

Efficacy of Banana Peel in Reduction of Aflatoxin Toxicity in Rats

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ABSTRACT

An experiment was conducted to determine the efficiency of banana peel to reduce the toxic effect of aflatoxins (AFS) in male albino rats. Seventy eight male albino rats were assigned to 13 experimental diets. The first one served as control received basal diet only, the second served as control DMSO (basal diet and 5ml DMSO /kg B.W.) daily, but the third and fourth one were received 1mg and 2mg AFs/ml respectively. The three following groups were received basal diet only supplemented with banana peel in percentage of 1,2 and 3%, then three groups received basal diet and orally (1mg AFS suspended in 5ml DMSO /kg B.W.) low dose of aflatoxins with the same levels of banana peel. The last three groups received basal diet and orally (2mg AFS suspended in 5ml DMSO /kg B.W.) high dose of aflatoxins with the same levels of banana peel. The duration of this experiment was 6 weeks. Results showed that, albino rats received 38mg and 76mg/kg aflatoxins revealed a significant elevation in the serum levels of AST, ALT, ALP, creatinine, urea, cholesterol and triglyceride, while decrease in total protein (TP) and albumin. Results of hematological parameters showed decrease in red blood cells (RBCs), hemoglobin (HB), packed cell volume (PCV), mean Corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) and platelets while increase in total leucocyte count (TLC). Body weights and organ weights were determined to study its changes. The treatments with banana peel can play a practical role in detoxification of aflatoxins in albino rats.

Keywords: Aflatoxins (AFS), Banana Peel (BP), Adsorption, Albino rats.

INTRODUCTION

About 50 years ago after an outbreak of Turkey X disease in England aflatoxins were discovered in *Aspergillus flavus* (Klich *et al.*, 2000). Aflatoxins (AFS) are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* (Romagnoli *et al.*, 2007 and Saladino *et al.*, 2016). Both fungal growth and aflatoxins production depend on a variety of physical, chemical, and biological factors (Bacaloni *et al.*, 2008). These metabolites have high toxicity including carcinogenic, teratogenic and mutagenic properties (Ali *et al.*, 2005; Cho *et al.*, 2007 and Saladino *et al.*, 2016). Among 20 types of aflatoxins, 4 types of AFs including B₁, B₂, G₁, and G₂ commonly occur in the natural environment (Bacaloni *et al.*, 2008 and Mallakian *et al.*, 2017). International Agency for Research on Cancer (IARC, 1993) and the world health Organization (WHO, 2000) were classified aflatoxin B₁ as Group 1 carcinogen (carcinogenic to humans). Aflatoxins especially AFB₁, AFG₁ and AFM₁ are the most toxic, naturally occurring carcinogens known with AFB₁ being the most hepatocarcinogenic compound, which caused various cancers of the liver and other body organs in humans and animals (INCHEM 1993; Kitya *et al.*, 2009; WHO, 2008; Beckingham, 2001 and Bujaidar *et al.*, 2015). Several methods were reported for the removal of mycotoxins from contaminated commodities, including physical separation, extraction with solvents and adsorption. Adsorption, a very common treatment of mycotoxin reduction, involves binding the toxin to absorbent compound during the digestive process in the gastrointestinal tract. A growing demand in developed countries for safer foods and cleaner production processes has been observed in recent times. The processing of agro product results in the formation of waste materials in high amount (Martin *et al.*, 2012) and commonly are in the form of peels, seeds and oilseed meals (Stanikova *et al.*, 2005), however accumulation of the wastes poses a problem to the environment as they are prone to microbial spoilage (Garcia *et al.*, 2006). Agro waste may be a source of high-added value products potentially useful as

beneficial food constituents, food flavors and antioxidants or as cosmetics, chemo preventive agents, drugs or drug adjuvants. Therefore, efforts have been made by researchers to explore possibility of reusing plant wastes as the source of organics. Agricultural wastes such as peel of various fruits and vegetables nowadays is applied in food and different industries (Mohamed *et al.*, 1994). Banana peels are agricultural waste that discarded all over the world as useless material; it causes waste management problems although they have some compost and cosmetics potentiality (Hossain *et al.*, 2012). Banana peel contains high potassium and phosphorus, which prove to be helpful in the compost; it could be used for medicine as well as personal care and known for anti-fungal and antibiotic properties, loaded with lot of vitamins, minerals and fiber that benefit for skin care and for healing the wound (Sakaltar, 2011), banana peels have absorbent potentiality (Hossain *et al.*, 2012). It has absorption capabilities for some elements and ions in liquid or solution, such as absorption capacities to remove chromium from wastewater (Memon *et al.*, 2008), copper (Hossain *et al.*, 2012) and also some dyes (Velmurugan *et al.*, 2011).

Banana peel is mainly composed of fiber and lignin whose percentage value changes for stages of maturity. It also contains 6-9% protein and 20-30% fiber (based dry matter). Green plantain peels contain 40% starch that is transformed into sugars after ripening. Green banana peels contain much less starch (about 15%) when green than plantain peels, while ripe banana peels contain up to 30% free sugars. It also contain lots of vitamins, minerals and fiber that has proved beneficial for skin care and healing the wound, they also have been used as a substrate for the production of fungal biomass. Besides medicinal properties it also possesses good natural adsorbent of heavy metals like chromium, copper and some dyes from wastewater because of it is very useful for purification and refining processes John B. *et al.*, (2017).

The aim of this research was the using of Banana peel as an agro waste for reducing the toxic effect of aflatoxins.

MATERIALS AND METHODS

1-Materials

All chemicals and standard aflatoxins(B1, B2, G1 and G2) were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA). Solvents were purchased from Merck (Darmstadt, Germany).

Aspergillus flavus strain used in this study was isolated from raw peanuts samples which collected from local markets.

Banana peels were obtained from local juice stores in Cairo, Egypt.

2-Methods:

Aflatoxins production and assays:

Preparation of media for *Aspergillus flavus* growth:

Two types of media were used in this purpose. The following are the components of each medium.

a) Potato-Dextrose-Agar Media (PDA):

This medium was prepared according to Shotwell *et al.* (1966) as follows Flask 1 contained: 20g Dextrose, 0.2g CaCO₃, 0.2g MgSO₄.7 H₂O, and 100 ml distilled water. Flask 2 contained: 15g agar and 400ml distilled water. Flask 3 contained: 200g potato (spilled and sliced) and 500ml distilled water. The contents of flask 3 brought shortly to 121°C in autoclave and filtrated through cheesecloth. The solution was brought up to original volume simultaneously. The agar in flask 2 was melted and the solution in flask 1 was heated to boiling. Contents of three flasks were mixed together and autoclaved for 15min. at 121°C.

b) Yeast Extract Sucrose Media (YES):

This medium yields high amount of aflatoxin especially B₁ and G₁ with *A. flavus*. It was prepared according to method of Davis *et al.* (1966) as follows: 20g yeast extracts, 200g sucrose, 10mg FeSO₄.7H₂O, 5mg ZnSO₄.7H₂O, 1mg MnSO₄.4H₂O, and 1litre distilled water all contents were dissolved, mixed and autoclaved for 15 min at 121°C.

Aflatoxin production:

Aspergillus flavus Inoculum was maintained on Potato Dextrose Agar (PDA) slant tubes for 7 to 21 days at 28 °C. The spores of tested organism (7-days old) were scraped by adding 3ml sterile distilled water to the surface growth on agar slant. An aliquot amount from the resulting spore suspension (1ml) added to conical flasks (2L) containing (1L) of yeast extract media. Mycelia mats after 10-day incubation at 30°C were broken with a glass rod and collected by filtration through filter paper. Culture filtrates (mother solution) were extracted with chloroform (1:2, v/v). (Abd El-Mageed, 1987). The chloroform extract was evaporated until obtaining dry film in rotary evaporator. Then the obtained dry film containing AFS was reconstituted with DMSO (Dimethyl sulfoxide) and used for quantitative analysis of AFS and then preparation of AFS doses.

Determination of aflatoxins concentration

The concentration of AFS in DMSO solution was determined using HPLC technique (Agilent 1200 Series U.S.A with column C18, Lichrospher 100 RP-18, 5µm×25cm) as follows: The mobile phase constituted of water: methanol: acetonitrile (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wavelengths for all aflatoxins were 362 and 460nm (Fluorescence detector), respectively (Roos *et al.*, 1997).

Preparation of aflatoxins dose for rat experiment

The DMSO containing AFS solution consisted of a mixture of aflatoxin B₁, B₂, G₁ and G₂ at a total concentration of 38mg AFS/ ml as a ratio of 8: 2: 4: 1, respectively was used to prepare 2 final concentrations of AFS, 1mg AFS /5ml DMSO (low dose) and 2mg AFS/5ml DMSO (high dose).

Preparation of Banana peels adsorbent

Peel was purchased from juice stores. Sun drying process was done within 3-4 days in an average temperature of 28°C. Then oven drying was done in a cabinet oven with air circulation at 60°C, for 16h. Whereas dried banana peels was refined by laboratory mill to pass a 1.0 mm-size to produce banana peels flour (Adejuitan *et al.*, 2008).

Analysis of banana peel:

The proximate analysis of banana peel for measuring the levels of protein using Dumas method AOAC (2012), crud lipid analyzed using Vogel's text book method, silica by AOAC (2005), total dietary fiber using Instrument instruction of ANKOM 2000 fiber analyzer, ash using AOAC (2005), cellulose, lignin, hemicellulose determined by AOAC (2012), magnesium, calcium, sodium and potassium were analyzed according to Analytical methods for ICP optima 2000 (Perkin Elmer) AOAC (2012),

Table 1. Composition (%) and calculated chemical composition (%) of the control diet

Ingredient g/kg	Basal diet %	Basal Diet supplemented with banana peel at levels		
		1%	2%	3%
		Casein	20	200
Sucrose	50	490	480	470
Starch	15	150	150	150
Fiber	05	50	50	50
Corn oil	05	50	50	50
Mineral mixture	3.5	35	35	35
Vitamin mixture	1.0	10	10	10
Choline Bi-Tartarate	0.2	02	02	02
DL-Methionine	0.3	03	03	03
Banana Peel	0.0	10	20	30
Total	100	1000	1000	1000

Animal experiment:

Seventy eight male albino rats weighing about 115±5g (provided by the Laboratory Animal Center, Faculty of Veterinary Medicine, Cairo University) were housed in stainless steel cages in animal house in Regional Center for Food and Feed, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt under controlled light and temperature conditions (12 hours light: dark cycle, 22 – 33

°C). During the acclimation period (1 week) and experimental period (6 weeks), the normal basal diet and tap water were supplied *ad libitum*.

Animal Diets

The standard diet for tested animals was formulated as shown in table (1) according to Pang *et al.*, (1992) and NRC, (1995).

Mineral mixture (g/Kg):- calcium phosphate dibasic 500.00; potassium citrate, monohydrate 220.00; sodium chloride 74.00; potassium sulfate 52.00; magnesium oxide 24.00; ferric citrate (16-17% Fe) 06.00; manganous carbonate (43-48 Mn) 03.50; zinc carbonate

1.60; chromium potassium sulfate 0.55; cupric carbonate (53-55% Cu) 0.30; potassium Iodate 0.01 and sucrose, finely powdered 118.03.

Vitamin mixture (g/Kg):- nicotinic acid or nicotinamide 3.000; calcium d- pantothenate 1.600; pyridoxine- HCL 0.700 ; thiamin- HCL 0.600 ; riboflavin 0.600 ; folic acid 0.200 ; d- biotin 0.020 ; cyanocobalamin vitamin B₁₂ (Vit. B₁₂) 0.001; retinylpalmitate or acetate Vit. A 400.000 IU; α -tocopheryl acetate (vitamin E) 5.000 IU; cholecalciferol Vit. D₃ 0.0025; menaquinone Vit. K 0.005 and sucrose, fine powdered to complete 1 Kg.

Experimental Design:

The study was carried out on seventy eight male albino rats weighing about 115±5g. Rats were divided into thirteen groups and treated for 6 weeks as follows:

- G1 Basal diet
- G2 Served as vehicles treated control+5ml DMSO/ kg B. W. daily
- G3 Received orally low dose of AF_S (1mg AFS suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday
- G4 Received orally high dose of AF_S (2mg AFS suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday
- G5 Received basal diet supplemented with banana peel at level 1%
- G6 Received basal diet supplemented with banana peel at level 2%
- G7 Received basal diet supplemented with banana peel at level 3%
- G8 Received orally low dose of AF_S (1mg AF_S suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday +1% banana peel
- G9 Received orally low dose of AF_S (1mg AF_S suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday +2% banana peel
- G10 Received orally low dose of AF_S (1mg AF_S suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday +3% banana peel
- G11 Received orally high dose of AF_S (2mg AF_S suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday +1% banana peel
- G12 Received orally high dose of AF_S (2mg AF_S suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday +2% banana peel
- G13 Received orally high dose of AF_S (2mg AF_S suspended in 5ml DMSO/ kg B.W.) twice/ week on Monday and Thursday +3% banana peel

Blood sampling

Blood samples were collected from orbital plexus of rat's eye under CO₂ anesthesia by fine capillary glass tubes in accordance with the method of Schermer (1967), at the end of experiment. Each sample was collected into two tubes, non- heparinized and heparinized. The non-heparinized blood samples were allowed to coagulate then centrifuged at 5000 r.p.m. for 6 min. Serum was removed using a Pasteur pipette into Wassermann tube and stored at -20°C for subsequent analysis of ALT, AST and ALP activities as well as TP, albumin, urea, creatinine, total cholesterol and triglycerides.

The heparinized blood samples were used as fresh as possible for determination of complete blood count (RBCs, HB, PCV, MCV, MCHC, MCH, TLC, Platelets).

Tissue sampling:

Rats were dissected at the end of experiment the liver and kidney were immediately removed and weighed.

Statistical Analysis

Statistical analysis for the collected data was done in the following procedure outlined by Gomez and

Gomez (1984). The treatment means were compared using the least significant difference test (LSD) at the 5% level of probability as outlined by Waller and Duncan (1969). Using the Duncan test institute program used a computer in the statistical analysis.

RESULTS AND DISCUSSION

Data presented in Table (2) showed the chemical composition of dried banana peel these parameters showed that banana peel rich in protein, lipid, fiber, ash and minerals. Mosa and Khalil (2015) concluded that supplementation diet with fresh or dried banana peel may be useful for acute liver failure patients, and it could be develop the retarded liver function and lipid profile.

The *in vivo* study 13 groups of albino rats were investigated for 6 weeks. At the end of the experiment blood samples were collected to estimate the blood parameters ALT, AST and ALP activities, as well as TP, albumin, creatinine, urea, cholesterol and triglyceride. These parameters were determined to assess the effect of aflatoxin on either absence or presence of banana peel on liver and kidney function of the tested animals.

Table 2. Proximate Analysis of Banana Peel:

Ingredient	Content (g/100g dry matter)
Protein	9.8
Crud lipid	6.24
Silica	1.2
Total dietary fiber	14.38
Ash	17.68
Cellulose	10.14
Lignin	15.38
Hemicellulose	2.64
Minerals	
Mn.	14.8
Ca.	4.9
Fe.	54.19
Na	186.0
K	8.71

Table 3. Serum biochemical activity of ALT, AST and ALP and concentration of total protein and albumin of rats fed on aflatoxin-contaminated diet supplemented with banana peel

Parameters Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	albumin(g/dl)
G1	51.66 ^D	121.66 ^D	448 ^{EF}	7.51 ^{AB}	4.27 ^A
G2	51.33 ^D	122.66 ^D	449 ^{EF}	7.55 ^{AB}	4.25 ^{AB}
G3	64.66 ^{CD}	154 ^{CD}	591 ^{BCD}	6.00 ^E	3.46 ^G
G4	100 ^A	254 ^A	684 ^A	5.81 ^E	3.40 ^G
G5	52 ^D	136.33 ^{CD}	436 ^F	7.75 ^A	4.29 ^A
G6	57 ^D	140 ^{CD}	460 ^{EF}	7.47 ^{ABC}	4.25 ^{AB}
G7	59.33 ^{CD}	149.66 ^{CD}	525 ^{DE}	7.16 ^{BCD}	4.18 ^{ABC}
G8	53 ^D	144.66 ^{CD}	383.3 ^F	7.05 ^{BCD}	4.07 ^{BCD}
G9	61 ^{CD}	147 ^{CD}	466.66 ^{EF}	6.99 ^{CD}	4.02 ^{CD}
G10	65.66 ^{CD}	152 ^{CD}	576.6 ^{CD}	6.96 ^{CD}	3.93 ^{DE}
G11	58 ^D	158 ^C	637.66 ^{ABC}	6.92 ^D	3.79 ^{EF}
G12	76.33 ^{BC}	168 ^C	668 ^{AB}	6.81 ^D	3.72 ^F
G13	91.33 ^{AB}	205 ^B	680 ^A	6.67 ^D	3.65 ^F
LSD	17.94	32.6	95.5	0.49	1.91

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at P<0.05, the least significant difference (LSD) was calculated at 95% confidence interval.

The main aflatoxin action mechanism is alteration in activity of serum biochemical parameters which indicated impaired functions, decreased activity or degenerative changes in particular organ producing the biochemical constituents. ALT, AST and ALP, lysosomal enzymes are present in high concentration within the hepatocytes; their detection in blood is usually considered as first sign of liver injury. These results are in agreement with those obtained by Bankole and Mabekoje, (2004), El-Bahr, *et al.*, (2015), Juma, *et al.*, (2015), Hussain, *et al.*, (2016), and Marwa, *et al.*, (2015).

Data revealed in table (3) that low and high doses of AFS (G3 and G4) caused a significant decrease in values of TP and albumin when compared with control group G1. The lowest values of TP and albumin were obtained at high dose of AFS in G4 (5.81 and 3.40g/dl respectively). There is no significant changes noticed in TP and albumin levels in rats treated with BP alone (1%, 2% and 3%) in all experimental periods corresponding to control group G1 (P<0.05). On the other hand supplementation of 1%, 2% and 3% banana peel in groups with low and high doses of AFS(G8 to G13) showed a significant increase in TP and albumin when compared to AFS control groups (G3 and G4).

Results in Table (3) clearly indicated that rats administrated the different two doses of AFS (1mg or 2mg/ B.W. twice /week) showed a significant increase in serum ALT, AST and ALP activities comparing with control group G1 and G2 (P<0.05). The highest values of ALT and ALP activities were 100 and 684 (U/L) which was obtained in G4 group of high dose of AFS. There is no significant changes noticed in ALT, AST and ALP activities in rats treated with BP alone (1%, 2% and 3%) (G5, G6 and G7) in all experimental periods corresponding to control group G1 (P<0.05). While the co- administration of BP with 1mg or 2mg of AFS (G8 to G13) resulting in minimizing toxic effect of AFS on their levels especially with low AFS dose comparing to AFS control group.

Simultaneous administration of rats with the three percentage levels of BP with low and high doses of AFS resulted in reduction of elevated activities of ALT, AST and ALP with elevation in levels of TP and albumin (comparing to groups treated by AFS alone and control group). The ameliorating effect of banana peel on liver function was obvious on low AFS dose. These results were in close relation with Chibanga, *et al.*, (2014) on broiler chicken as they found a reduction in the concentration of serum metabolites including total proteins and albumin. The decrease in serum protein observed might be due to less protein synthesis by injured liver and increased protein loss by injured renal tubules (Benjamin, 1978). These observations agreed by that of Shahat, *et al.*, (2017) who used ozone to reduce the toxicity of aflatoxinscontaminated soybean. Also El-Desouky *et al.*, (2017) studied the use of ozone gas to reduce and /or removal of AFB1.

Serum biochemical parameters data are presented in Table (4) Clearly indicated that rats treated by different two doses of AFS (1mg and 2mg/ B.W. twice /week) showed significant increase in serum levels of creatinine and urea comparing to that of control group(P<0.05). The increase of their levels was parallel to dose of AFS. The highest value of serum

creatinine was obtained on treatment with high dose of aflatoxin G4 (0.87 mg/dl). There is no significant changes noticed in, urea and cholesterol concentration in rats treated with BP alone (1%, 2% and 3%) in all experimental periods corresponding to control group G1 (P<0.05). Supplementation of 1%, 2% or 3% banana peel in groups with low dose AFS (G8, G9, and G10) showed a significant reduction in increased levels of urea, cholesterol, and triglyceride comparing with low AFS control (G3). Similarly, groups G11, G12, and G13 showed a significant reduction in serum levels of creatinine, urea, cholesterol, and triglyceride comparing with high AFS control (G4). These observations are agreed by that of Ayoub *et al.*, (2011); Abdel-Wahhab, *et al.*, (2016) and Sobhy, *et al.*, (2016) that used Jojoba

oil and Nigella cake protein as feed supplement. Urea is formed in the liver and represents the principal end product of protein catabolism. While creatinine is a metabolic byproduct of muscle metabolism. They are filtered from the blood and excreted in the urine by the kidneys. Results revealed that diets containing ≥ 5.0 mg AFB1/kg significantly increased the serum urea nitrogen compared to those fed the control determination has a reputation of being a more specific test for the diagnosis and prognosis of progressive renal disease than the serum urea nitrogen, as there are fewer non-renal factors that may influence creatinine. Same results obtained by Al-Masri, (2017) who found antioxidant activity of ascorbic acid against aflatoxin in contaminated nuts (almonds and walnuts) on rat.

Table 4. Creatinine, urea, cholesterol and triglyceride concentration in serum of rats fed on aflatoxin-contaminated diet supplemented with banana peel

Parameters	Creatinine	Urea	Cholesterol	Triglyceride
Groups	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
G1	0.69 ^E	33.33 ^E	38.66 ^F	56.33 ^G
G2	0.70 ^{DE}	34.00 ^E	39.00 ^F	56.66 ^G
G3	0.82 ^{AB}	46.00 ^A	57.66 ^{AB}	109.66 ^{AB}
G4	0.87 ^A	50.33 ^A	62.00 ^A	114.00 ^A
G5	0.65 ^E	35.00 ^{DE}	40.33 ^F	60.66 ^{FG}
G6	0.76 ^{BC}	35.6 ^{DE}	41.00 ^{EF}	69.00 ^{EF}
G7	0.76 ^{BC}	36.33 ^{CDE}	44.33 ^{DEF}	71.00 ^{DEF}
G8	0.77 ^{BC}	37.00 ^{BCDE}	48.66 ^{CDE}	75.66 ^{DEF}
G9	0.77 ^{BC}	39.30 ^{BCD}	50.60 ^{BCD}	80.66 ^{DE}
G10	0.78 ^{BC}	40.66 ^{BC}	50.66 ^{BCD}	86.33 ^{CD}
G11	0.78 ^{BC}	40.66 ^{BC}	52.00 ^{BCD}	86.66 ^{CD}
G12	0.79 ^{BC}	41.00 ^B	52.33 ^{BCD}	88.33 ^{CD}
G13	0.79 ^{BC}	41.33 ^B	53.66 ^{BC}	98.00 ^{BC}
LSD	0.05	4.49	7.98	15.51

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at P <0.05, the least significant difference (LSD) was calculated at 95% confidence interval.

The data in Table (5) represented the changes in hematological parameters in rats treated with the different two doses of AFS (1mg and 2mg/ B.W. twice/ week) all parameters (RBCs, HB, PCV, MCV, MCHC, MCH, and platelets) except TLC showed significant

decreased values. The decrease of their level was parallel to dose of AFS. While supplementation of BP to AFS groups (G8 to G13) resulting in minimizing toxic effect of AFS on comparing to AFS control groups (G3 and G4).

Table 5. Hematological parameters RBCs, HB, PCV, MCV, MCHC, MCH, and TLC in blood of rats fed on aflatoxin-contaminated diet supplemented with banana peel

Parameters	RBCs	HB	PCV	MCV	MCHC	MCH	TLC	Platelets
Groups	(x10 ⁶ cmm)	(g/dl)	(%)	(fl)	(%)	(pg)	(x10 ⁶)	
G1	6.50 ^A	39.1 ^{AB}	36.9 ^A	58.0 ^A	135.5 ^A	74.2 ^A	48.6 ^H	625 ^A
G2	6.55 ^A	39.6 ^A	37.1 ^A	57.6 ^A	136.0 ^A	74.5 ^A	49.0 ^H	627 ^A
G3	5.08 ^E	33.5 ^F	26.2 ^F	48.0 ^{FG}	108.0 ^F	56.2 ^{EF}	87.3 ^D	439.3 ^H
G4	4.63 ^G	25.0 ^I	13.5 ^I	45.0 ^I	62.2 ^I	36.2 ^H	172.5 ^A	277.6 ^J
G5	6.25 ^B	38.8 ^{AB}	33.6 ^B	54.6 ^B	134.1 ^A	72.6 ^A	57.2 ^G	582.3 ^B
G6	5.91 ^C	38.1 ^{BC}	33.0 ^B	52.6 ^C	133.2 ^A	71.6 ^{AB}	62.3 ^{FG}	558.6 ^C
G7	5.54 ^D	37.2 ^{CD}	31.1 ^C	52.6 ^C	132.4 ^A	68.8 ^B	65.8 ^F	545.6 ^{CD}
G8	5.49 ^D	37.1 ^{CD}	29.5 ^D	52.0 ^{CD}	128.1 ^B	65.4 ^C	67.0 ^F	531.0 ^{DE}
G9	5.12 ^E	36.4 ^D	28.7 ^{DE}	50.6 ^{DE}	124 ^C	62.3 ^{CD}	72.5 ^E	519.0 ^{EF}
G10	5.09 ^E	35.0 ^E	28.0 ^{DE}	49.3 ^{EF}	119.9 ^D	59.3 ^{DE}	75.5 ^E	503.3 ^F
G11	5.01 ^{EF}	34.0 ^{EF}	27.4 ^{EF}	49.0 ^{EF}	111.8 ^E	58.6 ^E	83.6 ^D	464.3 ^G
G12	4.89 ^{EF}	29.7 ^G	22.5 ^G	47.3 ^{GH}	100.8 ^G	54.3 ^F	95.0 ^C	424.0 ^H
G13	4.79 ^{FG}	26.9 ^H	18.4 ^H	46.0 ^{HI}	89.7 ^H	49.2 ^G	138.1 ^B	350.6 ^I
LSD	0.232	1.295	1.450	1.750	3.226	3.314	5.089	17.877

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at P <0.05, the least significant difference (LSD) was calculated at 95% confidence interval.

On another hand there was non-significant difference of RBCs, and MCV values in rats fed with high AFS dose and supplemented with 3% BP when compared with high dose AFS control group. The same finding with PCV value at supplementation 1% BP.

The decrease in hematological parameters may be due to many factors such as inhibition of protein synthesis as evidenced by lower serum albumin and hematopoietic cellular defects of aflatoxin (Hanif, et al., 2006). These results were in agreement with those obtained by Blomhoff et al., (2006) & Reddy et al., (1984) & Huff et al., (1986) and Mohiuddin et al., (1986) which reported that aflatoxin caused hematopoietic suppression and anemia observed as decreases in total erythrocytes, packed-cell volume and hemoglobin, also in agreement with Hasan, (2014) who studied the acute and chronic effects of aflatoxin on the liver of rats during the storage of walnuts.

Our results are in agreement with Aravind, et al., (2003) as they indicated that toxins present in the contaminated feed significantly depressed performance, organ morphology, and most of the serum biochemical parameters. The effects of the exposure of six –week old broiler to doses of aflatoxin correspond to a marked decrease in the red blood cell (RBC), hemoglobin (Hb), packed cell volume (PCV), and white blood count (WBC) heterophils, and monocytes counts. However there was increase in percentage of lymphocytes and eosinophils, studied by Jeff-Agboola, (2014).

Data illustrated in Table (6) showed the effect of feeding AFS contaminated diet (low and high doses), singly or in combination with different concentrations of banana peel on rats body weights during the experiment period (6 weeks). The results recorded revealed that body weight gain of administrated rats with the different two doses of AFS (1mg and 2mg/ B.W. twice/ week of groups 3 and 4 significantly).

Decreased in relation to control group G1 during the experiment period. Also results at day 14, 28, and 45 showed that there was no significant difference between groups with high dose of aflatoxin and 1%, 2% and 3% banana peel (G11, G12 and G13) compared to control single high dose of aflatoxin (G4). Generally, there was a significant decrease in body weights at all week observations this decreasing in growth rate by aflatoxin supplementation which may be due to disturbance in one or more of basic metabolic processes (carbohydrate, lipid, and protein metabolism) in liver (Cheeke and shull, 1985). Similar results were also obtained by Motawe, et al., (2014) in broiler chickens; Shehata and Mohamed, (2012) in Nile Tilapia fish; Shehata, (2012) in rabbits; Abdel-Fattah, et al., (2010); Saad and Abdel-Fattah, (2008); Rawi, and Waggas, (2013) in rats administrated with aflatoxin. Basmacioglu, et al., (2005) reported that the depression in body weight gain through aflatoxicosis is due to the disruption of protein synthesis. Jeff-Agboola, (2014) concluded that reduction in weight of birds fed with feed containing the aflatoxin moulds and associated with increase in concentration of the toxin. Similar results obtained by Justin, et al., (2015) who concluded that chick body weights were reduced with consumption of diets containing aflatoxin. These observations are agreed by that of El-Desouky et al., (2017) who study the use of ozone gas to

reduction and or removal AFB1 is biologically safe and does not produce any secondary compounds that may cause toxicity.

Table 6. Effect of aflatoxin with or without supplementation of banana peel on rats body weight changes during experimental period.

Parameters Groups	day 14 (g)	day 28 (g)	day 45 (g)
G1	169.33 ^A	216 ^A	252.33 ^A
G2	169 ^A	217.33 ^A	253 ^A
G3	141.33 ^D	177.66 ^{CDEF}	199 ^{CD}
G4	126.66 ^E	158.66 ^G	176.66 ^D
G5	168 ^{AB}	196.66 ^B	235 ^B
G6	165.33 ^{AB}	195 ^{BC}	233.33 ^B
G7	164 ^{AB}	195.33 ^{BC}	226.33 ^B
G8	160.33 ^{AB}	190 ^{BCD}	215 ^{BC}
G9	158.66 ^{AB}	184.33 ^{BCDE}	212.66 ^{BC}
G10	156.33 ^C	183 ^{BCDE}	206.33 ^{BC}
G11	137.33 ^{DE}	176.33 ^{DEF}	186.66 ^{CD}
G12	135 ^{DE}	168.66 ^{EF}	181 ^{CD}
G13	130.33 ^{DE}	165 ^{FGW}	180.66 ^D
LSD	10.62	16.09	15.78

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at P <0.05, the least significant difference (LSD) was calculated at 95% confidence interval.

The data in Table (7) showed significant increase in rat organ (liver and kidney) weights administrated with different two doses of AFS (1mg and 2mg/ B.W. twice/ week) at the end of experimental period comparing with control group (G1). The increases of their weights were parallel to dose of AFS. The highest value produced values of 9.60g and 0.86g obtained in (G4) high dose AFS for liver and kidney respectively. There was no significant effect of liver and kidney weights in groups supplemented with BP at 1%, and 2%, whereas significant increase liver and kidney weights were found in rats fed with 3% BP.

Table 7. Effect of aflatoxin with or without supplementation of banana peel on rats' organ weights at the end of experimental period

Parameters Groups	liver weights(g)	kidney weights(g)
G1	5.83 ^F	0.50 ^F
G2	5.83 ^F	0.50 ^F
G3	9.36 ^A	0.83 ^A
G4	9.60 ^A	0.86 ^A
G5	6.46 ^{EF}	0.56 ^{EF}
G6	6.60 ^{EF}	0.60 ^{DE}
G7	6.83 ^{DE}	0.66 ^{CD}
G8	6.86 ^{DE}	0.70 ^C
G9	7.13 ^{DE}	0.70 ^C
G10	7.56 ^{CD}	0.70 ^C
G11	8.16 ^{BC}	0.73 ^{BC}
G12	8.76 ^{AB}	0.80 ^{AB}
G13	8.96 ^{AB}	0.80 ^{AB}
LSD	0.94	0.087

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at P <0.05, the least significant difference (LSD) was calculated at 95% confidence interval.

Supplementation of BP with percentage 1% and 2% and 3% to both doses of AFS groups (G8 and G13) resulting in minimizing toxic effect of AFS on comparing to AFS control groups (G3 and G4). The results in accordance with the study of Mosa and Khalil (2015) who found that the relative liver and kidney weights of rats fed with banana peel decreased significantly than the positive control groups.

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فعالية قشر الموز في تقليل سمية الأفلاتوكسين في الجرذان البيضاء

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أجريت التجربة لتحديد كفاءة قشر الموز لتقليل التأثير السام للأفلاتوكسينات على ذكور الجرذان البيضاء وذلك بإضافة مستويات مختلفة من قشر الموز. استخدم في هذه الدراسة عدد (٧٨) من ذكور الجرذان البيضاء قسمت إلى ١٣ مجموعته متساوية تحتوى كل مجموعة على ٦ جرذان. تم تغذية المجموعة الأولى على العليقة الأساسية فقط، وغذيت المجموعة الثانية على العليقة الأساسية مضافا إليها ثنائي ميثيل سلفوكسيد (DMSO) (العليقة الأساسية + ٥مل DMSO/كيلوجرام من وزن الجسم) يوميا. غذيت المجموعتان الثالثة والرابعة على تركيزات (١ملجم/مل، ٢ملجم/مل) للأفلاتوكسين على التوالي. المجموعتين الثالثة والرابعة غذيت على علائق تحتوى على نسب مختلفه من قشر الموز بتركيزات ٢ و ٣% على التوالي، أما المجموعتين الثالثة والرابعة غذيت على العليقة الأساسية بالإضافة الى التركيز المنخفض من الأفلاتوكسينات (١ملجم أفلاتوكسين مذاب في ٥مل DMSO/كجم من وزن الجسم) مع نفس نسب الإضافة من قشر الموز. كما غذيت المجموعتين الثالثة والرابعة على العليقة الأساسية مع التركيز المرتفع من الأفلاتوكسينات (٢ملجم أفلاتوكسين مذاب في ٥مل DMSO/كجم من وزن) مع نفس نسب الإضافة من قشر الموز. وقد استمرت هذه التجربة لمدة ٦ أسابيع، وتم تقدير مستويات كل من الإنزيمات التالية: ALT, AST, ALP, البروتينات الكلية، الألبومين، الكرياتينين، الكوليسترول والجليسريدات الثلاثية. وأيضا تقدير تغيرات الدم مثل كرات الدم الحمراء (RBCs)، الهيموجلوبين (Hb), PCV, MCV, MCH, MCHC, TLC, والصفائح الدموية (Platelets). تم قياس اوزان الجسم والأعضاء الداخلية للفران لدراسة تغيراتها. أظهرت النتائج أن المجموعات التي تم معاملتها بالتركيز المنخفض والمرفع (٣٨ملجم/كجم، ٦٧ملجم/كجم) للأفلاتوكسين بها ارتفاع معنوى فى أنزيمات الكبد الأساسية: ALT, AST, ALP, البروتينات الكلية، الألبومين، الكرياتينين، الكوليسترول والجليسريدات الثلاثية. وهذا يدل على أن إضافة قشر الموز تلعب دورا مؤثرا فى إزالة سموم الأفلاتوكسينات فى الجرذان البيضاء.