

Reprotoxicological Ameliorating Effect of Camel Milk against Aflatoxicosis in Male Rats

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Abstract

This study was performed to cover the toxic effects of aflatoxin B1 on the reproductive system in male rats and to evaluate the improving effects of camel milk. A total of 120 adult male rats (aged at 90 days) were randomly assigned into four groups (30 rats each); control (C), received distilled water, camel milk group (Cm), received camel milk (10 ml/kg bw/day), Aflatoxin group (Af), received AFB1 (0.3 mg/kg bw/day), and combination treated group (CmAf), received camel milk and AFB1 for 42 days. At days 21 and 42 of the experiment, 10 males from each group were weighted, anesthetized, and blood samples were collected for assessment of the serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone (T). Epididymis tails were dissected for evaluating the semen quality. Pituitary and testicular tissue samples were obtained for analysis the gene expression levels of pituitary FSH β and LH β and testicular FSHR and LHR. The remaining 10 males, from each group, were mated with experienced females to evaluate the fertility index. Significant ($p < 0.05$) reductions of hormonal levels, semen quality, expression level of LHR, and fertility index were shown in Af group. However, the combination group revealed a significant ($p < 0.05$) improvement in these parameters. In conclusion, the high toxicity of aflatoxin B1 on the reproductive system was demonstrated, while the administration of camel milk reduced these toxic effects.

Keywords: Aflatoxin B1, Camel milk, Reproduction, Infertility, Toxicity

INTRODUCTION

Difuranocoumarin complexes with the bifuran group linked with the coumarin nucleus and either the pentanone or lactone ring are known as aflatoxin (AF). When it comes to AF contamination, the most commonly discovered items include a range of nuts and spices, as well as figs and dried fruits (1). A single AF type was established to have contaminated 37.6% of the grains examined and in milk and cooking butter (2). Despite the notion that rice is not particularly susceptible to AF contamination, AFB1 was found in rice from India, Egypt, Iran, the United Kingdom, and the United States. Because AFs are absorbed through contaminated food, they are harmful to human health (3).

An extensive group of heme-binding cytochrome enzymes known as cytochrome P450s is contributed in the synthesis and metabolism of chemicals derived from the body and from external sources, including xenobiotics and Afs. Hepatocytes need CYP1A2 and CYP3A4 mostly for the first two electron transfer oxidation pathways of Af degradation. It is CYP3A4 that converts AFB1 into an active product and subsequently a less harmful molecule (4).

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Each species, gender, age group, and nutritional status has its own unique level of toxicity when it comes to AF ingestion. Hepatic damage and decreased milk and egg production are also consequences of toxic exposure. AF produces gastrointestinal problems in animals, as well as anemias and icterus as a result of the toxicity effects. Studies on the potential carcinogenic effects of AFs are well-documented. According to the International Agency for Research on Cancer AFB1 has been designated as the toxin having sufficient evidence of being a carcinogenic substance (5).

Reduced Red blood cells (RBCs) count and low packed-cell volume are two signs of hemolytic anemia that may be brought in by the AF, as can spleen obstruction from RBC debris and high levels of inorganic iron. When lymphopenia occurs, the total leucocyte count often rises. When it comes to serum biochemical qualities, the total protein level drops, along with a fall in lipoproteins, triglycerides and cholesterol levels. As a result of the Af toxicity, there are increases in the activities of liver enzymes, such as alkaline phosphatase and aspartate aminotransferase (6).

Aflatoxins can affect Leydig cells directly, affecting important functions of enzymes and hormones. AFB1, in special, can decrease testosterone levels in a dose-reliant manner while also inhibiting the expression of certain dehydrogenase enzymes. Since steroidogenesis relies on both enzymatic and hormonal mechanisms, a thorough knowledge of how mycotoxins affect both systems is essential (7).

Because of camel milk's high contents of vitamin C and some important proteins, such as lactoferrin, camel milk was employed for medical purposes in a variety of civilizations for a long time (8). Therefore, the current study was carried out to identify the toxicological effects of AFB1 on male rat's reproductive functions, and the role of camel milk in reducing these side effects, when used for 42 days.

MATERIALS AND METHODS

Aflatoxin B1

It was purchased from (Sigma Aldrich, USA), and the dose was 0.3 mg/kg BW/ day (9).

Camel milk

It was obtained fresh from a camel breeder in Al-Hamza district in Al-Diwaneyah city. It was transported in a cool box.

Animal ethics and care

All animals were handled according to international and national animal care and use. The period of the experiment was during July 19 to October 20, 2020. A total of 120 adult

male rats (aged at 90 days and weighted 170±3.8 g) were adapted to the animal house environment at the College of Veterinary Medicine, University of Al-Qadisiyah, before the start of the experimental procedures. A room (proper ventilation, temperature at 22±2°C, light from 06:00 A.M. to 06:00 P.M, and ad libitum standard chow and water) was utilized to hosted the rats.

Experimental design

The rats were randomly assigned into four groups (30 each). Control (C) group received distilled water orally, Cm group received camel milk (10 ml/kg BW/day), Af group received AFB1 (0.3 mg/kg BW/day), and CmAf group received a combination of camel milk and AFB1. The treatment period was 42 days. At day 21 and 42 of the experiment, 10 males from each group were weighted, anesthetized, and blood samples were collected for assessment of the serum levels of LH, FSH, and T. Epididymis tails were dissected for evaluating the semen quality. Pituitary and testicular tissue samples were obtained for analysis the gene expression levels of pituitary FSH β and LH β and testicular FSHR and LHR. The remaining 10 males, from each group, were mated with experienced females to evaluate the fertility index.

Assessment of serum LH, FSH, and T levels: Rat LH, FSH, and T ELISA kits (CUSABIO, China) were employed. Protocol steps were based on the kit manufacturer's procedures.

Analysis Of Gene Expression Of Pituitary Fsh β And Lh β And Testicular Fshr And Lhr

Extraction of total RNA

Pituitary and testicular tissues were utilized to extract the total RNA via the use of Total RNA Extraction solution AccuZol® Kit (Bioneer, Korea).

cDNA synthesis

cDNA was synthesized according to M-MLV Reverse Transcriptase kit (Bioneer, Korea). A total volume of 10 μ l was used that contained 8 μ l (100ng/ μ l) RNA, 1 μ l random hexamer primer, and 1 μ l DEPC water. For the master mix, a total volume of 20 μ l was utilized that was composed of 10 μ l Step 1 RT master mix, 1 μ l (200 μ) M-MLV RTase, 4 μ l 5X M-MLV RTase reaction buffer, 2 μ l 100mM DTT, 2 μ l dNTP, and 1 μ l RNase inhibitor. The thermocycler conditions were 60min-42°C for the RT step (cDNA synthesis) and 5min-95°C for the heat inactivation.

Quantitative Real-Time PCR

The qRT-PCR that targeted the FSH β , LH β , FSHr, and LHR genes and normalized by the GAPDH, as a housekeeping gene, was performed based on methods described by Burow *et al* (10). The data of the qPCR were analyzed using the fold

change method for the relative expression of the target genes

and GAPDH, which is described by Livak and Schmittgen (11).

GraphPad Prism V5

Statistical analysis

(USA) with the use of Mean \pm standard deviation (SD) to display the data was utilized. Significant data (at $P < 0.05$) based on the use of one-way ANOVA (Newman-Keuls) were calculated (for each time point) between groups, while Significant data (at $P < 0.05$) based on the employment of student t-test were measured (for each group) between time points (12).

RESULTS

Reproductive hormones

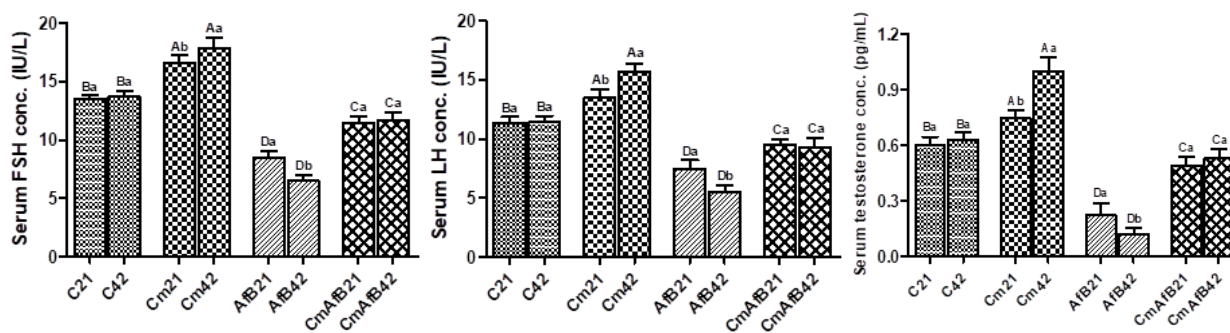


Figure 1: Effect of camel milk on serum FSH, LH, and testosterone concentrations in Af-treated male rats.

Values denote Mean \pm SD of 10 observations. Different uppercase letters denote the significant ($p < 0.05$) difference between groups (for each period), and different lowercase letters denote the significant ($p < 0.05$) difference between the two time points (for each group).

Semen quality

At 21 day period, Sperm motility of Af and CmAf group male rats revealed significant decrease ($p < 0.05$) and Cm group male rats revealed significant increase ($p < 0.05$) in comparison with control male rats. At 42 day period, the percentage of motile sperms of CmAf group male rats restored to the control levels, whereas that of Af and Cm groups remains as that of 21 day period. In comparison between the two point time for each group, control and Cm groups showed no significant differences ($p > 0.05$), whereas Af group recorded significant decrease ($p < 0.05$) and CmAf group showed significant increase ($p < 0.05$) at 42 day period in comparison with 21 day period (Table 1).

At 21 day period, sperm count and viability of Af and CmAf group male rats revealed significant reduction ($p < 0.05$),

Based on the results, illustrated in figure (1), Af group male rats showed significant ($p < 0.05$) decrease in the serum levels of FSH, LH, and T, whereas, restoration was occurred in CmAf group male rats. However, significant ($p < 0.05$) elevations were revealed in the Cm group male rats among the experimental groups. When the two time points were compared, control and CmAf groups showed no significant differences between the ($p > 0.05$), whereas Cm group increased significantly ($p < 0.05$) at 42 day period than 21 day period and Af group decreased significantly ($p < 0.05$) at 42 day period than 21 day period.

whereas Cm group males showed significant elevation ($p < 0.05$) compared with control males. At 42 day period, the two parameters were restored in CmAf males to the control level, whereas that of Af and Cm groups remains as that of 21 day period. In comparison between the two periods for each group, Cm and CmAf groups showed significant increase ($p < 0.05$) and Af group recorded significant decrease ($p < 0.05$) at 42 day period in comparison with 21 day period (Table 1).

At 21 day period, the percentage of abnormal sperms Af group was the highest and that of Cm group was the lowest ($p < 0.05$) among the experimental groups, whereas CmAf group recorded significant increase ($p < 0.05$) than control. At 42 day period, the percentage of abnormal sperms was restored in CmAf group to the control level, whereas that of Af and Cm groups remains as that of 21 day period. In comparison between the two periods for each group, Cm and CmAf groups showed significant decrease ($p < 0.05$) and Af group recorded significant increase ($p < 0.05$) at 42 day period in comparison with 21 day period (Table 1).

Table 1: Effect of camel milk on semen quality in aflatoxin-B1 treated male rats.

| Semen parameters | Period | Groups | | | |
|--------------------------|--------|-------------------|--------------------|-------------------|-------------------|
| | | C | Cm | Af | AfCm |
| Sperm motility (%) | 21 day | 76.24±5.322 Ba | 93.726±4.101 Aa | 46.19±3.463 Da | 66.55±4.170 Cb |
| | 42 day | 74.74±6.130 Ba | 94.349±4.799 Aa | 35.13±3.366 Cb | 76.83±4.164 Ba |
| Sperm count (million/mL) | 21 day | 62.87±5.193 Ba | 81.76±4.050 Ab | 31.77±4.598 Da | 51.98±4.379 Cb |
| | 42 day | 63.17±6.990 Ba | 94.96±4.756 Aa | 21.94±5.284 Cb | 63.59±7.864 Ba |
| | 42 day | 74.83±4.529 Ba | 84.99±4.780 Ab | 24.98±4.552 Da | 65.28±5.008 Cb |
| Sperm viability (%) | 21 day | 74.33±5.824 Ba | 93.08±6.062 Aa | 4.935±1.219 Cb | 75.03±4.772 Ba |
| | 42 day | 23.98±1.806 Ca | 15.97±1.189 Da | 73.41±1.491 Ab | 36.38±1.583 Ba |
| | 42 day | 23.28±1.430 Ba | 5.038±1.058 Cb | 92.96±2.213 Aa | 25.31±1.129 Bb |

Values denote Mean ± SD of 10 observations. Different uppercase letters denote the significant (p<0.05) difference between groups (for each period), and different lowercase letters denote the significant (p<0.05) difference between the two time points (for each group).

Molecular Analysis

Pituitary Fshβ And Lhβ Genes

At both experimental periods, the expression levels (fold

changes) of pituitary FSHβ and LHβ genes of Af showed no significant (p>0.05) difference in comparison with control, whereas that of Cm and CmAF groups revealed significant increase (p<0.05), where that of Cm group was significantly higher (p<0.05) than that of CmAf group. When comparing the two periods for each group, only Cm group recorded significant elevation (p<0.05) at 42 period than 21 period, whereas other groups showed no significant differences (p>0.05) between the two periods (Figure 2).

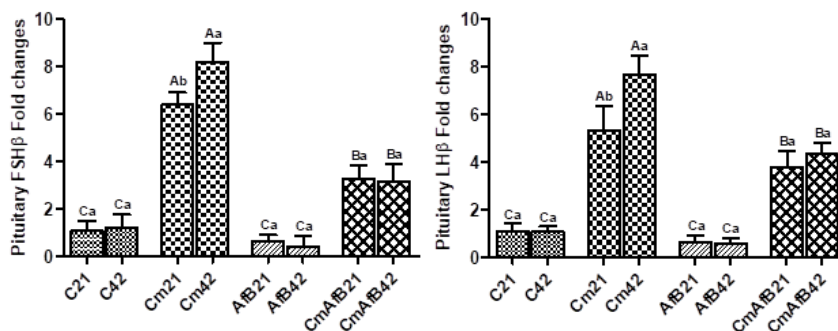


Figure 2: Effect of camel milk on the expression levels of pituitary FSHβ and LHβ genes in Af-treated male rats.

Values denote Mean ± SD of 10 observations. Different uppercase letters denote the significant (p<0.05) difference between groups (for each period), and different lowercase letters denote the significant (p<0.05) difference between the two time points (for each group).

Testicular FSHR and LHR

At both experimental periods, the expression levels (fold changes) of testicular FSHR gene of Af group showed no

significant (p>0.05) difference and LHR showed significant decrease (p<0.05) in comparison with control and LHR gene, whereas Cm and CmAF groups revealed significant increase (p<0.05), where that of Cm group was significantly higher (p<0.05) than that of CmAf group. When comparing the two periods for each group, Cm and CmAf groups recorded significant elevation (p<0.05) at 42 period than 21 period, whereas control and Af groups showed no significant differences (p>0.05) between the two periods (Figure 3).

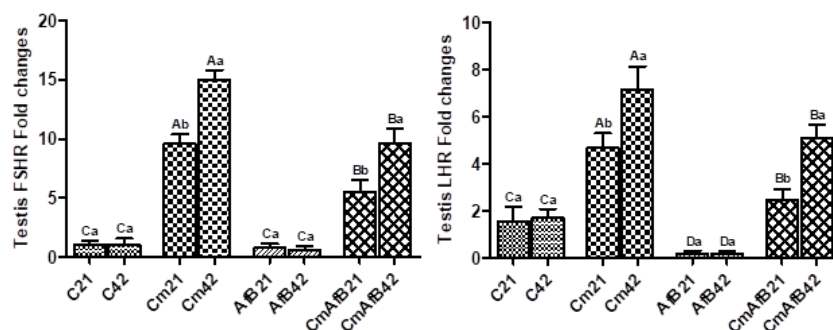


Figure 3: Effect of camel milk on the expression levels of testicular FSHR and LHR genes in Af-treated male rats.

Values denote Mean \pm SD of 10 observations. Different uppercase letters denote the significant ($p < 0.05$) difference between groups (for each period), and different lowercase letters denote the significant ($p < 0.05$) difference between the two time points (for each group).

Fertility index

As illustrated in figure (4), the offspring number of female rats that mated with Cm group male rats was the highest ($p < 0.05$) and those mated with AF was the lowest ($p < 0.05$) among experimental groups. As for group CmAf, the number was significantly higher ($p < 0.05$) than that of Af group and

significantly lower ($p < 0.05$) than that Cm and control groups.

Duration of pregnancy

The pregnancy duration of female rats that mated with control Cm male rats showed no significant difference ($p > 0.05$), which was significantly higher ($p < 0.05$) than that of the females mated with Af and CmAf male rats, where CmAf group recorded significant increase ($p < 0.05$) than that of Af group (Figure 4).

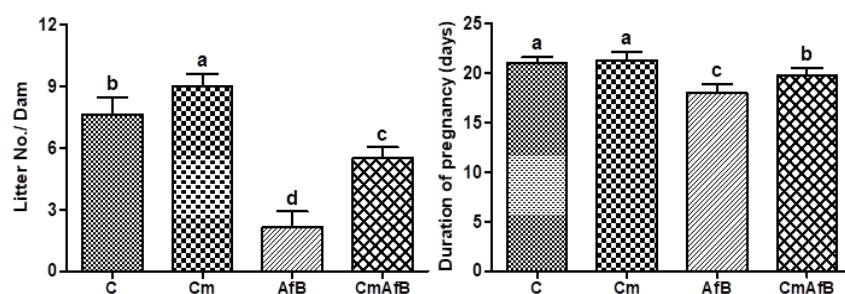


Figure 4: Effect of camel milk on the litter number and duration of pregnancy in female rats mated with Af-treated male rats. Values denote Mean \pm SD of 10 observations. Different uppercase letters denote the significant ($p < 0.05$) difference between groups (for each period), and different lowercase letters denote the significant ($p < 0.05$) difference between the two time points (for each group).

DISCUSSION

The AFB1-treated rat levels of FSH were significantly reduced. Using AFB1 lowered FSH concentrations in rats, as documented by Owumi *et al* (7), which is in agreement with our results. Sex hormone levels and sperm production in animals are controlled by the hypothalamus pituitary testis axis (HPTA). The introduction of AFB1 contributed in considerably reduced blood levels of pituitary hormones, including LH, FSH, prolactin, and testosterone, as a consequence of the toxin's negative effect on the

reproductive axis. Testicular steroidogenesis is suppressed by AFB1, and this has been connected to its toxicity to Leydig cells as indicated by previous studies (13).

AFB1's deleterious effects on spermatogenesis also was shown by the functioning of enzymes essential for sperm synthesis in testicles. When AFB1 is administered to rats, the activity of the testicular lactate dehydrogenase (LDH) and the glucose-6-phosphate (G6PD) enzymes in the spermatogenic cells are decreased, which implies alterations in the activities of spermatogenic cell metabolism of Lactate and NADPH synthesis, which are important for the sperm

production. When camel milk was administered in combination with AFB1 treatment, the concentrations of

FSH were adjusted to a better degree than in the AFB1-only treated animals. These results may imply that NADPH and lactate metabolism have been corrected (14).

After providing AFB1, the LH levels significantly decreased. The HPTTA seems to have been disrupted by the AFB1. AFB1 has been shown to suppress LH in adult rats and nursing buffalos, and our findings support that finding (14,15). Also in accordance with the present study are the findings of Owumi *et al* (7) who found reduced levels of LH in rats given AFB1. The toxic effects to the LH was repaired when camel milk was used in combination with AFB1, and LH concentrations were increased to a higher degree than those in the AF group. This might be because the ingredients of camel milk eliminated the AFB1 as a disrupting factor to hormones and enzymes. According to Mohamed *et al* (16), camel milk prevented and recovered male rats from the effects of fenprothrin, a synthesized pyrethroid.

AfB1 exposure decreased the testosterone levels in the rats of the AFB1 group. Low testosterone concentrations have also been linked to AfB1, according to some research, as the duration of exposure is prolonged, these effects may become more prominent (17). Testosterone concentrations in the rats given camel milk recovered to increased amounts than those in the AF group, suggesting the milk had favorable effects on testosterone concentrations. As a result of the CM's elevated antioxidant micronutrients, minerals, and other elements, the serum antioxidant enzymatic components also returned to their normal levels (18).

The lower sperm motility that was shown in the males treated with AfB1 is in agreement with Supriya *et al* (19) results. The sperm motility is highly dependent on the availability of energy, as variety of functions are carried out by mitochondria, including ATP production, the synthesis of ROS, calcium signaling, and the induction of cell death. Systemic dysfunction, including infertility has been linked to changes in mitochondrial activity (20,21). A combination treatment group resulted in restoration of normal motility. Immobilization stress substantially decreased sperm count, sperm survival percentage, mass motility frequency, and antioxidant characteristics measured, as did previous research by Zakaria *et al* (8), who found an improvement of antioxidant capacity as a result of camel milk supplementation, however it didn't reach the control levels.

The higher levels of FSH β and LH β gene expressions, as well as the increased serum levels of FSH, LH, and testosterone Cm group male rats indicating a positive effect of camel milk. These alterations could be attributed to the high expression levels of obestatin gene (ghrl gene) in the gastric mucosal cells. Obestatin plays an important role in the control of the gonadotrophic axis activities because of its involvement in the complicated gut-brain neuro-hormonal networks (8). Therefore, camel milk might be modified the expression of this gene to high levels, since camel milk is

rich in proteins and peptides which can activate the effect of obestatin.

The AFB1 had no impact on the levels expression levels of all genes, except LHR. Because aflatoxin function as a genotoxic element; possibly after persistent exposure; therefore, the gene level remained unaffected. In the case of LHR, significant decline in its expression was seen in the current work. There are several beneficial components in the camel milk, including lactoferrin, vitamins, and zinc, which may be responsible for the increased gene expression (21).

The decreased number of litters delivered by females mated with the AFB1 treated males could be due to the poor quality of the semen, resulting in higher rates of non-conceiving females and high levels of congenital defects, abortions, and less duration of pregnancy. Males exposed to *A. flavus* invasion (22) or toxicants like tetra-chloro-di-benzo-p-dioxin (23) were shown to be impacted by substantial alterations in the composition of their semen. Camel milk, on the other hand, increased the frequency of born litters per female rat and restored normal duration of pregnancy. Zakaria *et al* (24) observed that camel milk enhanced testicular characteristics, such as testosterone, in males whose mothers had been subjected to intentional lead exposure during pregnancy (25,26).

CONCLUSION

The present study reveals findings that indicate that aflatoxin B1 is highly toxic to the reproductive system of male rats, as uncovered by the facts that serum concentrations of sex hormones were affected as is so for the fertility index of females mated with those affected males. The study, also indicates that camel milk may be helpful in the restoration of these alterations.

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