig. 3B).

Intracellular accumulation of reactive oxygen species can arise from toxic insults and can perturb the cell's natural antioxidant defense system resulting in damage to all major classes of biological macromolecules. During the last decades, the oxidative stress has been pointed out as major component of several biological and pathological processes like aging, inflammation, carcinogenesis and several other diseases (Halliwell and Gutteridge, 1999). Additionally, some reports suggest that oxidative stress is a key determinant of ZEA induced toxicity *in vivo* and *in vitro* (Abid-Essefi et al., 2009, 2011; Ben Salah-Abbes et al., 2008; Hassen et al., 2007; Salah-Abbes et al., 2009a).

In this context, both enzymatic and non-enzymatic antioxidant defenses are fundamental to prevent oxidative stress and may also indicate the level of protection against foreign agents. In the present study, we found that acute ZEA treatment significantly increased catalase activity in the kidney and SOD activity in the liver, kidney and testes, suggesting compensatory increases in antioxidant enzyme activity in attempt to prevent oxidative damage to cells and macromolecules. In this context, such assumption could be supported by the fact that levels of non-enzymatic antioxidant defenses (NPSH and ascorbic acid) and of a marker of lipid peroxidation (TBARS) did not

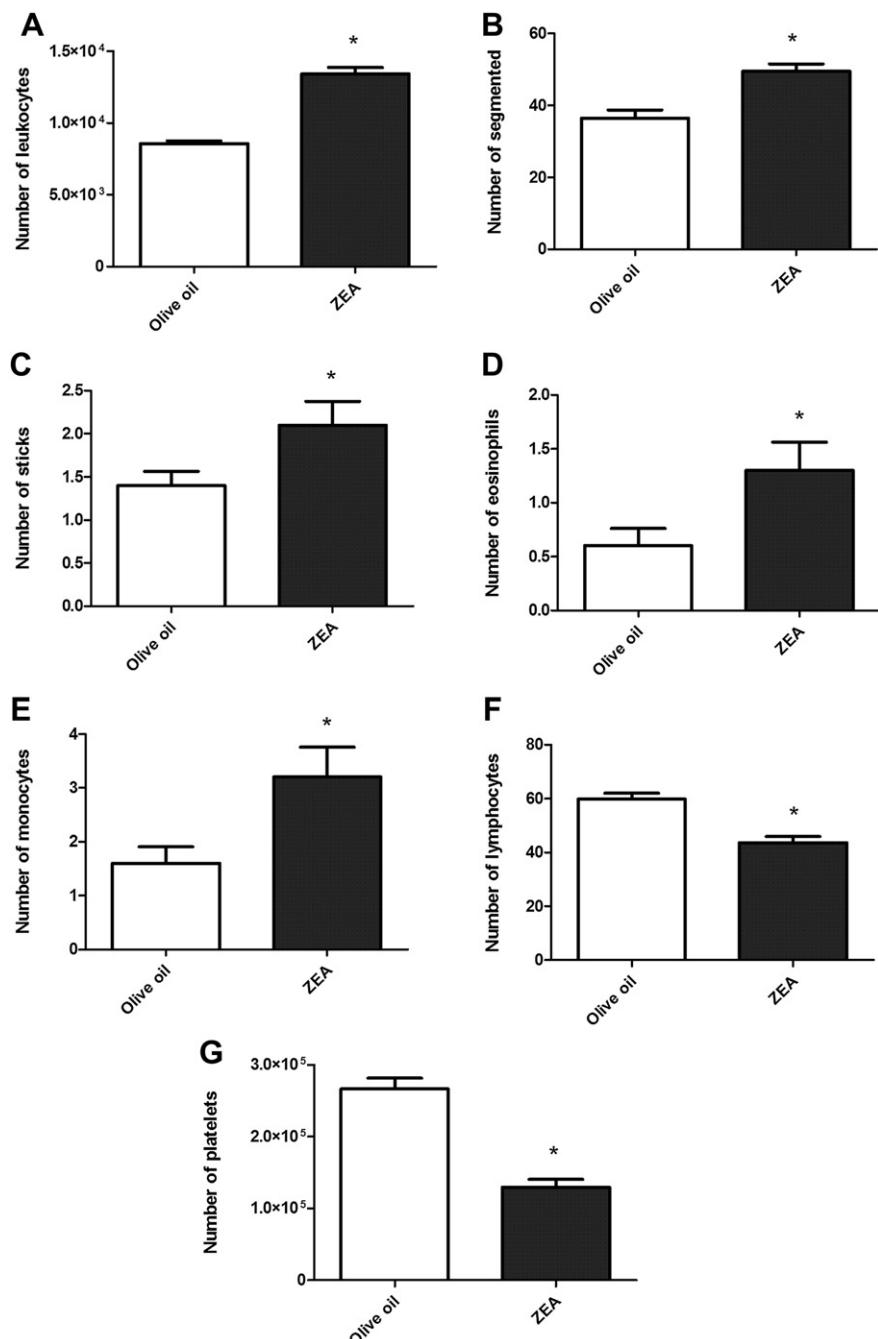


Fig. 1. Effect of ZEA (40 mg/kg, p.o.) on blood parameters 48 h after a single oral administration. A: Number of leukocytes ($t(18) = 9.552, P < 0.0001$), B: segmented neutrophils ($t(18) = 4.354, P = 0.0004$), C: sticks ($t(18) = 2.178, P = 0.043$), D: eosinophils ($t(18) = 2.278, P = 0.0352$), E: monocytes ($t(18) = 2.530, P = 0.021$), F: lymphocytes ($t(18) = 5.050, P < 0.0001$) and G: platelets ($t(18) = 7.409, P < 0.0001$). Data are mean + S.E.M. for $n = 10$ animals in each group.
* Indicates a significant difference ($P < 0.05$) compared with olive oil group.

change significantly in liver, kidney or testes after acute ZEA administration. Altogether, these results may suggest that ZEA affects enzymatic rather than non-enzymatic markers of oxidative stress, and that increased SOD and CAT activities in fact may counteract oxidative damage and depletion of non-enzymatic antioxidant defenses. In agreement with our results, Stadnik et al. (2010) have shown increased SOD

activity in the liver after 10 days of ZEA (200 and 500 μ g/kg, p.o.) administration, and that ascorbic acid content in rat liver was unchanged after 24 h or 10 days of ZEA administration. In addition, catalase activity increased in the liver and kidney of mice 24 h after ZEA (40 mg/kg, i.p.) administration (Zourgui et al., 2008). On the other hand, orally treated male Balb/c mice treatment for 28 days with ZEA

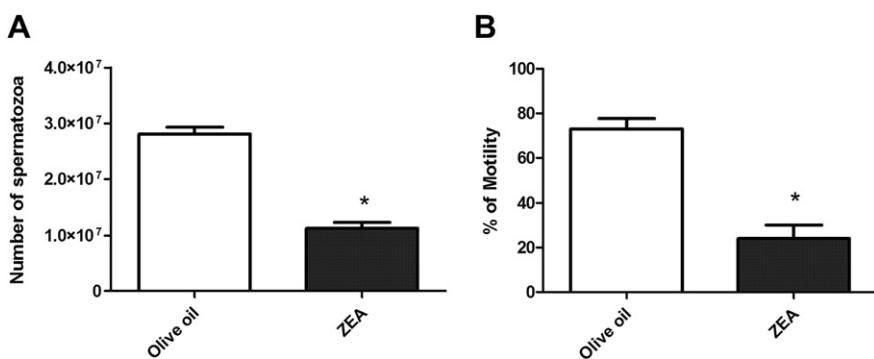


Fig. 2. Effect of ZEA (40 mg/kg, p.o.) on number of spermatozoa in epididymis (A ($t(18) = 10.35, P < 0.0001$)) and percentage of motile spermatozoa (B ($t(18) = 6.385, P < 0.0001$)) 48 h after a single oral administration. Data are mean + S.E.M. for $n = 10$ animals in each group. * Indicates a significant difference ($P < 0.05$) compared with olive oil group.

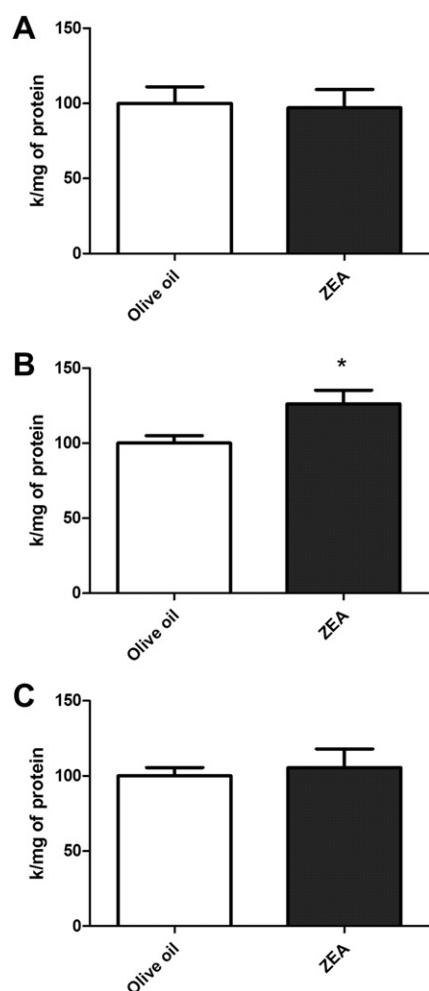


Fig. 3. Effect of ZEA (40 mg/kg, p.o.) on CAT activity in liver (A), kidney (B ($t(17) = 2.603, P = 0.0185$)) and testes (C) 48 h after a single oral administration. Data are mean + S.E.M. for $n = 9$ –10 animals in each group. * Indicates a significant difference ($P < 0.05$) compared with olive oil group.

(40 mg/kg, i.p.) decreased glutathione peroxidase, SOD and CAT activities in testes (Salah-Abbes et al., 2009a). These apparent conflicting data can be explained by the differences in animal species, strain, sex as well as routes, schedules and doses of ZEA used. Regarding this point, Malekinejad et al. (2006) has reported differences between species in hepatic biotransformation of ZEA in pig, sheep, cattle, chicken and rat. In addition, some studies showed that ZEA increases the weight of testis, epididymis, prostate and seminal vesicle reinforcing that more studies are necessary to elucidate the effects of mycotoxin intoxication in a variety of species, strains and tissues (Salah-Abbes et al., 2009a; Yang et al., 2007).

Studies in various female species (rodents, rabbits, pigs, monkeys) including man have shown that ZEA has estrogenic activity and impairs reproduction, including reproductive organs and their function, leading to hyperestrogenism. As well as in the female reproductive system, estrogens exist in the male reproductive system (Claus et al., 1987) and are involved in stimulating spermatogenesis and steroid synthesis by binding to estrogen receptors (ERs), including ER α and ER β (Rago et al., 2006; Stabile et al., 2006). Furthermore, testicular spermatozoa count is an important indicator for investigators to detect the adverse effects of various factors on male reproductive system (Ban et al., 1995). However, to the present moment it is not possible to point out whether the target for ZEA toxicity are cells undergoing spermatogenesis, or fully mature spermatozoa, or both. In our study, there was a significant decrease in spermatozoa count in epididymis homogenates as well as reduced spermatozoa motility. Kim et al. (2003) have reported that a single dose of ZEA (5 mg/kg, i.p.) is able to induce testicular germ cell apoptosis in rats in a time-dependent and stage-specific pattern. Yang et al. (2007) shows that the treatment with ZEA or α -ZOL at 0, 25, 50 and 75 mg/kg i.p. once a day for 7 consecutive days, in Kunming male mice decreased the number of live spermatozoa, and increased the number of abnormal spermatozoa. In addition, low pregnancy rate was observed when females were mated with ZEA or α -ZOL exposed males. Salah-Abbes et al. (2009a) showed that in a chronic protocol (40 mg/kg, p.o. for 28 consecutive days) the number and motility of spermatozoa decreased in Balb/c mice. These studies

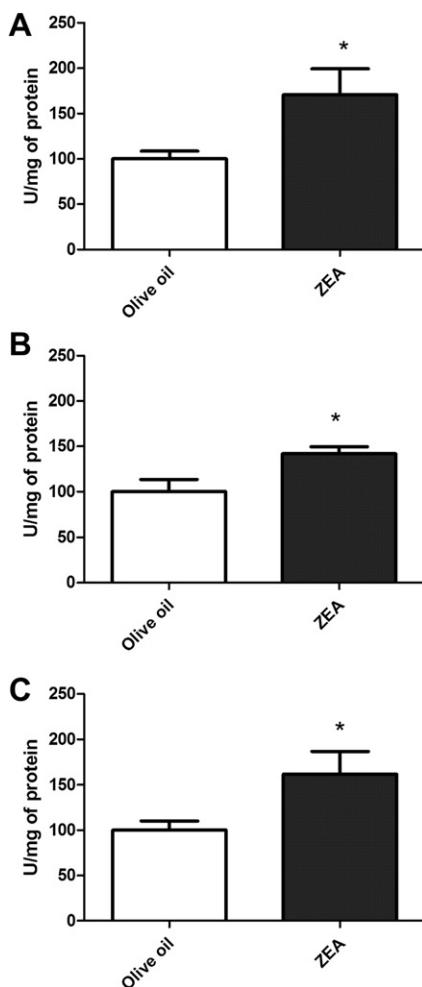


Fig. 4. Effect of ZEA (40 mg/kg, p.o.) on SOD activity in liver (A ($t(18) = 2.502, P = 0.0228$)), kidney (B ($t(18) = 2.632, P = 0.0175$)) and testes (C ($t(17) = 2.285, P = 0.0347$)) 48 h after a single oral administration. Data are mean + S.E.M. for $n = 9$ –10 animals in each group. * Indicates a significant difference ($P < 0.05$) compared with olive oil group.

suggest that ZEA reduces the number and motility of spermatozoa independently of the experimental protocol and mice strain. Furthermore, it is plausible that the same factor responsible for reduced number and motility of spermatozoa induced by ZEA administration could lead to alterations on SOD activity, rather than the second-named consequence producing the first.

Although is difficult to point out the exactly mechanisms underlying the toxicity of ZEA to spermatozoa, it is interesting to note that GST activity seems to be a critical factor. To our knowledge, this is the first study that shows a decreased GST activity in testes after ZEA administration. Glutathione S-transferase plays an important role in the biotransformation and detoxification of many xenobiotics, and semen contains significant amount of GST, important for sperm protection against oxidative stress (Mann et al., 2000). Reduced activity of GST and increased ROS levels lead to sperm membrane damage (Gopalakrishnan and Shaha, 1998). It has been also demonstrated that GST has

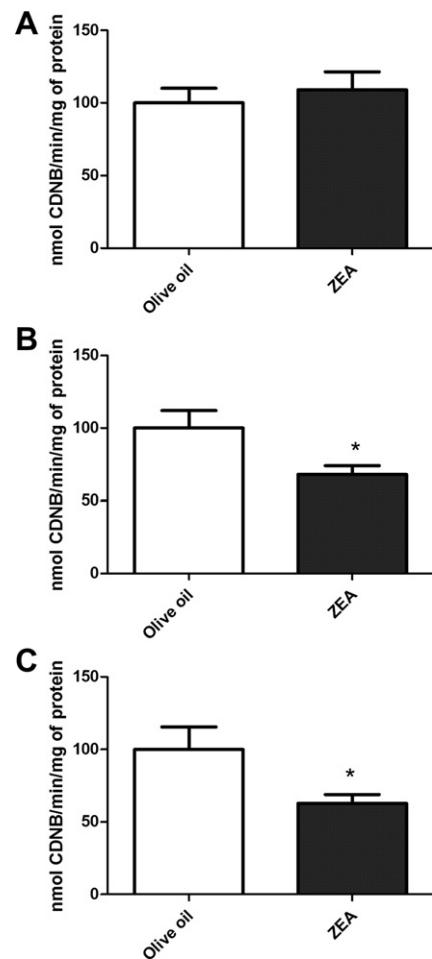


Fig. 5. Effect of ZEA (40 mg/kg, p.o.) on GST activity in liver (A), kidney (B ($t(17) = 2.277, P = 0.036$))) and testes (C ($t(17) = 2.151, P = 0.0462$))) 48 h after a single oral administration. Data are mean + S.E.M. for $n = 9$ –10 animals in each group. * Indicates a significant difference ($P < 0.05$) compared with olive oil group.

a relevant protective role during spermatogenesis (Castellon, 1999) and that GST Mu-1 gene (GSTM1) is a critical isozyme in the prevention of oxidative stress in sperm (Chen et al., 2002). In fact, GSTM1, GSTM3 and GSTM5 gene polymorphisms have been shown to predispose to male infertility after varicocele, by decreasing spermatozoa motility and concentration and causing oxidative damage to spermatozoa DNA (Chen et al., 2002; Okubo et al., 2005). In addition, a decrease in spermatozoa count and motility and an increase in dead spermatozoa in GSTM1 null humans was observed (Vani et al., 2010), further suggesting a critical role for GST activity in infertility and oligozoospermia.

Regarding the effects of ZEA on blood cell counts, it has been demonstrated that ZEA is hematotoxic, immunotoxic and genotoxic in Balb/c mice (Abbes et al., 2007, 2006). In addition, Forsell et al. (1986) and Pestka et al. (1987) have shown similar effects of ZEA on hematological parameters of the immune system in B6C3F1 mice. In the present study ZEA increased leukocytes number

concomitantly to a decrease in lymphocyte counts, reinforcing the ZEA potential to cause acute immune toxicity. Regarding this point, Berek et al. (2001) has shown that ZEA caused immunosuppression by depressing T or B lymphocyte activity. Our results are also in agreement with those by Swamy et al. (2004), who have demonstrated that ZEA-contaminated diet linearly reduced B-cell count in broiler chickens. In addition, a single intravenous administration of ZEA (15 mg/ml) led to the formation of pronounced abnormalities in lymphocyte membrane phospholipid metabolism in rats (Karagezian, 2000). Notwithstanding, the decline in platelets count suggests a possible detrimental effects of ZEA on blood coagulation process, as previously suggested by Maaroufi et al. (1996).

In summary, we showed that mycotoxin ZEA induces acute reproductive toxicity in male Swiss albino mice, as demonstrated by changes in spermatozoa count and motility. Although the effect of ZEA on sperm count and motility can not be solely credited to changes in the testicular redox system, it is possible that decreased GST activity is involved in this effect, because semen contains significant amounts of GST, which is important enzyme for sperm protection against oxidative stress (Mann et al., 2000). In addition, reduced GST activity leads to increased ROS levels and subsequent sperm membrane damage (Gopalakrishnan and Shah, 1998), a 14% decrease in GST enzymatic activity in testes has been related to impaired spermatogenesis and sperm motility in knockout mice for the transcription factor NRF2 (Nakamura et al., 2010). In conclusion, our present results show that a single administration of ZEA may cause deleterious effects on the male reproductive system, and suggest that GST activity may be a potential target to attenuate ZEA reproductive toxicity.

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Conflict of interest statement

The authors of this manuscript have no financial or personal relationship with any organization which could influence the work on the compound in this manuscript.

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