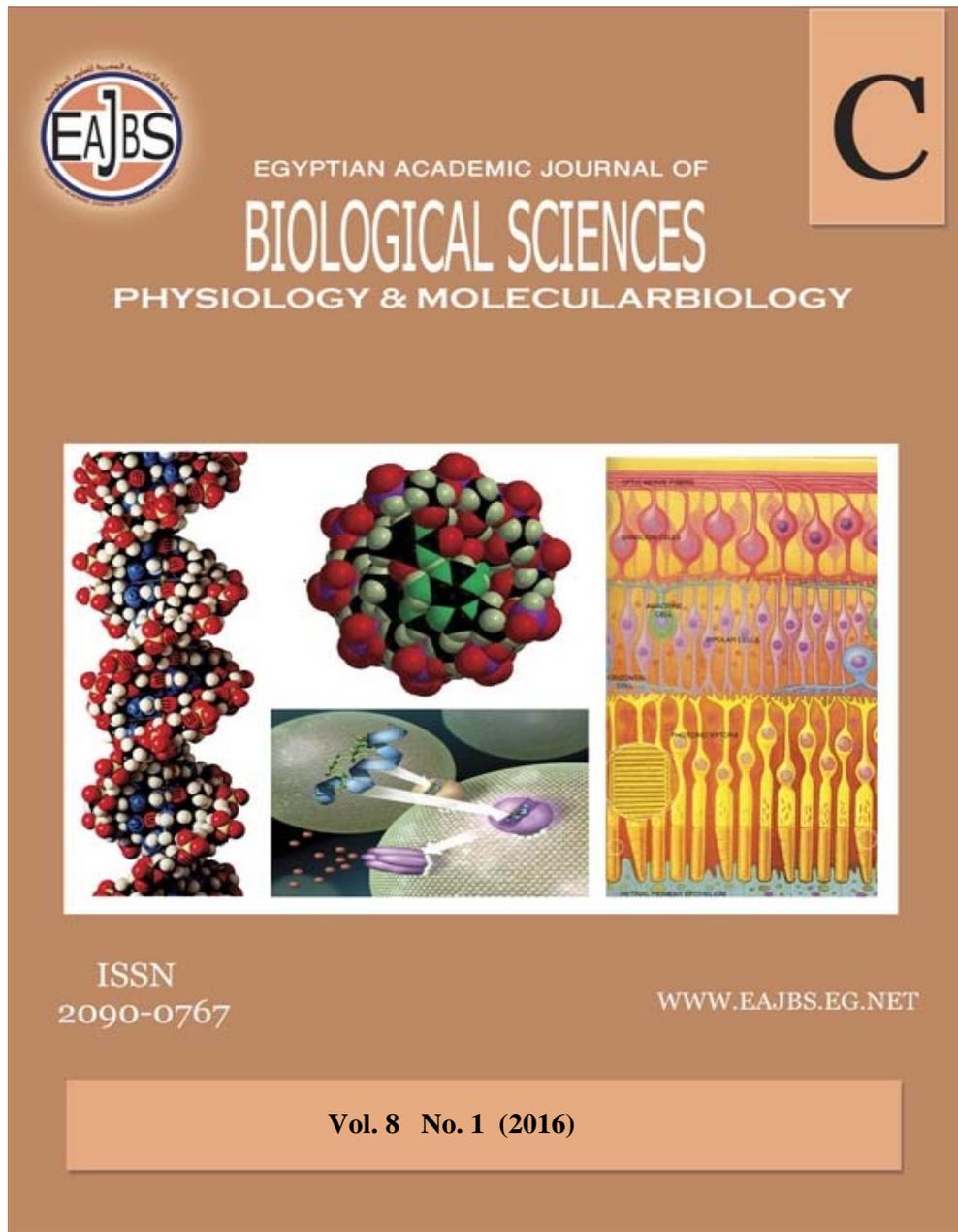


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Hormonal Changes in BALB/c Mice During Aflatoxins Exposure produced From *Aspergillus flavus* and the Protective Effects of *Panax ginseng* Extract.

Madlen Qassm abbas¹, Khalil Muoload Wahab² and Anmar Sael Hussein³

1- College of dentistry, Tikreet University.

2- Institute of medical technology/ Al Mansour.

3- College of medicine , Faluja university.

*Corresponding author: Madleen_qasim@yahoo.com

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ABSTRACT

The aim of the present study to identify the influence of aflatoxins (9mg/kgb.w) in the sum of hormone levels Total thyroxin, Testosterone and Luteinizing hormonein BALB/c mice and the protective effects of pretreatment of the three concentrations of *Panax ginseng*(150mg / kg b.w) , (100mg / kg b.w) and (50mg / kg b.w) At the end of administrated interval, the laboratory animals had weighted and killed to obtain blood samples in order to identify the changes in the level of thyroid hormones T4 as well as testosterone and Luteinizing hormone the results showed as follows:

- 1- Reducing in the level of hormone T4 , testosterone and Luteinizing hormone LH in the blood serum of the aflatoxins AFs (9mg/kgb.w) animals group when compared with control.
- 2- Increasing in the level of T4, testosterone and Luteinizing hormone LH of each Animals administrated pretreatment with *Panax ginseng* at the tree concentrations.
- 3- The best protective effects against Aflatoxins in current study done by the concentrations (150mg / kg b.w) of *Panax ginseng* extract.

INTRODUCTION

Aflatoxins have been described to interfere with the functioning of the number of endocrine glands by disrupting the enzymes and their substrates that are responsible for the synthesis of the various hormones. Aflatoxins and their metabolites as well as the generated ROS have been reported to cause various cancers in diverse endocrine glands like pituitary gland, granulosa cell tumors of the ovary and adenomas and adenocarcinomas of the adrenal gland, kidneys, thyroid gland, ovaries, testes, thyroid gland, parathyroid glands and endocrine pancreas (Gupta, 2011, Agag, 2004). The plasma testosterone and luteinizing hormone (LH) concentrations have been reported to be reduced in aflatoxin-fed birds (Lakkawar, and Johri, 2004). In laboratory animals, the maturation of both males and females have been delayed after exposure to aflatoxin (D°onmez, *et al.* 2012).

On the other hand, aflatoxicosis in white leghorn males chicken decreased feed consumption, body weight, testes weight, semen volume (Marin, *et al.* 2002) and decreased plasma testosterone values (Hasanzadeh, and Rezazadeh, 2012).

Exposure to aflatoxin can cause several health-related conditions including hormonal changes, food and feed contamination with aflatoxin is unavoidable because the absence of alternative food and feed resources.

When ingested, aflatoxin binds to liver proteins. The metabolic products may persist for 2 to 3 months or longer and can be detected through blood tests (Wagacha and Muthomi, 2008). Aflatoxins can cause disease in vertebrates animals when introduced *via* a natural route, ingested, absorbed through the skin or inhaled (Hussan, 2006). As well (Ko, 1998) reported Ginseng (*Panax ginseng*) is one of the most widely used medical herbals, particularly in traditional oriental medicine, also has a wide range of pharmacological and physiological actions. And previous studies have shown that ginseng control hormonal secretion (Kim, 1999).

MATERIALS AND METHODS

Aspergillus flavus Isolate: Pure *Aspergillus flavus* isolates were obtained from the Biotechnology Department, Science College/Baghdad University, they were cultured on rice medium, Aflatoxins production in rice medium, extraction and analysis using High Performance Liquid Chromatography (HPLC) (Omar, 2012). After incubation time, the moldy rice was soaked overnight with 75 ml of chloroform in dark place. Then the soaked medium was homogenized with electric homogenizer for 15 min. The extracted solution was filtered through gauze then was sieved through a Whatman No.1 filter papers.

The residues were washed by 50ml of chloroform, and then was filtered. Chloroform fractions were pooled and evaporated to dry at 50°C. Dried extracts stored at 4°C until use (Turner, 2009).

Detection and Quantification of aflatoxin

It was prepared by diluting in absolute methanol, 1mg /ml for B2 and G2, 10mg/ml for B1 and G1. Then was combined 100µl aliquot of each aflatoxin solution in a 2-ml vial and mixed well. This mixture was more diluted in series to 100,000 doublings in water: methanol (7:3v/v) then stored at -70°C in deep freeze until using (Al-Azawee, 2006).

Laboratory Animals and Experimental Design

Fifty six of Swiss albino mice (male) were purchased from the National Centre for Drug, their range ages were between (8-12) weeks age and weighting (25-30)g. The mice were acclimatized for two weeks before treatment. Animals were housed in plastic cages containing hard wood chips for bedding, the animal reared in house at 25± 2C°, 4/10 hour's light / dark cycle. So the animals fed with suitable quantity of complete diet, then the animals were divided into 8 groups and were fed orally administered, as shown below:

Group 1: Animals without any treatments (control).

The following groups were treated by oral gavage with:

Group 2: Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks).

Group 3: Methanol 10% two times in a week (for two weeks).

The pretreatment groups with crude extract of ginseng divided into three groups, each group contained (8) mice («1» refers to pretreated groups (which treated with ginseng aqueous crude extract for ten days then were treated with crude extract of aflatoxins (9mg/kg b.w) twice a week for two weeks). The

letters a, b and c refer to concentration of *P. ginseng* extract (150,100 and 50 mg/kg b.w) respectively.

Group 1-a: Animals were pretreated with (150mg / kg b.w) plant aqueous crude extract.

Group 1-b: Animals were pretreated with (100mg / kg b.w) plant aqueous crude extract.

Group 1-c: Animals were pretreated with (50mg / kg b.w) plant aqueous crude extract

Blood Collection and Hormonal study analysis:

The blood samples were collected at the end of the experiment from all groups. Mice were anesthetized with ethyl ether inhalation. About 0.5-1ml of blood was collected directly from heart through cardiac puncture using 1ml disposable insulin syringes. Blood was collected in a sterile eppendorf tube and left it for about 30 minutes at room temperature to clot, then were centrifuged at 3000 r/ 15 minutes. Serum was transferred to 0.5 ml eppendorf tube using micropipette, and then kept in deep freezer until biochemical analysis was performed. Clinical signs and indications were recorded and noted precisely all through the analysis. Blood tests were gathered and serum was segregated for the accompanying hormonal measure parameters:

1- Total thyroxin (TT4) assay (nmol/L): The standard assay (TT4-Kit) was used (Lazar A, 1993).

2. Testosterone assay (nmol/L): Testosterone was measured using standard assay (Testosterone -Kit) was used (Lazar A, 1993).

3. Luteinizing hormone assay (mlu/L): These rum level of LH was measured using standard assay (LH-Kit) (Lucas,1996). The LH biotic pack indicative Automation, INC. depends on the rule of a strong stage compound connected immunosorbent test which is the traditional sandwich ELISA strategy. Utilizing spectrophotometrically innovation to measured force of shading at 450 nm. The centralization of LH is specifically relative to shading power of test. Two way analysis of variance (ANOVA) were used statistically analyzed among of all data (SAS, 2004).

RESULTS AND DISCUSSION

In the present study, the measurably huge reductions were seen in the levels of blood TT4, Teststeron and LH in all trial bunches contrasted with the control bunch. Aflatoxins AFs effects in hormone level of Total thyroxin (TT4) at Animals exposed to Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks). Showed depression at the concentrations of T4 reach to 3.511nmol/L Whereas, no such symptoms were observed in control 8.511nmol/L as show in Table 1, while pretreated with *Panax ginseng*(150mg / kg b.w), (100mg / kg b.w) and (50mg / kg b.w) after aflatoxins exposure show protective effects against aflatoxins as represented in Table 1 where the T4 concentrations 9.357, 8.493, and 8.357nmol/L, respectively.

Table 1: The Total thyroxin (TT4) level in serum of BALB/c mice After exposure to Aflatoxins and the protective effects of *Panax ginseng* extract.

| Treatment | O.D | Concentrations nmol/L | Chi-square (χ^2) |
|---|-------|-----------------------|-------------------------|
| Animals were pretreated with (150mg / kg b.w) plant aqueous crude extract. | 0.739 | 9.357 | 40.00 |
| Animals were pretreated with (100mg / kg b.w) plant aqueous crude extract. | 0.635 | 8.493 | 57.69 |
| Animals were pretreated with (50mg / kg b.w) plant aqueouscrude | 0.780 | 8.357 | 62.50 |
| Animals without any treatments (control). | 0.881 | 8.511 | 40.00 |
| Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks). | 0.633 | 3.511 | 50.00 |
| Methanol 10% two times in a week (for two weeks). | 0.901 | 8.293 | 50.00 |
| Chi-square (χ^2) | --- | --- | 8.922 ** |

** (P<0.01).

Aflatoxins AFs effects in hormone level of Testosterone was significantly Decreased ($P < 0.001$) in treatment groups with aflatoxins (9 mg /kg b.w.) Whereas the levels of Testosterone at the group of

mice pretreated with *Panax ginseng* extract increase gradually with the increase of ginseng extract pretreatment as shown in Table 2.

Table 2: The Testosterone assay level in serum of BALB/c mice After exposure to Aflatoxins and the protective effects of *Panax ginseng* extract.

| Treatment | O.D | Concentrations gm/mL | Chi-square (χ^2) |
|---|-------|----------------------|-------------------------|
| Animals were pretreated with (150mg / kg b.w) plant aqueous crude extract. | 2.402 | 2.402 | 10 |
| Animals were pretreated with (100mg / kg b.w) plantaqueous crude extract. | 2.532 | 2.532 | 15 |
| Animals were pretreated with (50mg / kg b.w) plant aqueouscrude | 2.292 | 2.292 | 15 |
| Animals without any treatments (control). | 2.463 | 2.463 | 10 |
| Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks). | 0.870 | 0.986 | 10 |
| Methanol 10% two times in a week (for two weeks). | 2.879 | 2.607 | 60 |
| Chi-square (χ^2) | --- | ---- | 13.589 ** |

** ($P < 0.01$).

At the Animals exposed to Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks). Showed depression at the concentrations of Luteinizing hormone reach to 0.239 (IU/L) Whereas, no such symptoms were observed in control 0.649 (IU/L) as show in Table 3, while pretreated with *Panax*

ginseng (150mg / kg b.w), (100mg / kg b.w) and (50mg / kg b.w) after aflatoxins exposure show protective effects against aflatoxins as represented in Tables 3 where the Luteinizing hormone concentrations 0.907, 0.798, and 0.710 (IU/L) respectively.

Table 3: The Luteinizing hormone level in serum of BALB/c mice After exposure to Aflatoxins and the protective effects of *Panax ginseng* extract.

| Treatment | O. D | Concentrations (IU/L) | Percentage (%) |
|---|------|------------------------|----------------|
| Animals were pretreated with (150mg / kg b.w) plant aqueous crude extract. | 67.0 | 0.907 | 5.00 |
| Animals were pretreated with (100mg / kg b.w) plant aqueous crude extract. | 56.9 | 0.798 | 28.33 |
| Animals were pretreated with (50mg / kg b.w) plant aqueouscrude | 87.0 | 0.710 | 66.67 |
| Animals without any treatments (control). | 23.6 | 0.649 | 10.0 |
| Crude extract of aflatoxins (9 mg /kg b.w.), two times in a week (for two weeks). | 0.87 | 0.239 | 5.98 |
| Methanol 10% two times in a week (for two weeks). | 23.8 | 0.610 | 6.34 |
| Chi-square (χ^2) | --- | | 12.509 ** |

** ($P < 0.01$).

The mechanism concerning the decrease in plasma levels may be associated with the decrease in blood Aflatoxin AFs level. It is basically critical in the amalgamation of these hormones (Bankole and Adebajo,

2003). Interestingly, the decrease in both hormone levels were only observed in the group which received aflatoxin only, while groups pretreated with ginseng extract were not affected. The reason for these decline in the hormone levels in the

groups which received Aflatoxin material alone could be the irreversible binding of iodine by aflatoxins, subsequently, its disrupted absorption from the digestive tract. In fact, aflatoxin is a compound that possesses high binding capacity (Thrasher, 2012).

Epidemiological and clinical and trial examines uncovered that short presentation to vast measurements of aflatoxin delivered intense poisonous quality which might be deadly; while introduction to little dosages over an extended timeframe is cancer-causing most creators conjecture that aflatoxin may influence the regenerative framework by its dangerous impact on the liver, where the cell hepatic harm could repress compound amalgamation and/or chemical movement or restraint of lipid digestion system or unsaturated fat union, bringing on diminished combination of forerunner atoms for gonadal and additionally gonadotropic hormones, e.g. FSH, luteinizing hormone (LH) oestradiol, testosterone and progesterone (Handan and Güleray 2005). Aflatoxin may also affect the reproductive system by causing lysis of germ cells, as it was known that aflatoxin at graded doses induced severe oxidative damage in the testis and accessories that promote their apoptosis (Kawkab *et al.*, 2012). In vitro evidence from animal model and clinical studies suggest that reactive oxygen species (ROS) play a role in the aetiology of adverse reproductive events (Walsh *et al.*, 2000; Acevedo *et al.*, 2001; Sikka, 2001) causing the generation of radical species to exceed scavenging by antioxidants as a result of excessive production of ROS and/or insufficient admissions or expanded use of cancer prevention agents. Expanded ROS is connected with diminished steroidogenesis and their cyclic creations add to a declined ovarian capacity (Kodaman and Behrman 2001). Likewise changes create amid the vehicle of these hormones in the circulation

system. A case for that is a noteworthy diminishment of plasma protein content by AF, Aflatoxins targets protein union pathways particularly the DNA format, RNA layout (mRNA, tRNA, rRNA), proteins, interpretation, interpretation and cell metabolic responses (Hossam El-Din, 2013).

CONCLUSION

The investigations led to the conclusion that aflatoxins are severely toxic to male reproductive system and thyroid gland. The manifestations include severe changes in the levels of some reproductive hormones such as testosterone. *Panax ginseng* extract play important role in protective against aflatoxisis.

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