

## Effects of aflatoxin B<sub>1</sub> on profiles of gonadotropic (FSH and LH), steroid (testosterone and 17 $\beta$ -estradiol) and prolactin hormones in adult male rat

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### Summary

Aflatoxins (AFs) are natural contaminants of feed and feedstuffs, thus the study of the noxious effects of these agents on the male reproductive system is of outstanding importance. Our aim for this study is the evaluation of the effects of oral administration of aflatoxin B<sub>1</sub> on the reproductive hormonal changes in adult male rat. Twenty eight Wistar strain male rats were selected for this study. The rats were divided into 4 groups, viz, control (C), test groups (T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>). The toxin doses were prepared in sterile distilled water at 0.8 ppm, 1.6 ppm, 3.2 ppm, and administered through oral gavages, 1 ml/animal/day to groups T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>, respectively for 48 days and each animal of group C was gavaged with 1 ml/day sterile distilled water. The hormonal assays were carried out using ELISA biochemical kits for serum FSH, LH, prolactin testosterone and 17 $\beta$ -estradiol. Results showed that, the levels of serum LH and testosterone were lower (P<0.001), but conversely the levels of FSH and prolactin were higher (P<0.001) in the test groups. The level of 17 $\beta$ -estradiol was affected by significantly falling (P<0.01) only in group T<sub>3</sub>. We conclude that, the oral administration of the aflatoxin B<sub>1</sub> strictly alters the concentrations of FSH, LH, prolactin, and testosterone in male Wistar rats.

**Key words:** Male rat, Aflatoxin B<sub>1</sub>, Pituitary hormones, Testosterone, 17 $\beta$ -estradiol

### Introduction

Aflatoxins (AFs) are natural contaminants of feed and feedstuffs. The severity of poisoning by AFs depends on the age, sex and species of the animal, the amount being exposed to and duration of exposure (Gugnani, 2000). Aflatoxins (AFs) are poisonous compounds synthesized by genus of *Aspergillus* fungi (Blunden *et al.*, 1991). The species of the fungi, frequently isolated from the feed and feedstuffs, are *Aspergillus flavus* and *Aspergillus parasiticus* (Dutta and Das, 2001). Epidemiological as well as clinical and experimental studies revealed that short exposure to large doses of aflatoxin produced acute toxicity which may be lethal; while exposure to small doses over a protracted period of time is carcinogenic (Krishnamachari *et al.*, 1977). Little is

known about moderate concentrations of aflatoxin, which occur frequently in tropical and subtropical countries. Marvan *et al.* (1983) have experimentally studied distribution of AFB<sub>1</sub> in goslings and chickens, and according to the results of this study, AFB<sub>1</sub> concentrations on the organs and tissues were categorized as follows: gonads, parenchymatous organs-liver and kidney, lymphopoietic organs-spleen, bursa cloacalis, and thymus, followed by the endocrine glands and muscles; lungs have the low concentration, and the brain the lowest. In Chinese hamsters, Petr and Turek (1995) have shown that after a single intraperitoneal dose of 0.1 mg AFB<sub>1</sub>/kg body weight, free AFB<sub>1</sub> was detected in the blood, liver, kidney and testis from just minutes to up to 8-10 h after injection. The cell population of seminiferous tubules were scattered and disorganized in the testes of

mice administered 25 and 50 mg aflatoxin/animal/day for 45 days, and degenerative changes in Leydig cells as well as significant changes in cauda epididymal sperm count along with decreased sperm motility were also reported (Nair and Verma, 2000). Many investigators have reported a reduction in serum testosterone levels after aflatoxin treatment in male rats (Srivastava and Singh, 1985). Aflatoxins can be carcinogenic (Groopman *et al.*, 1996) and mutagenic (Mace *et al.*, 1997). Aflatoxins, thus, have become an issue in human and veterinary health. In general, at smaller concentrations the aflatoxins and, AFB<sub>1</sub> in particular, can affect aspects of male reproduction, namely spermatogenesis (Mace *et al.*, 1997), Leydig cell function (Egbunike *et al.*, 1980; Egbunike, 1982) and fertility (Ibeh *et al.*, 1994) as seen from sperm and seminal enzymes (Ibeh and Saxena, 1998).

Our aim for this study is the evaluation of the effects of oral administration of aflatoxin B<sub>1</sub> on the reproductive hormonal changes in adult male rat.

## Materials and Methods

Twenty eight Wistar strain male rats, 65–70-day-old, weighing  $200 \pm 15$  g were selected for this study. They were kept in 12 h light: 12 h dark period, 20–30°C temperature and 50–60% relative humidity conditions. The rats were divided randomly into 4 equal groups, namely control, test group 1, test group 2, and test group 3 (C, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>), then caged separately. The animals were fed on standard pellet diet (*ad libitum*). Aflatoxin B<sub>1</sub> was obtained from Sigma chemical company. Toxin doses (0.8 ppm, 1.6 ppm, 3.2 ppm) were prepared in distilled water. The groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> received aflatoxin B<sub>1</sub> at levels of 0.8, 1.6, 3.2 ppm/1 cc distilled water/animal/day, respectively for 48 days by gavaging. Control group (group C) was gavaged with 1<sup>cc</sup> distilled water/animal/day for the same period. At the end of the experiment, the animals were anesthetized with xylozine/ketamine, then blood samples were obtained directly from the heart and blood sera were separated. The hormonal assays were carried

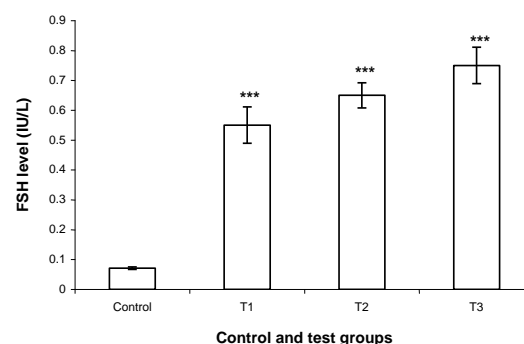
out using ELISA biochemical kits for serum testosterone (IBL, Flughafenstrasse, 52a, Hamburg D-22335, Germany), 17 $\beta$ -estradiol (Diametra 20090 Segrate Milano Italy), prolactin, LH and FSH (Pishtaz Teb, Zaman Diagnostics Tehran, Iran). In case of intra-assay and inter-assay of LH kit, if frequency of repetition is 24, the amount of SD (IU/L) will be 0.53, and 0.45, respectively. In the case of intra-assay and inter-assay of FSH kit, if the frequency of repetition is 24, the amount of SD (IU/L) will be 0.14, and 0.45, respectively. In the case of intra-assay and inter-assay of prolactin kit, if frequency of repetition is 24, the amount of SD (IU/L) will be 3.4, and 6.8, respectively. The ANOVA and Duncan's tests were used for statistical analyses of the data.

## Results

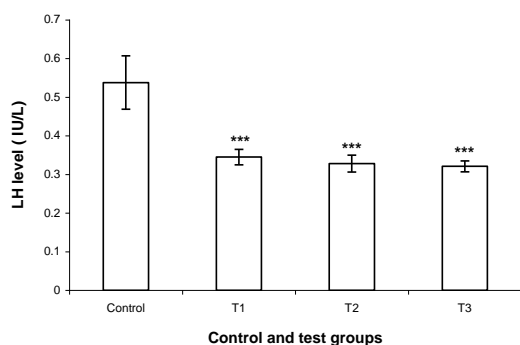
The level of FSH was significantly higher ( $P < 0.001$ ) in all treatment groups (Fig. 1), whereas the levels of LH and testosterone were lower ( $P < 0.001$ ) in all of the treatment groups in comparison to the non treated (control) group (Figs. 2 and 3). The level of prolactin was significantly higher ( $P < 0.001$ ) in all treatment groups (Fig. 4). The level of 17 $\beta$ -estradiol was significantly ( $P < 0.01$ ) decreased only in group T<sub>3</sub> (Fig. 5).

## Discussion

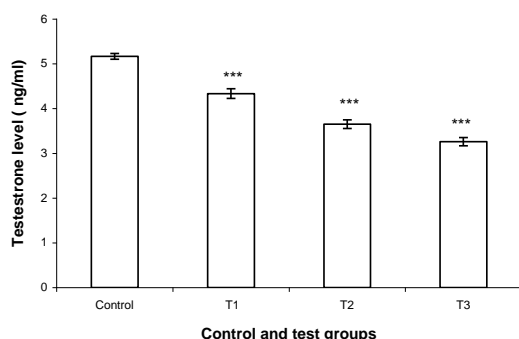
In this study LH level was significantly lower in the treatment groups compared to



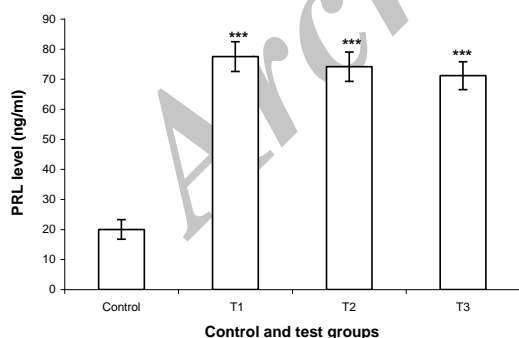
**Fig. 1:** The FSH levels in control, test group received 0.8 ppm (T<sub>1</sub>), test group received 1.6 ppm (T<sub>2</sub>) and test group received 3.2 ppm (T<sub>3</sub>) AFB<sub>1</sub>. Each test group was compared with the control group. \*\*\* =  $P < 0.001$



**Fig. 2:** The LH levels in control, test group received 0.8 ppm (T<sub>1</sub>), test group received 1.6 ppm (T<sub>2</sub>) and test group received 3.2 ppm (T<sub>3</sub>) AFB<sub>1</sub>. Each test group was compared with the control group. \*\*\* = P<0.001

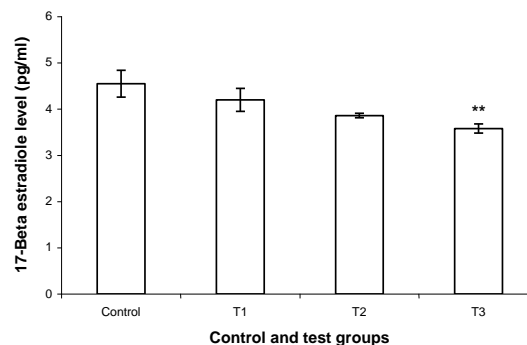


**Fig. 3:** The testosterone levels in control, test group received 0.8 ppm (T<sub>1</sub>), test group received 1.6 ppm (T<sub>2</sub>) and test group received 3.2 ppm (T<sub>3</sub>) AFB<sub>1</sub>. Each test group was compared with the control group. \*\*\* = P<0.001



**Fig. 4:** The prolactin levels in control, test group received 0.8 ppm (T<sub>1</sub>), test group received 1.6 ppm (T<sub>2</sub>) and test group received 3.2 ppm (T<sub>3</sub>) AFB<sub>1</sub>. Each test group was compared with the control group. \*\*\* = P<0.001

control group. The aflatoxin has a hypophysiotoxic effect, especially on adenohypophysis (Clarke *et al.*, 1987). Thus, decreases in levels of the LH could be



**Fig. 5:** The 17-β estradiol levels in control, test group received 0.8 ppm (T<sub>1</sub>), test group received 1.6 ppm (T<sub>2</sub>) and test group received 3.2 ppm (T<sub>3</sub>) AFB<sub>1</sub>. Each test group was compared with the control group. \*\* = P<0.01

related to the effect of the toxin on hypophysis. We observed an extreme increase in FSH level in serum of the treatment groups. Following administration of aflatoxin B<sub>1</sub> to the roosters, the degeneration and desquamation of the epithelium and a decrease in the size and thickness of the germinal layer in the seminiferous tubules occur (Ortatatli *et al.*, 2002). Serum FSH levels tend to be elevated when the testes are damaged and circulating inhibin-B is reduced (Attardi *et al.*, 1989; Jensen *et al.*, 2004). It is obvious that this degenerative effect of the toxin on germinal epithelium of the seminiferous tubules would breakout into sertoli cells, bringing about a decrease in inhibin B<sub>1</sub> level and, consequently, due to reduction of the inhibitory effect of the inhibin B<sub>1</sub> on the production and secretion of FSH, the level of this hormone increases. According to the results of a study in female rats, increase in levels of estradiol and inhibin B causes a decrease in the level of the FSH in the follicular phase (Erickson and Shimasaki, 2001; Padhy *et al.*, 2009). Thus in both the male and female rats, by decrease in the level of the inhibin B, the level of the FSH will increase. Our results revealed reduction in blood testosterone levels and this is due to the extreme damage to leydig's cells (Aydiner *et al.*, 1997). DNA binding and inhibition of nucleic acid synthesis is the most common mechanism suggested for aflatoxin B<sub>1</sub> action. This may explain the direct effect of AFB<sub>1</sub> on leydig cell. The indirect effect of AFB<sub>1</sub> on leydig cells and consequently on testosterone level can also

be due to hypophysotoxicity. With decreasing LH level by means of the effect of AFB<sub>1</sub> on hypophysis, leydig cells will reduce testosterone secretion, i.e. indirect effect of toxin. A previous report stated that, the activities of 3 $\beta$ - and 17 $\beta$ -hydroxysteroid dehydrogenases and serum testosterone levels were significantly reduced in aflatoxin-treated mice as compared with the controls (Verma and Nair, 2002).

Oral administration of aflatoxin (25 and 50  $\mu$ g/animal/day) for 45 days to adult mice caused, as compared with control, a dose-dependent significant rise in cholesterol content.

Oral administration of aflatoxin B<sub>1</sub> to male *Coturnix coturnix japonica* breed quails brings about significant decrease in the testosterone level in the blood (Gokhan *et al.*, 2006). Aflatoxin B<sub>1</sub> inhibits prolactin secretions by rat pituitary cells in culture, (Abdel-Haq *et al.*, 2000), but our results revealed that oral administration of this toxin brings about tremendous increase in serum prolactin level in male rat.

The results of this study showed that, the concentration of 17 $\beta$ -estradiol has a tendency to decline after 45 days administration of aflatoxin B<sub>1</sub> in all treatment groups, but this reduction was significant only in the T<sub>3</sub> group (group which received 3.2 ppm). It is well established that 17 $\beta$ -estradiol is synthesized by Leydig cells in response to luteinizing hormone (LH) and by Sertoli cells in response to follicle-stimulating hormone (FSH) in male mammals. A previous paper has indicated that germ cells are also a possible source of 17 $\beta$ -estradiol in the male rat (Carreau, 2001). With reference to the results of our experiment and the reports of the previous studies, we conclude that, due to either the direct effects of the aflatoxin B<sub>1</sub> on the Leydig cells, Sertoli cells (Gopal *et al.*, 1980; Wanzhu *et al.*, 2005), Germ cells or indirect effects i.e., on the hypophysis (Clarke *et al.*, 1987), the level of 17 $\beta$ -estradiol in the male rat decreases, but as the results of this study show, this happens only by administration of a relatively high dose of the toxin.

In conclusion, the present study clearly demonstrated that, oral administration of aflatoxin B<sub>1</sub> to male rat, causes alterations in

the serum concentrations of the gonadotropic (FSH, LH and prolactin) as well as gonadal (testosterone and 17 $\beta$ -estradiol) hormones in adult male rat and these alterations include decrease in LH, testosterone and 17 $\beta$ -estradiol, but elevation in FSH and prolactin levels.

## Acknowledgement

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