

## Biochemical and Histopathological Study on the Effect of *Nigella Sativa* and Curcumin on Aflatoxin Residues in Quails

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### Abstract

Current study investigated the effects of *Nigella sativa* (NS) and Curcumin on Aflatoxins (AFs) residues on some hepatic and renal functions in quails. A total 120 unsexed Japanese quails chicks one-week-old were equally divided for one month of experiment into 4 groups; G1, G2, G3 and G4. G1; was considered control group and was given AFs free diet, G2; was given 2.5 mg/kg diet AFs, G3; was given AFs 2.5 mg/kg diet and 1.5% NS and G4, was given 2.5 mg/kg diet AFs with Curcumin 400 mg/kg diet, respectively. Total cholesterol (TC), triacylglycerol (TG), high density lipoprotein- cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), creatinine, uric acid, and Aflatoxin residues were determined. Also the histopathological examination on liver was performed. TC, TG and LDL-c were significantly ( $P \leq 0.5$ ) increased in G2 than G1, while reduced in G3 and G4 than G2. However, HDL-c was significance ( $P \leq 0.05$ ) decrease in G2 than G1. While, increased in G3 and G4 than G2. Creatinine and uric acid were significantly ( $P \leq 0.5$ ) elevated in G2 than G1, while it was significantly ( $P \leq 0.5$ ) decreased in G3 and G4 than G2. AFs residues in liver tissue was significantly ( $P \leq 0.05$ ) elevated in G2 than G1 and significantly ( $P \leq 0.05$ ) reduced in G3 and G4 than G2. This study indicated that Addition of NS and Curcumin to quails diets; improved via modulating lipid profile, creatinine, uric acid and reduction of AFs residues with respect to hepatic and renal function.

**Key words:** quails, AFs, *Nigella sativa*, Curcumin, liver, Lipid profile

### Introduction

Stored foods could be contaminated by toxigenic fungi and mycotoxins secreted by them. Antimicrobial and

mycotoxin inhibitory are needed for control measures to ensure the safety of stored foods (*Prakash et al., 2015*). Where in tropical and

subtropical areas are mostly chronic due to their favorable environmental conditions for mold growth and toxin production (*Prakash et al., 2012*).

Aflatoxins are common contaminants of field crops like corn and nuts, AFs are considered the etiology of acute and chronic forms of lotoxicosis in consumers (*Elmore et al., 2014*). They are produced from the fungus *Aspergillus* (*Bankole and Adebajo, 2003 ; WHO, 2000*). AFs are various and approximately 20 related fungal metabolites primarily produced by the *Aspergillus flavus* and *Aspergillus Parasiticus* (*Cortés et al., 2010; Thrasher and Crawley, 2009*). They are classified as a class one carcinogen by The World Health Organization (*WHO, 2000*). AFs and its metabolites accumulate in animal tissues after AFs consumption (*Deng et al., 2010*) and their residues remain inside animal tissues and cause health impairments (*Boonyaratpalin et al., 2001; El-Sayed and Khalil, 2009*) revealed that residues of AFs. So, AFs contamination is considered a critical threat on animal's health especially with the increasing of using plant ingredients in animals feed (*Agag, 2004*). The AFs induce hepatotoxic effects as hepatomegaly, histopathological alteration and inhibition of enzymes activity due to elevation of AFs residue in liver .The highest levels of AFs residue had been found in

the liver, followed by the muscle and gizzard tissue (*Bintvihok and Kositcharoenkul, 2006*). The liver rather than the muscles was observed to be the main targeted organ in aflatoxicosis (*Deng et al., 2010*).

*Nigella sativa* L. (Ranunculaceae) may called as 'NS', is an erect herbaceous annual plant. NS contains both fixed, essential oils, proteins, alkaloids and saponins. Much of the biological activity of the NS is due to thymoquinone, the major component of the essential oil. The fixed oil is composed mainly of unsaturated fatty acids (*Piras et al., 2013*). NS has an inhibitory effect on growth and AFs production by *Aspergillus Parasiticus* (*Al-Qurashi et al., 2007*), the inhibitory effect of NS on AFs production ability of *Aspergillus flavus* might be related to several components have some biological activities, as  $\alpha$ -pinene and thymol and high phenolic content (*Shakya and Sallal, 2007*). After storage thymoquinone produces di-thymoquinonene and higher oligo condensation products, which provides the spice with its aromatic flavor (*Nickavar et al., 2003*).

Many times ago, Curcumin is considered one of the spices, it uses in preservation of food through its antioxidant mechanism, coloring agent in food as it's yellow dye for textiles. It also has a therapeutic agent in traditional Indian medicine to treat a wide variety of diseases,

with no known side effects (*Duke, 2002; Chattopadhyay et al., 2004*). Many researches in the second half of the 20<sup>th</sup> century have identified Curcumin has the responsible for biological activity of turmeric (*Aggarwal et al., 2007*). Curcumin had a protective effect on liver tissues, as it showed potential effect against bacteria, fungi and cancer (*Hatcher et al., 2008*). Moreover, it is appeared the safest with oral administration even at high doses, as proven in various animal experiments (*Duvoix et al., 2005*). The pharmacological properties of curcumin make it a powerful compound for treatment and prevention of a wide range of animal diseases (*Maheshwari et al., 2006*).

#### **Materials and methods:-**

##### **Experimental birds and management:**

A total number of 120, one- week-old apparently healthy unsexed Japanese quails obtained from Agricultural Technological Center, Faculty of Agriculture, Cairo University, Giza, Egypt. Chicks were kept for one week to acclimatize, in the Lab Animal House of Faculty of Veterinary Medicine, Suez Canal University, before the start of the experimental period that was continued for 30 days of experiment. The chicks randomly divided into four groups of average body weight (30.5 g). Chicks were housed in wire battery cages (86×50×25 cm) and equally

partitioned into three pens (29×50×25cm) according to *Hassan et al., (2003)*. The batteries were contained the feeders and drinker equipments, the chicks were allowed *ad-libitum*, free access to feed and water. Ventilation and temperature (22°C-31°C), were controlled electrically with 24 hours of lighting.

##### **Basal experimental diet:**

Diet was formulated to fulfill the nutritional requirements of growing quails and according to *NRC, (1994)*, that contained 24% CP and 2900 kcal ME/kg. Fresh feed were mixed weekly. Neither antibiotic growth promoters nor anti-coccidials were added to any one of the experimental diets.

##### **Standard toxigenic strains:**

Toxigenic strain of *Aspergillus parasiticus* NRRL 2999 (ATCC) were obtained from Mycology Department, Animal Health Research Institute (AHRI), Doki, Egypt. AFs were prepared on rice according to *Shotwell et al., (1966)*. AFs levels in rice powder were measured by HPLC in the Mycotoxins Central Lab and Food Safety of the National Research Center, Doki, Egypt. According to *Nabney and Nesbit, (1965)*. Milled rice was added to the basal diet to provide 2.5 mg AF /Kg diet (2500 ppb) (*Eraslan et al., 2004*).

##### **Treatment material**

**Nigella sativa seeds** were purchased from Isis company, Egypt. They were crushed and added freshly every week to the feed in a dose rate 1.5%

(*Tahan and Bayram, 2011*). **Curcumin powder** ( $C_{21}H_{20}O_6$ ) was purchased from research Lab Company, India. And added to the diet in a dose rate 400 mg/kg diet (*Tarasub et al., 2012*).

#### **Blood sampling:**

Blood samples were collected from overnight fasting 45 days old quails by slaughtering the birds from each group. They were collected into a clean and dry screw capped centrifuge tubes without anticoagulant and left to clot at room temperature, then centrifugated at 3000 rpm for collection of clear serum. Sample used for the biochemical analysis of serum lipids profile, creatinine and uric acid.

#### **Serum lipid profile assay:**

The levels of serum total cholesterol (TC), triacylglycerol (TG) and high-density lipoprotein cholesterol (HDL-c) were measured using enzymatic calorimetric kits (ELITech Diagnostic, France) according to *Ellefson and Caraway, (1976)*, *Fossatiet al., (1980)* and *Lopez-Virella et al., (1977)*, respectively. Serum low density lipoprotein cholesterol (LDL-c) *Friedewald et al., (1972)* was measured using enzymatic calorimetric kits (QCA Co., Spain) following the instructions of the corresponding reagent kit (*Galler et al., 2007*).

#### **Serum creatinine and uric acid:**

The levels of serum creatinine and uric acid, were measured using

commercial kits (Diamond, Egypt), following the instructions of the corresponding reagent kit and according to (*Murray, 1984*).

#### **Aflatoxins residues:**

Liver samples were collected immediately after sacrificing the quails from each group, then washed in normal saline and stored at  $-80\text{ }^{\circ}\text{C}$  until use. The AFB1 in the tissues was extracted, purified using an immuno-affinity column and estimated by a HPLC-fluorescent detection method with pre-column derivatization (*Tavčar-Kalcher et al. 2007* and *Hussain et al., 2010*).

#### **Histopathological examination:**

Part of each segment of liver were taken immediately after slaughter and fixed in 10% formalin saline for 24 hours. Routine histological laboratory methods including dehydration, clearing and paraffin embedding were applied. Then, 5- $\mu\text{m}$  thickness sections were stained with the standard hematoxylin and eosin method (*Gridley, 1960*).

#### **Statistical analysis:**

All collected data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 16, USA) (*Coakes et al., 2009*). Differences between means of different groups were carried out using one way ANOVA followed by Duncan Multiple Range tests. Differences were to be significant at ( $P \leq 0.05$ ).

**Table (A):** Composition of quail experimental diet

Ingredient	Concentration (kg/100 kg diet)
Ground yellow corn	55.780
Soya bean meal	31.960
Fish meal	1.000
Corn gluten	7.450
Bran	1.000
Dicalcium Phosphate (22% Ca & 19% P)	0.710
Limestone (38% Ca)	1.300
Lysine (purity 98%)	0.170
DL – Methionine (purity 98%)	0.070
Iodized sodium chloride	0.300
Mineral & Vitamin premix	0.300

**Table (1):** Aflatoxins content in milled rice.

Sample	AFs (mg/kg)				
	B1	B2	G1	G2	Total
AFs level in rice	4.65	0.02	0.08	0.06	4.81

**Experimental Design:**

Groups	No of quail	Age of quail/week	AFs 2.5mg/kg/diet	NS 1.5%	Curcumin 400mg/kg/diet	Time of experiment
G1	30	2	-	-	-	30 days
G2	30	2	+	-	-	30 days
G3	30	2	+	+	-	30 days
G4	30	2	+	-	+	30 days

Table keys: + = treated - = non treated

**Results:-**

Table (1) demonstrated that TC and TG were significantly ( $P \leq 0.05$ ) elevated in G2 and G4 than G1. However, HDL-c showed significantly ( $P \leq 0.05$ ) decreased in G2 and G4 than G1. Although, LDL-c was significantly ( $P \leq 0.05$ ) increased. TC and TG showed a significant ( $P \leq 0.05$ ) reduction in G3 and G4 than G2. However, HDL-c was significantly ( $P \leq 0.05$ ) increased in G3 and G4 than G2.

LDL-c showed significant decrease in G3 and G4 ( $P \leq 0.05$ ) reduction when compared to the G2.

G2 induced a significantly ( $P \leq 0.05$ ) increase in creatinine and uric acid concentration than G1. While creatinine and uric acid concentration were significantly ( $P \leq 0.05$ ) decreased in G3 and G4 than G2 (Table1).

Table (1) demonstrated that there was no AFs residues detected in G1. AFs residues revealed significant

( $P \leq 0.05$ ) increase in G2 than G1. While G3 and G4 were significantly ( $P \leq 0.05$ ) increased when compared with G2.

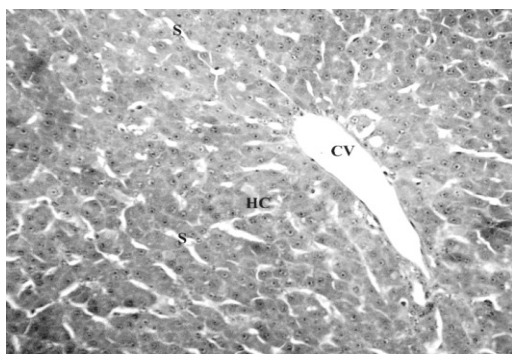
Liver histopathology showed that G2 displayed sever macrovesicular

fatty degeneration and ballooning degeneration of the hepatocytes Fig. (2), while the Liver of G3 and G4 showed normal liver architecture (Fig. 3 & 4).

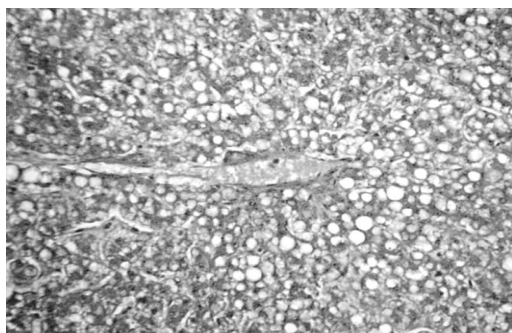
**Table (1):** Effect of dietary aflatoxin on serum concentration of TC, TG, HDL-c and LDL-c, Creatinine, Uric acid and Tissue Aflatoxin residues.

Groups	G1	G2	G3	G4
TC (mg/dl)	154.35±6.01 <sup>c</sup>	236.54±15.83 <sup>a</sup>	155.01±1.43 <sup>c</sup>	197.80±14.50 <sup>b</sup>
TG (mg/ dl)	110.72±2.97 <sup>c</sup>	181.68±5.75 <sup>a</sup>	121.30±2.57 <sup>c</sup>	153.59±1.46 <sup>b</sup>
HDL-c (mg/ dl)	103.60±2.60 <sup>a</sup>	55.68±2.97 <sup>c</sup>	96.60±4.94 <sup>a</sup>	70.20±1.56 <sup>b</sup>
LDL-c (mg/ dl)	28.61±5.31 <sup>c</sup>	144.57±14.34 <sup>a</sup>	34.16±6.3 <sup>c</sup>	96.88±15.33 <sup>b</sup>
Creatinine (mg/ dl)	0.28±0.02 <sup>d</sup>	6.18±0.66 <sup>a</sup>	1.74±0.06 <sup>c</sup>	3.46±0.36 <sup>b</sup>
Uric acid (mg/ dl)	2.09±0.03 <sup>c</sup>	6.78±0.55 <sup>a</sup>	3.43±0.25 <sup>b</sup>	4.08±0.07 <sup>b</sup>
AF residues (ppp)	0.00±0.00 <sup>d</sup>	9.00±0.57 <sup>a</sup>	3.00±0.57 <sup>c</sup>	6.00±0.57 <sup>b</sup>

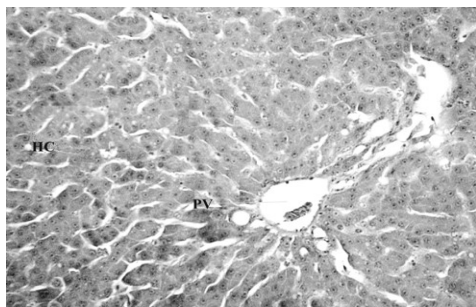
Values are means SE. means in the same row with different superscripts differ significantly at ( $P \leq 0.05$ ).



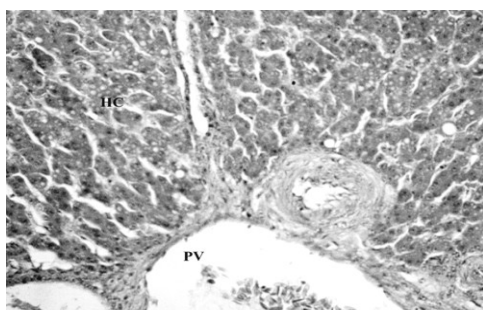
**Fig. (1):** Liver of control gr. displayed normal architecture of central area. showing central vein (CV), cords of hepatocytes (HC) and sinusoids (S). H&E. magnification (200x).



**Fig. (2):** liver of Aflatoxin treated group at a dose 2.5 ppm showed sever macrovesicular (arrow) fatty degeneration and ballooning degeneration of the hepatocytes. H&E.magnification (200x).



**Fig. (3):** liver of NSgr. received daily single oral dose of NS at a dose 1.5 % showed normal liver architecture H&E.magnification (200x).



**Fig. (4):** liver of Curcumin gr. showed Normal histological appearance of the liver. H&E. magnification (200x). (HC) hepatic cord, (PV) portal vein.

### Discussion

AFs contaminated diets are considered a worldwide issue especially in poultry farms. AFs produces toxin on both living and stored plants especially in high moisture environment, AFs is produced mainly by toxigenic fungi (genus *Aspergillus*, particularly *A. flavus* and *A. parasiticus* (*Huwig et al., 2001* and *Verma, 2004*)). AFs makes many problems to animals that it may lead to death sometimes, which causes severe losses to the poultry farm's worldwide (*Mariam et al., 2013*). The main clinical signs of AFs toxicity are chronic in nature, including impaired liver function, lower growth rate, loss of body weight, increased disease susceptibility, internal organ dysfunction and increased mortality (*Santacroce et al., 2008*).

Results of the current study, revealed a significant ( $P \leq 0.05$ ) elevation in serum TC and TG in G2 when compared with G1. On the other hand, significant ( $P \leq 0.05$ ) decrease in HDL-c in G2 than G1. Although, a significance ( $P \leq 0.05$ ) increase in LDL-c in G2 when compared with G1, these finding are in agreement with the results of *Madheswaran et al., (2004)*; *Kasmani et al., (2012)*. While TC and TG were significantly ( $P \leq 0.05$ ) reduced in G3 and G4 when compared by G2. However, showed significant ( $P \leq 0.05$ ) increase in G3 and G4 in HDL-c when compared to G2 but, showed significant ( $P \leq 0.05$ ) increase in G3 and G4 in LDL-c than G2 similar observation were recorded by *Ayoub et al., (2011)* and *Arafa , (2005)* Table (1). *Kaneko et al., (1997)*. Hypercholestermia in serum of G2

might be attributed to the hepatic damage observed in this study, as liver is the main organ of cholesterol metabolism, also AFs induces hepatic hyper-lipemia (*Huff et al., 1986*).

The target organ of AFs toxicity is the liver where it turns into reactive forms that bind to mitochondrial DNA leading to liver damage (*Yunus et al., 2011*). Aflatoxicosis causes depletion in HDL-c and elevation in LDL-c, due to inhibition of protein biosynthesis in liver tissues (*Wu et al., 2009*). The decrease in plasma cholesterol level in G3 may be attributed to the high content of NS from unsaturated fatty acids which may stimulate the cholesterol excretion into the intestine and the oxidation of cholesterol to bile acids (*Khodary et al., 1996*). The mechanism by which Curcumin decreased serum cholesterol and TAG in previous study are not known. There is theory that Curcumin decrease blood cholesterol due to inhibition of its absorption in the diet, while it was noticed that it cause slight increase in serum HDL-c (*Arafa, 2005*). Curcumin suppressed LDL-R receptor gene in activated liver cell so it decrease LDL-c level in serum (*Kang and Chen, 2009*). Current results pointed that creatinine and uric acid levels in G2 were significant ( $P \leq 0.05$ ) increase than G1. However a significance ( $P \leq 0.05$ ) decrease occurred in G3 and G4 in compared with G2. The elevation in creatinine and uric acid

in G2 is confirmed by *Selim et al., (2014)*, while the reduction in G3 and G4 are coincided by *Al-Ghasham et al., (2008)*. Due to decrease the utilization of phosphocreatinine in contraction of muscle that leads to elevation of transformation of phosphocreatine into creatinine which also leads to increase of creatinine level in animal's serum, histopathological changes in aflatoxicated kidney support that opinion (*Soliman et al., 2012*). Uric acid is the primary end product of protein metabolism in birds. It is synthesized in the liver and excreted through the kidney tubules. Increased blood uric acid concentrations along with increased urea and creatinine suggest impaired kidney function in both birds and mammals (*Hochleithner, 1994*). NS protects the liver and kidney from AFs and its free radical reaction (*Mona et al., 2002*). Curcumin administration showed a significant ( $P \leq 0.05$ ) decrease in the levels of serum creatinine and uric acid. The curative effect of curcumin on the kidney markers can be attributed to its antioxidant property as it has been found that reactive oxygen species may be involved in the impairment of glomerular filtration rate (GRF) (*Hughes et al., 1996*).

The present study showed that there was a significant ( $P \leq 0.05$ ) increase in AFs residues in G2 than G1. While, a significant ( $P \leq 0.05$ ) reduction in those G3 and G4 **Table (1)**. The increase in AFs residues in



G2 is confirmed by *Selim et al., (2014)*, although the reduction in G3 and G4 are in according to *El-Nagerabi et al., (2012)* ; *Reddy et al.,(2009)*, respectively. Animal tissues can retain AFs residues leading to suspected public health risks due to human consumption of contaminated food (*Puschner, 2002* and *Murjani, 2003*). Prolonged exposure to AFs produced accumulation of toxic residues in liver tissues (*El-Sayed and Khalil, 2009*). AFs residues were detected in the liver after exposure to even smaller doses of toxin (less than 2 mg/kg) (*Deng et al., 2010*). *Abdelhamid et al., (2004)* reported that AFs residues in flesh showed a cumulative effect based on the dietary AFs exposure level and duration. The inhibitory effect of *Nigella sativa* on AFs production ability of *Aspergillus* might be related to several components known to have biological activities, such as  $\alpha$ -pinene and thymol and high phenolic content (*Shakya and Sallal, 2007*). The inhibition of the ternary steps of AFs biosynthesis involving lipid peroxidation and oxygenation is the mechanism of Curcumin action in AFs production inhibition (*Ferreira et al., 2013*).

This theory goes in agreement with our histopathological results as liver tissue of AFs treated quails revealed severe macrovesicular fatty degeneration and ballooning degeneration of the hepatocytes **Fig. (2)**. These results were supported with the results of *Miazzo et al.,*

*(2005)* ; *Ibrahim, (2013)*, Meanwhile, the liver of G3 and G4 showed normal liver architecture that's according to *Ayoub et al., (2011)* and *Sharma et al., (2011)*, respectively.

AFs toxic metabolite's combine with nucleic acids and nucleoprotein, which is necessary to cell bioactivity, lead to formation of hepatic lipids and hepatomegaly. In high dose exposure of AFs which causes liver destruction due to accumulation of fat droplets as a clear vacuoles in hepatocyte's cytoplasm (*Ibrahim, 2013*). Liver's lymphoid aggregation in the sixth week are induced by NS then end by total liver regeneration by the eighth week (*El-Gazzar et al., 2006*). *El-Agamy, (2010)* demonstrate the potential beneficial effects of Curcumin to counteract the oxidative stress induced by long-term AFs administration. Curcumin by its ability to scavenge free radicals, interacting with oxidative cascade, quenching oxygen radicals, inhibiting oxidative enzymes and chelating metal ions inhibits lipid peroxidation and restores the antioxidant status (*Wei et al., 2006*).

## Conclusion

It could be concluded that, addition of *Nigella Sativa* and Curcumin to AFs contaminated diets of quails had hepatoprotective and renoprotective effects. They exerted their effect through improving serum lipid profile, creatinine and

uric acid beside lowering the AFs residues in the Liver.

#### Acknowledgments:

My great thanks for **Dr. Mohammed S. Yusuf**, lecture of Nutrition and Clinical Nutrition, Faculty of Vet. Medicine, Suez Canal University for his help and effort with me. I feel also it duty extend my sincere gratitude to **Dr. Hend M. Tag**, Lecturer of Zoology dept. Faculty of science, Suez canal university, who has given me many useful histopathological comments.

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## الملخص العربي

### دراسة كيميائية حيوية وهستوباثولوجية عن تأثير حبة البركة والكرم علي بقايا الأفلاتوكسن في السمان

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أجريت هذه التجربة لدراسة تأثير حبة البركة والكرم علي بقايا الأفلاتوكسن علي بعض وظائف الكبد والكلبي في السمان. تم تقسيم عدد ١٢٠ من السمان الياباني غير محدد الجنس بالتساوي الي ٤ مجموعات: مجموعة ١، مجموعة ٢ مجموعة الأفلاتوكسن ٢,٥ ملجم/كجم عليقة، مجموعة ٣ مجموعة الأفلاتوكسن ٢,٥ ملجم/كجم عليقة مضافا لها حبة البركة ١,٥% ومجموعة ٤ مجموعة الأفلاتوكسن ٢,٥ جزء من المليون مضافا اليها الكرم ٤٠٠ ملجم / كجم عليقة، بالترتيب. و استمرت التجربة لمدة شهر. تم قياس الكوليستيرول الكلبي، الجليسيريدات الثلاثية والبروتينات الدهنية العالية الكثافة، البروتينات الدهنية قليلة الكثافة، الكرياتينين، حمض اليوريك وبقايا الأفلاتوكسن. أيضا تم عمل فحص هستوباثولوجي للكبد. كان هناك زيادة معنوية في الكوليستيرول الكلبي، الجليسيريدات الثلاثية والبروتينات الدهنية قليلة الكثافة في مجموعة الأفلاتوكسن عن المجموعة الضابطة، بينما كان هناك تحسن معنوي في مجموعتي حبو البركة والكرم عن مجموعة الأفلاتوكسن. بالرغم من أنه كان نقص معنوي في البروتينات الدهنية عالية الكثافة في مجموعة الأفلاتوكسن عن المجموعة الضابطة، بينما كان هناك زيادة معنوية في مجموعة حبة البركة والكرم عن مجموعة الأفلاتوكسن. و أظهرت التجربة أيضا زيادة معنوية في الكرياتينين وحمض اليوريك في مجموعة الأفلاتوكسن عن المجموعة الضابطة، بينما أوضحت نقص معنوي في مجموعة حبة البركة والكرم عن مجموعة الأفلاتوكسن. مجموعة الأفلاتوكسن أظهرت زيادة معنوية في بقايا الأفلاتوكسن في خلايا الكبد عن المجموعة الضابطة. هذه الدراسة أوضحت أن إضافة حبة البركة والكرم الي علائق السمان؛ يحسن ويعزز صحة الدواجن عن طريق تحسين صورة الدهون، الكرياتينين، حمض اليوريك وتقليل بقايا الأفلاتوكسن مع ما يتعلق بالهستوباثولوجي للكبد.