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# Lycopene protects against acute zearalenone-induced oxidative, endocrine, inflammatory and reproductive damages in male mice

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ABSTRACT

Male mice received lycopene for 10 days before a single oral administration of zearalenone (ZEA). After 48 h testes and blood were collected. Mice treated with lycopene/ZEA exhibited amelioration of the hematological changes. Lycopene prevented the reduction in the number and motility of spermatozoa and testosterone levels, indicating a protective effect in the testicular damage induced by ZEA. Lycopene was also effective in protecting against the decrease in glutathione-S-transferase, glutathione peroxidase, glutathione reductase and δ-aminolevulinic acid dehydratase activities caused by ZEA in the testes. Exposure of animals to ZEA induced modification of antioxidant and inflammatory status with increase of reduced glutathione (GSH) levels and increase of the oxidized glutathione, interleukins 1β, 2, 6, 10, tumor necrosis factor-α and bilirubin levels. Lycopene prevented ZEA-induced changes in GSH levels and inhibited the processes of inflammation, reducing the damage induced by ZEA. Altogether, our results indicate that lycopene was able to prevent ZEA-induced damage in the mice.



## 1. Introduction

Zearalenone (ZEA) is a nonsteroidal estrogenic mycotoxin produced by several species of *Fusarium* and is present in many food products for both humans and animals [1]. As far as human exposure is concerned, a European study by Scoop [2] reported ZEA to be present in 32% of food samples ($n = 5018$), which were almost all cereal products. In fact, the fusariotoxicoses are a great public health problem. ZEA, particularly, is stable and not easily degraded by common food and feed processing procedures [3].

Several reproductive disorders in farm animals, as well as some hyperoestrogenic syndromes in humans, have been attributed to ZEA [4]. ZEA binds to estrogen receptors *in vitro*, with similar affinity for both forms: ERα and ERβ [5] disrupting the endocrine balance by having effects similar to estrogen, and consequently inducing harm to the reproductive system and its development. Numerous studies revealed that testes are sensitive to ZEA [6,7]. In male mice, ZEA affect sperm parameters such as number and motility [8,9], decrease fertility, change weight of endocrine glands [10] and inhibit testosterone biosynthesis in Leydig cells [11].

In addition to endocrine dysfunction, several studies suggest that oxidative stress appears to be a key determinant of toxicity induced by ZEA *in vivo* and *in vitro* [11,12], and the enzymes of the glutathione system seem to have an important role in spermatogenesis [13]. Reduced glutathione (GSH) itself is a critical factor in maintaining the cellular redox balance and has been demonstrated to be involved in the regulation of cell signaling and repair pathways [13]. The cellular levels of GSH are controlled by multiple enzyme systems such as glutathione peroxidase (GPx) and glutathione reductase (GR). ZEA produces several effects on the antioxidant system, such as a significant decrease in the GSH level in human hepatocytes Hep G2 cells [12]. Furthermore, the GSH system is unbalanced by ZEA, resulting in a decline in sperm count [14]. It is suggested that ZEA operates in male germ cells through the induction of oxidative stress causing the inhibition of glutathione-S-transferase (GST) [8,9].

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ZEA has also been shown to be immunotoxic [15] in different animal species [15,16]. In blood of weanling piglets, ZEA increased the inflammatory cytokine (tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) synthesis [17]. In chickens, ZEA presented potent effects on the expression of splenic lymphocyte cytokine interleukin-2 (IL-2) and interleukin-6 (IL-6) and interleukin-10 (IL-10) [15]. ZEA seems to work as both suppressor and inductor of the production of inflammatory cytokines [17]. In mice, only few studies have been carried out regarding the effects of ZEA on the immunologic system, specifically on plasmatic cytokine levels.

Lycopene, a red–orange carotenoid pigment present in tomatoes, has received particular attention in recent years as a result of studies indicating that it is a highly efficient antioxidant, possessing a singlet-oxygen and free radical scavenging capacity [18,19]. This carotenoid has many biochemical functions, some of which include it being an antioxidant scavenger, antihyperlipidemic agent and inhibitor of proinflammatory, as well as having prothrombotic factors [20] and antiapoptotic effects [21]. Recent experimental studies demonstrated that lycopene may act as a chemopreventive agent against certain types of cancers such as prostate cancer [18,19], in addition to the possibility of serving as a promising intervention to testicular toxicity associated with oxidative stress [9,18]. Moreover, there are a few studies correlating the toxicity of ZEA in the δ-aminolevulinic acid dehydratase (δ-ALA-D) activity, which is an enzyme that catalyzes the biosynthesis of heme.

Based in our previous findings that shows the preventing effects of lycopene on ZEA toxicity [8,9] in this study we further elucidate the mechanism of lycopene's protective effects following ZEA exposure. We have investigated the parameters of oxidative stress, testosterone levels and inflammation parameters on testicular tissue and blood after an oral exposition to ZEA in male mice.

## 2. Material and methods

### 2.1. Animals and reagents

Male Swiss albino mice (25–30 g in weight and 90 days old) were used. Animals were housed in groups of 5 in Plexiglas cages (41 × 34 × 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 #068/2012) of the Federal University of Santa Maria, Brazil.

ZEA, GSH, 1-chloro-2,4 dinitrobenzene (CDNB), epinephrine bitartrate salt, glycine, trizma base, were purchased from Sigma (St. Louis, MO, USA). Lycopene was obtained from commercially available capsules (Equaliv Licopeno®). All other chemicals were analytical grade from local suppliers. ZEA and lycopene were dissolved in olive oil, immediately before administration.

### 2.2. Experimental design and sampling

#### 2.2.1. Treatment

Treatment schedule is depicted in Fig. 1. Mice were weighed and randomly divided in two groups receiving lycopene (20 mg/kg) or olive oil (10 ml/kg) by oral route (*per* oral, p.o., by intragastric gavage administration) for 10 days. In the eleventh day, each group was again randomly divided in two groups receiving ZEA (40 mg/kg – 8% of $LD_{50}$) or olive oil (10 ml/kg) by oral route. The choice of the doses of ZEA and lycopene were based on preliminary experiments in our laboratory [8,9]. The regime of administration of lycopene (10 days, p.o., by gavage) did not interfere in the metabolism of ZEA [8,9]. Forty-eight hours after ZEA or vehicle administration the animals received a dose of pentobarbital (180 mg/kg, intraperitoneal, i.p.), and blood was collected by cardiac puncture into tubes containing heparin (1 UI/μl) and EDTA. The testes were removed, weighed, and homogenized in Tris–HCl 50 mM pH 7.4 for the determination of enzymatic indicators of oxidative stress and GSH. One of the testes was used to analysis of activity of enzymes and GSH levels and another to western blot assay. The epididymides were weighed and used for determining the number and motility of spermatozoa.

### 2.3. Blood cells

The first blood sample for each animal was collected in a tube containing EDTA as anti-coagulant and was used for hematological determinations using a blood counter (Mindray, BC-2800 Vet model) with adapted dilutions. These included red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), platelets (PLTs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean platelet volume (MPV).

### 2.4. Number and motility of spermatozoa

Assessment of spermatozoa count and motility was performed according to Freund and Carol [22] to confirm the oligozoospermia induced by ZEA. Briefly, the cauda epididymides were homogenized in 2 ml of warmed (37 °C) saline solution (0.9% NaCl). An aliquot (10 μl) of the diluted spermatozoa suspension was transferred to a standard hemocytometer counting chamber and was allowed to stand for 5 min. Cells settled in the 5-min period were counted with the help of light microscope (Nikon Eclipse 50i) at 200× magnification.

### 2.5. Testosterone levels

Plasma testosterone levels were assayed by radioimmunoassay (RIA), using commercial reagent (Total testosterone, Coat-A-Count ®, DPC, Los Angeles, CA, USA) with the hormone marked with I (radioactive tracer). The radioactivity was measured by automatic counter for determining the standard curve and the concentration of the samples. Results were expressed an ng/ml serum.

### 2.6. IL-1β, IL-2, IL-6, IL-10 and TNF-α levels

The second blood sample was used for determination of the following serum inflammatory parameters: IL-1β, IL-2, IL-6, IL-10 and TNF-α. Levels of IL-1β, IL-2, IL-6, IL-10 and TNF-α were determined using commercially available ELISA assays, following the instructions supplied by the manufacturer (DuoSet Kits, R&D Systems; Minneapolis, USA). Results are shown as pg/ml tissue.

### 2.7. Enzyme assays

#### 2.7.1. GST activity

GST activity was assayed spectrophotometrically at 340 nm by the method of Habig et al. [23]. The reaction mixture contained a 50 μl aliquot from supernatant (1000 × g/10 min/4 °C) of testes, 0.1 M potassium phosphate buffer (pH 7.4), 100 mM GSH and 100 mM CDNB. GST activity was expressed as nmol CDNB/min/mg of protein.

#### 2.7.2. GPx activity

GPx activity was assayed spectrophotometrically according to the method of Wendel [24], through the glutathione/NADPH/GR