Enhancement biochemicals levels and improve histological tissues of kidney and liver organs for duckling's received aflatoxin B1 by using propolis extract

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Abstract:

The aim of the present study was focused to see the effect of propolis as a natural product on duckling's health when exposed to aflatoxin B_1, to enhancement body weight of duckling's received feed contaminated aflatoxin B_1, to enhancement blood function and to improve kidney and liver tissues. The present study show that, birds began to show symptoms of toxicity after 2 weeks. Aflatoxin B_1 alone was found to reduce feed efficiency and poor health can cause by an imbalance of nutrients. Abnormalities symptoms were found with all ducklings received aflatoxin B_1. These birds showing slow eating, leg paralysis, slow moving as result of loss functional movement, inflammatory edema of the eyelids (affect eyes), hair loss, and changed in the color when compared with control. All ducklings fed 0.018 ng / ml aflatoxin B_1 diet had significantly ($P < 0.01$ & $P < 0.05$) lower body weight compared with the control group (Un-treated). Whereas, all ducklings received Propolis extracts plus aflatoxin B_1 were enhanced and appear comparable to the control. Propolis extract was found improve significantly ($P < 0.01$ & $P < 0.05$) all body weight gain of all ducklings in the different treatment groups. On the other hand exposure to aflatoxin B_1 can cause several damage to organ systems, increase significantly ($P < 0.01$ & $P < 0.05$) all tested biochemical parameter determined as Urea m mol/L, Creatinine mg/dl, SGPT U/L and SGOT U/L compared with un-treated control. Whereas, all ducklings received Propolis extracts plus aflatoxin B_1 were improve all these parameters. In the affected birds metabolic changes lead to enlargement of liver, kidney and spleen as well as decrease in the size of bursa of fabricus as a result, liver is greatly enlarged, yellow and friable (easily broken), fat accumulates inside the cell of liver as clear vacuoles. Data show that enlargement in kidney weight in contaminated groups . Whereas, all ducklings received Propolis extracts plus aflatoxin B_1 were improved and appear comparable to the control. Also, microscopic examination of liver and kidney tissues showing, changes of histopathological tissues with all ducklings received aflatoxin B_1. Whereas, all ducklings received Propolis extracts plus aflatoxin B_1 were improved and appear comparable to the control.

Key words: Feed**,** *Aspergillus flavus***,** ducklings, kidney, liver

1. Introduction

Feed is the single most important input in increasing chicken and fish culture production and profits. Information about fungi associated with food and feeds is important in assessing the risk of mycotoxin contamination. Feeds are frequently contaminated simultaneously by several moulds. Mould contamination of food and the environment has a potential to produce fungal toxins (mycotoxins) that are harmful to chickens, turkeys, ducks, fish etc. According to WHO, about 25% and 40% of the world's food is contaminated by mycotoxins. These toxins pose serious health concerns to animals as well as human beings [1,2]. The consumption of these mycotoxin-contaminated feedstuffs by animals leads to an adverse effect on animal health and the effects are more serious in monogastric animals depending on the species and the susceptibility to toxins within the species [3]. The carryover of toxins from animal food may have severe consequences on human health. The economic impact of lowered productivity reduced weight gain, reduced feed efficiency, damage to body organs, interference in reproduction is many times greater than that of immediate mortality and morbidity [4]. Mycotoxins in food and feedstuffs affect both the organoleptic characteristics and the nutritive value of feed, leading to the risk of toxicosis. However, the biological effects of mycotoxins depend on the amount ingested by the host, the age, sex and strain of the animal, varieties of occurring toxins, time of exposure to mycotoxins, animal sensitivity and its condition. Mycotoxins can induce health problems that are specific to each toxin or suppress the immunity power of animals, favouring infections. This is the major reason for the difficulty of diagnosing mycotoxicoses [5]. Commercial feedstuffs are an important component in modern animal husbandry, but there is no information available about fungal contaminations [6]. Since feed accounts for about 50-60% of the variable costs of production, feed quality is crucial to the success of farming operations. Major problems that may result from low-quality feeds are poor appetite, slow growth, high feed conversion ratio, and low survival. These usually develop as a result of problems on quality of raw materials, feed formulation, processing technology, storage, and feed manage-merit. The most serious problems on feed quality are those involving rancidity, aflatoxin contamination, and nutrient loss [7]. Aflatoxicosis represents one of the serious disease of poultry, livestock and other animals. the cause of this disease in poultry and other food-producing animals has been attributed to the ingestion of various feeds contaminated with *Aspergillus flavus*. This toxegenic fungus is known to produce a group of extremely toxic metabolites, of which aflatoxin B_1 (AFB_1) is most potent. Avian species especially chickens; Gosling, duckling and turkey poults are most susceptible to AFB_1 toxicity. The incidence of hepatocellular tumours. Particularly in duckling, is considered to be one of the serious consequences of aflatoxicosis [8]. Propolis is a resinous substance collected by worker bees (*Apis mellifera*) from the bark of trees and leaves of plants. Propolis shows a complex chemical composition. Propolis shows pharmacological activities, such as antifungal, antibacterial, anticancer or anti-inflammatory to name a few, among other activities, have attracted the researchers' interest [9,10,11].

Because aflatoxigenic fungi and aflatoxins are the most spread, most dangerous and the most effects on health and economics, the aim of the present study was focused to see the effect of propolis as a natural product on duckling's health when exposed to aflatoxin B_1, to enhancement body weight of duckling's received feed contaminated aflatoxin B_1, to enhancement blood function and to improve kidney and liver tissues.

2. Materials and Methods

2.1 Fungal producing toxin

Aspergillus flavus was propagated as a pure culture in 100 ml yeast extract sucrose (YES) broth containing 2% yeast extract and 20% sucrose/ litre distilled water according to [12]**.** One disc 5mm of *A. flavus* was inoculated into each flask (250ml) having 100ml of sterile Y.E.S with 2, 5, 10 and 20% of 'methanol extract of propolis' (MEP). Another flask free 'methanol extract of propolis' (MEP) was used as a control. Three replicates for each treatment. All treatments were incubated at 26±2 ^{o}c. in the dark for 14 days.

2.2 Preparation of Propolis Extract

The hand-collected propolis was stored in a brown bottle and away from exposure to light until further processing. Propolis extracts were prepared as described by [13]**,** propolis was prepared by adding 100 g of the collected propolis to 900 mL of 70% methanol to give 10% methanolic extract of propolis (MEP) which extracted and heating for evaporating methanol (at 50 °C for 5 hours) and agitating. Water was then added. To

optimize purification, centrifugation at high speeds (4,000rpm) was proposed. All Samples were centrifuged for 25 minutes. The supernatant was stored overnight at ambient temperatures. The supernatant was further filtered through filter paper (Whatman no. 1) and stored at ambient temperatures in a bottle according to [14]. The final solution was termed 'methanol extract of propolis' (MEP) to produce a final solution from various propolis samples [15]. Kept at 4 °C in dark storage until use.

2.3 AFB1-Contaminated feed and propolise treatments

Upon analysis, the AFB_1-contaminated feed contained the following aflatoxins 0.018 µg/ml of AFB_1 with 2, 5, 10 and 20% of 'methanol extract of propolis' (MEP). AFB_1 was under the detection limit [16].

2.4 The Toxicity of aflatoxins and its control

2.4.1 Experimental design, Birds, the toxicity of *Aspergillus flavus* toxins on body weight and it's improved

A total of 24 ducklings (7-day-old av. 417 g) (mixed sex) were purchased from Fac. of Veterinary Med, Benha Univ. Ducks were weighed and randomly allotted to 6 dietary treatments. The experimental groups were described in Table (1) according to [17]. The experimental diets were formulated based on the [18] recommendations for 2 wk duck starter diets standard. Ducks were housed on a commercial farm. The temperature was maintained at 34°C to the end of the experiment. The overhead light was provided continuously for the entire period of the experiment. Ducklings were randomly divided into 6 treatment groups of ducks and there were 4 replicates for each treatment, then treated for two weeks and fed according to the indicated experimental diets as follows: group 1, received Basal Diets (BD) plus 0.018 ng/ml AFB_1 plus 2% of propolis' (MEP); group 2, treated orally with BD + 0.018 ng / ml AFB_1 +5% of MEP; group 3, treated orally with BD + 0.018 ng / ml AFB_1 +10% of MEP; group 4, received BD + 0.018 ng / ml AFB_1 +20% of MEP; group 5, treated orally with BD + 0.018 ng / ml AFB_1 (as a positive control); group 6, control which received Basal Diets (BD) free from AFB_1 (as a negative control). The ducklings were raised in battery cages, and warm air was used for brooding ducklings during the trial period. The ducks were observed daily for signs of toxicity. Body weights (BW) and growth uniformity, which was expressed as CV of BW were recorded at zero time, and at the end of the feeding experiment after 2 weeks of the experimental period. Mortalities and health status were observed and recorded daily throughout the entire experimental period as described by [19].

Table 1: Description of the experimental groups

Experimental group	Details
G1	Basal Diets (BD) + 0.018 ng / ml AFB_1 +2% of propolis' (MEP)

G2	BD + 0.018 ng / ml AFB_1 +5% of MEP
G3	BD + 0.018 ng / ml AFB_1 +10% of MEP
G4	BD + 0.018 ng / ml AFB_1 +20% of MEP
G5	BD + 0.018 ng / ml AFB_1 (as a positive control) free MEP
G6	Basal Diets (BD) free from AFB_1 (as a negative control)

2.4.2 Blood samples

At the end of the experiment, birds fasted for 12 h. (All ducks were not fed on the day of the sacrifice). After the experimental period (14 days), a 5-mL blood sample was collected from a jugular vein in a test tube without anticoagulant (using 1 ml syringes) for biochemical analysis. Blood samples were incubated at 37°C for 2 h, centrifuged at 1,500 × g for 10 min, and serum was separated and stored in 1.5-mL centrifuge tubes at −20°C until analysis. Blood samples were stored frozen at -20°C. Blood samples obtained from treated and untreated ducklings were analysis according to [19]**.**

2.4.2 Biochemical studied (Serum analysis)

All the biochemical determinations were carried out using commercial kits according to the kits manufactures unless explained as follow:-

2.4.2.1 Determination of Urea

Determination of urea level in serum by using the enzymatic colourimetric method of [20] by using kit obtained from BioMérieax SA (France).

2.4.2.2 Determination of Creatinine

Creatinine was determined in serum using commercial kits purchased from Stanbio Laboratory, Inc. (San Antonio, Texas, USA) according to the methods of [21].

2.5 Histopathological studies

After bleeding, birds were killed by cervical dislocation for organ weight calculation, including liver and kidney, and their relative weights were calculated as (organ weight/ live weight) × 100 [19]**.** Livers and Kidney specimens from all ducks were dissected immediately after death and fixed in 10% neutral-buffered formal saline for 72 h. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6 μm thick were cut and stained with Haematoxylin and eosin for histopathological investigation according to [22,23] then, examined by light microscopy [22,23,24]**.** Images were captured and processed using Adobe Photoshop version 8.0.

2.6. Statistical analysis: Obtained data were subjected to statistical analysis of variance according to [25]**,** and means separation were done according to [26].

3. Results and Discussion

3.1 Enhancement of duckling health received aflatoxin B1

The present study shows that control group (free aflatoxin B_1) was in normal ducklings (Healthy) as showing in **Fig. (1.a).** Abnormalities symptoms were found with all ducklings received aflatoxin B_1. Data also show that aflatoxin B_1 alone was found to reduce feed efficiency and poor health can be caused by an imbalance of nutrients either by deficiency. All ducklings fed 0.018 ng/100g feed aflatoxin B1 diet had lower body weight compared with the control group (Un-treated). The birds began to show symptoms of toxicity after 2 weeks, external examination of these birds showing birds hazeled together indicating abnormal behaviour, slow eating, slow movement, loss of functional movement, hair loss and change in the colour when compared with control Fig. (1.a&b.). [27] found that Affected ducklings displayed a delay in development, hyperkeratosis of the cornea and the oral mucosa, malformation and bone fragility, leg paralysis, inflammatory oedema of the eyelids, dermatitis, and scarce feathering. The ducklings suffered massive a vitaminosis and deficiency in calcium, phosphorus and manganese absorption. [27,28] stated that in the field, animals experiencing a mycotoxicosis may exhibit a few or many of a variety of symptoms, including digestive disorders, reduced feed consumption, un-thriftiness, rough hair coat or abnormal feathering, undernourished appearance, low production, poor production efficiency, impaired reproduction and/or a mixed infectious disease profile. Mycotoxins can increase the incidence of disease and reduce production efficiency. Some of the symptoms observed with a mycotoxicosis may, therefore, be secondary, resulting from an opportunistic disease, present because of mycotoxin-induced immune suppression. Immunotoxic effects of mycotoxins are reviewed. [29] concluded that the toxegenic *Aspergillus flavus* fungus is known to produce a group of extremely toxic metabolites, of which aflatoxin B_1 (AFB_1) is most potent. Avian species especially chickens; Gosling, duckling and turkey poults are most susceptible to AFB_1 toxicity. The incidence of hepatocellular tumours. Particularly in duckling, is considered to be one of the serious consequences of aflatoxicosis. [19] found that bill colour of ducks receiving contaminated corn was discoloured in contrast to the yellow colour of the ducks receiving uncontaminated corn. [30] concluded that moulds are capable of reducing the nutritional value of feedstuff as well as elaborating several mycotoxins. Mycotoxin-contaminated feed has adverse effects on animal health and productivity. Regarding nutritional quality, lipids, proteins, and minerals are of essential importance for the proper development and growth of farm animals. [31] stated that *Aspergillus* causes different forms of aspergillosis. The most common form of *Aspergillus* mould infection is brooder pneumonia, a lung and air-sac disease of chicks. less – common forms of aspergillosis affect eyes, skin, brain, or bones. Chicks affected by brooder pneumonia gasp, lose their appetite, and look sleepy. The disease does n't spread from chick to chick, but the mould can infect many chicks in a group at once, and up to half may die from the infection.

Fig. (1.a) Control duckling group showing normal symptoms of ducklings (Healthy);

Fig. (1.b) Duckling with serious aflatoxicosis problem, Aflatoxin B_1 alone showing abnormality symptoms, slow moving and was found to decrease the seize and body weight gain (weight loss), leg abnormalities (leg weakness) and lethargy, appearance are common symptoms of aflatoxin poisoning.

3.2 Improve body weight gain of duckling exposed aflatoxin B_1

Effect of aflatoxin B_1 produced by *Aspergillus flavus* and detoxification of their effects by using a natural product as Propolis extract on feed intake and changes in body weight gain of ducklings in the different treatment

groups were recorded in **Table (2).** The presented data show that aflatoxin B_1 alone was found to reduce feed efficiency and poor health can be caused by an imbalance of nutrients either by deficiency. All ducklings fed 0.018 ng /100g aflatoxin B_1 diet had significantly ($P < 0.01$ & $P < 0.05$) lower body weight compared with the control group (Un-treated). Visual examination revealed that bill colour of duckling receiving 0.018 ng/100g aflatoxins B_1 contaminated diet was discoloured. Whereas, all ducklings received Propolis extracts in combination plus aflatoxin B_1 were improved and appeared comparable to the control. Propolis extract was found significantly ($P < 0.01$ & $P < 0.05$) improve all body weight gain of all ducklings in the different treatment groups. Also, data show that significantly ($P < 0.01$ & $P < 0.05$) enhanced body weight gain of all ducklings with increasing the concentration of Propolis extract. The average of body weight gain in control group was 770 g then decreased to 631 g which loosed 139 g equal 18.1 reduction percent with the first group which received feed contaminated *A. flavus* toxin (aflatoxin B_1) and 2% Propolis. While ducklings of the second group which received feed contaminated with *A. flavus* toxin (aflatoxin B_1) and 5% Propolis loosed 114 g equal 19.1 % reduction. Group 3 was found to reduce from 770 g to 671 g which loose 99 g equal 12.9% reduction when treated with 10% Propolis extract, group 4 loosed 87 g and gave 11.3% reduction when treated with 20% Propolis extract. Group 5 free Propolis extract which received feed contaminated with *A. flavus* toxin (aflatoxin B_1 only) was found to decrease from 770 g to 500 g and gave 95.59% reduction. [32] said the economic impact of lowered productivity, reduced weight gain, reduced feed efficiency, damage to body organs, interference in reproduction is many times greater than that of immediate mortality and morbidity. [34] Concluded that in animals, adverse effects of AF also include a reduction in growth rate and feed efficiency, decreased egg production and hatchability, and increased susceptibility to disease. In addition, residues of AF from animals can appear in edible animal products for human consumption, which raises public health concerns. [35,36] found that in chicken, B_1 is metabolized into M_1 and B_2 in the liver and NADP linked enzyme system reduces B_1 and B_2 to cyclopentanol and aflatoxin. Aflatoxin B_1 impairs all-important production parameters in poultry including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, and male and female reproductive performance. [19] said all ducklings fed 100% M diet had lower growth performance ($P < 0.05$) ADG compared with 0%. [36,37,38] reported that dietary AF exposure reduces weight gain and feed intake, and worsens feed efficiency. The response of animals to AF-contaminated feed depends on the AF concentration, animal species, and age and sex. The reduced growth rate because of AF ingestion in the diet is primarily due to the reduction in feed intake. Generally, 0.95 mg/kg AF in the diet reduces weight gain by 11 percent because of, in part, reduced feed intake and metabolic inefficiencies from liver and GIT damage. [38,39,40] found that most mycotoxicoses of poultry are caused by an intake of low concentration of contaminants over a long whit the typical chronic symptoms of poor growth, poor feed efficiency, and suboptimal production. Ingestion of higher concentration, however, leads to acute clinical symptoms associated with specific vital organs, the immune system, and other aspects of avian physiology as well as mortality. [41] reported that aflatoxins contained in the mould-exposed diet significantly reduced daily weight gain and feed intake of male ducks. Compared with normal-diet group, presence of AFs in the diet significantly reduced weights of meat male ducks at 7, 14, 21, 28, and 35 days of age, significantly reduced daily weight gain and daily feed intake at different periods (1e7 days of age, 15e21 days of age, 22e28 days of age, 29e35 days of age, and significantly increased their F:G at different ages (except for days 8e14; $P < 0.05$).

Table (2): Effect of aflatoxin B1 on body weight gain of ducklings and their improvement after two weeks by Propolis

Groups	BW (g)		L. (g)	%L	%R
	Zero time	After 14 days			
G1	400.00	631.00	139	81.9	18.1
G2	406.00	622.67	114	80.9	19.1

G3	404.00	671.00	99	87.1	12.9
G4	417.00	683.00	87	88.7	11.3
G5	466.00	500.00	34.00	4.41	95.59
G6 (control)	410.00	770.00			
L.S.D@1%	2.164	24.193			
L.S.D@5%	1.488	16.629			

*BW=Body Weight ; L=Loss; %L = Loss percent; %R=Reduction

3.3 Improve biochemical function (improve blood function)

Effect of aflatoxin B_1 on blood function as result of biochemical analysis presented that, aflatoxin B_1 can cause damage to organ systems, increase significantly ($P < 0.01$ & $P < 0.05$) all tested biochemical parameter determined as Urea m mol/L, Creatinine mg/dl, SGPT U/L and SGOT U/L compared with un-treated control group 6 (Free aflatoxin B_1) as shown in **Table (3).** The effects of aflatoxin B_1 for two weeks on serum biochemical parameters of ducklings compared with control group indicated that the control group was in the normal limit, but were found to the differentiation between biochemical parameters i.e. urea, Creatinine, SGPT U/L and SGOT U/L when ducklings received aflatoxin B_1 for two weeks. Higher significant ($P < 0.01$ & $P < 0.05$) was found in between aflatoxin B_1 alone group 5 and the control group 6 (Free aflatoxin B_1). On the other hand analysis of liver function resulted that, significantly ($P < 0.01$ & $P < 0.05$) increase SGPT from 34.33 U/L to 56.67 U/L with 65.1% reduction for group 1 (duckings received aflatoxin B_1 plus 2% propolis extract), group 2 received aflatoxin B_1 plus 5% propolis extract record 52.67 U/L equal 53.4% reduction, group 3 received aflatoxin B_1 plus 10% propolis extract gave 49.00 U/L and 42.7% reduction, group 4 received aflatoxin B_1 plus 20% propolis extract gave 41.0 U/L and 19.4% reduction while, group 5 received aflatoxin B_1 contaminated diet feed had increase ($P < 0.01$ & $P < 0.05$) serum SGPT U/L and SGOT U/L concentrations as well as increase ($P < 0.01$ & $P < 0.05$) Urea m mol/L, and Creatinine mg/dl concentration compared with concentrations in those fed diet free aflatoxin B_1. it also showed that an increase of SGOT U/L increased from 63.67 to 79.00 in control (free from aflatoxin) with 24.1% reduction while, group 2 received aflatoxin B_1 plus 5% propolis extract record 76.33equal 19.9% reduction group 3 received aflatoxin B_1 plus 10% propolis extract gave 72.00 U/L and 13.1 reduction, group 4 received aflatoxin B_1 plus 20% propolis extract gave 65.33and 2.6% reduction while group 5 received aflatoxin B_1 only (free propolis extract) gave 84.00 equal 31.9% reduction. On the other hand analysis of kidney function resulted that, significantly ($P < 0.01$ & $P < 0.05$) increase creatinine from 0.45 to 0.55 with 22.2% reduction for group 1 (duckings received aflatoxin B_1 plus 2% propolis extract), group 2 received aflatoxin B_1 plus 5% propolis extract record 0.53equal 17.8% reduction, group 3 received aflatoxin B_1 plus 10% propolis extract gave 0.52 and 15.6 reduction, group 4 received aflatoxin B_1 plus 20% propolis extract gave 0.45 and 00% reduction while, group 5 received aflatoxin B_1 only (free propolis extract) gave 0.56 equal 24.4% reduction. the table also showed Urea conc which increase from 14.67 to 18.7 with 27.5% reduction for group 1 (duckings received aflatoxin B_1 plus 2% propolis extract), group 2 received aflatoxin B_1 plus 5% propolis extract record 17.3 equal 17.9% reduction, group 3 received aflatoxin B_1 plus 10% propolis extract gave 16.0 and 9.1 reduction, group 4 received aflatoxin B_1 plus 20% propolis extract gave 15.0 and 2.2% reduction while, group 5 received aflatoxin B_1 only (free propolis extract) gave 19.0 equal 29.5% reduction. [42,43] reported that mycotoxins can cause damage to organ systems, reduce production and reproduction, and increase diseases by reducing immunity. Some mycotoxins are carcinogens, some target liver, kidney, digestive tract or the reproductive system. [33] stated that, in contrast, recent literature reported adverse effects at concentrations as low as 0.02 mg/kg. A plausible explanation of these differences between earlier and more recent reports could be that modern broilers have more efficient nutrient conversion demanding faster hepatic metabolism, which in turn results in a higher metabolism of AFB_1. [34,38] reported that the lower tolerance of ducks also could be explained by a lower activity of hepatic enzymes responsible for cellular detoxification and excretion of a variety

of toxic substances. In contrast, recent literature reported adverse effects at concentrations as low as 0.02 mg/kg. A plausible explanation of these differences between earlier and more recent reports could be that modern broilers have more efficient nutrient conversion demanding faster hepatic metabolism, which in turn results in a higher metabolism of AFB_1. [45,38] concluded that enzyme activities are modulated following AF consumption. An increased release of enzymes from the pancreas to the intestinal tract might be a consequence of pancreatic damage. Very low doses of AFB_1 (0.02 and 0.04 mg/kg) have reduced the apparent digestibility of crude protein by 8-13 percent in ducks. Similarly, it has been suggested that dietary AF increases the amino acid requirements, and it appears to negatively impact ducks more than chickens.

Tabl Table (3): Improve biochemical changes (improve kidney and liver functions)

Groups	Blood functions (Enzymatic analysis)							
	Liver functions				Kidney functions			
	SGPT U/L	R%	SGOT U/L	R%	Creatinine mg/dl	R%	Urea m mol/L	R%
G1	56.67	65.1	79.00	24.1	0.55	22.2	18.7	27.5
G2	52.67	53.4	76.33	19.9	0.53	17.8	17.3	17.9
G3	49.00	42.7	72.00	13.1	0.52	15.6	16.0	9.1
G4	41.00	19.4	65.33	2.6	0.45	0	15.0	2.2
G5	60.00	74.8	84.00	31.9	0.56	24.4	19.0	29.5
G6 (control)	34.33		63.67		0.45		14.67	
L.S.D@1%	13.327		20.521		0.048		2.485	
L.S.D@5%	9.160		14.105		0.033		1.533	

* SGPT = Serum glutamic pyruvic transaminase; SGOT= Serum glutamic oxaloacetic transaminase

3.4 Effect of aflatoxin B_1 exposure on intestinal morphology and its improved

Effect of aflatoxin B_1 exposure on intestinal morphology consumption of aflatoxin B_1 cause a few or many of a variety of symptoms, including: opportunistic disease, present because of mycotoxin-induced immune suppression, however leads to acute clinical symptoms associated with specific vital organs, Liver as a key player of AF toxicity and sensitivity within Poultry. Liver damage occurred. In the affected bird's metabolic changes lead to enlargement of liver, kidney and spleen as well as a decrease in the size of bursa of fabrics as shown in **Figs. (2-4)** as a result, the liver is greatly enlarged, yellow and friable (easily broken) compared with the control group (free aflatoxin B_1). Fat accumulates inside the cell of the liver as clear vacuoles compared with control group (free aflatoxin B_1). While duckling received aflatoxin B_1 plus propolis extract was enhanced. Continuous enhancement with increasing propolis extract comparable to control. Similar results were obtained by [46,47] they reported that decreased feed intake along with increased liver weights in birds is consistent with earlier reports. [48] concluded that an average AF concentration of 0.95 mg/kg reduced both feed intake and daily weight gain by 11 percent, and worsened feed conversion by 6 percent. In the affected bird's metabolic changes lead to enlargement of liver, kidney and spleen as well as a decrease in the size of bursa of Fabricius, thymus and testes. With high dose exposure, fat accumulates inside the cell of the liver as clear vacuoles. As a result, the liver is greatly enlarged, yellow and friable (easily broken). There is general agreement that dietary AF reduces weight gain and feed intake, and worsens feed efficiency. Previous research indicated that the reduced growth rate because of AF ingestion in the diet is primarily due to the reduction in feed intake.[48,49] found that, aflatoxicosis represents one of the serious diseases of poultry, livestock and other animals. *Aspergillus flavus* is known to produce a group of extremely toxic metabolites, of which aflatoxin B_1 (AFB_1) is most potent. Avian species especially chickens; goslings, ducklings and turkey poults are most susceptible to AFB toxicity. External examination of carcass did not show any disease changes. On post-mortem examination, the carcass revealed large cirrhotic pale friable liver with perihepatitis. Heart, Lungs, Spleen and kidney were normal. Salphingitis, impaction of the oviduct with cheesy material and egg peritonitis were also noted. The case was diagnosed as aflatoxicosis. Case reports of aflatoxicosis in ducks are rare. Chronic exposure to low levels of aflatoxin can result in cancer and immunosuppression. Decreased feed intake along with increased liver weights in birds is consistent with earlier reports. Even though prevention and avoidance are the best way to control aflatoxicosis, natural contamination of crops with *A. flavus* is some times unavoidable. [46,19] Aflatoxin at a dietary concentration of 1 mg/kg or more caused severe reductions in growth and immune response in broilers, whereas 2 mg/kg increased the relative weight of the liver and decreased the relative weight of the bursa of Fabricius.

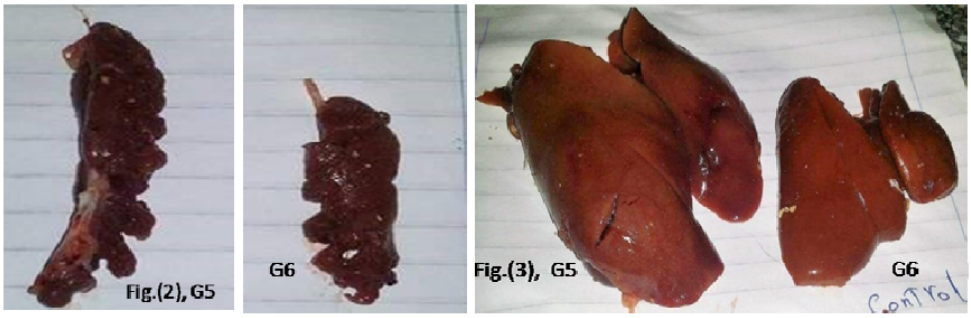


Fig.(2): Showing enlarged kidneys of duckling treated with only aflatoxin (group 5) in relation to control one (G 6).

Fig.(3): Showing enlarged liver and conjunction of duckling treated with only aflatoxin (group 5) compared to control one (G 6).

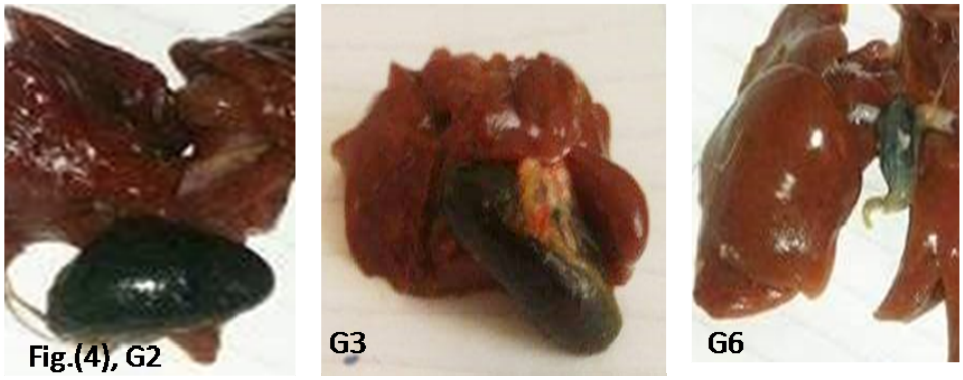


Fig.(4): Showing small size gall bladder in control group and enlarged gall bladder of duckling treated with aflatoxin plus propolis group 2 (5% conc.) as well as group 3 (10%conc.) in relation to control one (Control G6).

3.5 Improve Kidney weights of ducklings given feed contaminated with aflatoxin B_1

Effect of aflatoxin B_1 exposure on average Kidney weight of duckling and its control were studied. Data were tabulated in **Table (4)** the presented data showed that enlargement in kidney weight in contaminated groups. Aflatoxin B_1 alone was found to significantly ($P < 0.01$ & $P < 0.05$) increase average kidney weight of all tested ducklings compared with the control group (Un-treated). It was increased from 4.5 g to 6.9 g when ducklings were given feed contaminated with 0.018 ng /100g of AFB_1. Whereas, all ducklings received Propolis extracts plus aflatoxin B_1 were improved and appeared comparable to the control. Propolis extract was found significantly ($P < 0.01$ & $P < 0.05$) improve all kidney weight. Continuous improve kidney weight with increasing the concentration of propolis extract at 2%, 5%, 10%. This improves was 6 g in the average kidney with 2%. While at 5% it gives 5.70 g , at 10% it gives 5.6 g Enhanced average kidney weight was 5.5 g which recorded with 20% propolis as compared with untreated group(Control G6).

Table (4): Improve average Kidney weight of ducklings exposed aflatoxin B_1 by using propolis extract

Groups	Kw.	I	% I	%R
G1	6.00	1.5	0.25	99.75
G2	5.70	0.73	16.2	83.8

G3	5.60	1.1	19.6	80.4
G4	5.50	1	18.2	81.8
G5	6.90	2.4	34.8	65.2
G6 (control)	4.50			
L.S.D@1%	0.581			
L.S.D@5%	0.381			

*Kw.=kidney weight; I=increased; % I =increased percent; %R=Reduction

3.6 Histological studies

The biochemical results reported in the current study were confirmed by the histological results. Microscopic examination of liver and kidney tissues showing, changes as well as enhanced of histological tissues were confirmed and photographed as shown in figs (1-38).

3.6.1 For control ducklings G 6 (free aflatoxin B_1)

No histopathological changes were detected in the examined livers and kidneys of these ducklings. The liver showed normal histological criteria of blood vessels, bile ducts and hepatic cords. Rarely small numbers of hepatocytes showed vacuolar and hydropic degeneration **(Fig. 5a).** The kidneys revealed the normal histological appearance of both cortex and medulla. The renal cortex showed the normal histological structure of glomeruli and convoluted tubules **(Fig. 5b).**

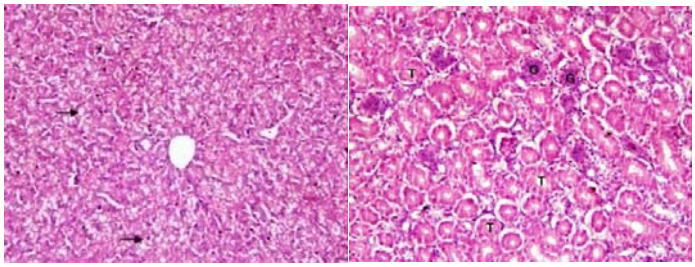


Fig. 5 a Liver of control duckling, and b showing normal histological criteria of hepatic tissue with vacuolar and hydropic degeneration of some hepatocytes (arrow). H&E stain x 200.

Fig. 5b kidney of control duckling, Group 6, showing normal histological criteria of glomeruli (G) and renal tubules (T). H & E stain x 200.

3.6.2 Effect of aflatoxin B_1 alone on liver and kidney tissue of ducklings (G 5)

Ducklings received feed contaminated with 0.018 ng/ml aflatoxin B_1 at rate 1 ml/100 g feed resulted that, the livers showed congestion of the central veins and portal blood vessels. Fibrin thrombi were noticed in the lumen of the central and portal veins **(Fig. 6).** Activation of Von Kupffer,s cells and the presence of small numbers of macrophages and lymphocytes were noticed in the dilated sinusoids **(Fig. 7).** Perivascular oedema mixed with inflammatory cellular infiltration mainly lymphocytes **(Fig. 8)** was occasionally observed. Multifocally, the perivascular interstitium was expanded by mononuclear cellular aggregates **(Fig.9)** mainly macrophages and lymphocytes **(Fig. 10).** Similar areas of mononuclear cellular aggregation displaced the hepatocytes were seen. Diffusely, the hepatic parenchyma was distorted by marked vacuolar and hydropic degeneration of the

hepatocytes characterized by swollen, pale, vacuolated cytoplasm **(Fig. 11).** Focal areas of coagulative necrosis characterized by retention of hepatic cord architecture and shrunken hepatocytes with homogenous eosinophilic cytoplasm and pyknotic nuclei. Occasionally, lytic necrosis of the hepatocytes characterized by loss of hepatic cord architecture and replaced by erythrocytes, fibrin and small numbers of inflammatory cells were noticed **(Fig.12).** Moreover, the portal areas revealed mononuclear cellular infiltration and hyperplasia of the biliary epithelium.

The examined kidneys revealed congestion of the cortical blood vessels and intertubular capillaries **(Fig. 13).** There were aggregates of mononuclear inflammatory cells, mainly macrophages and lymphocytes, separating surrounding and effacing variable areas of renal architecture in the cortex **(Fig. 14).** The glomeruli were enlarged and revealed either degeneration of glomerular tufts characterized by vacuolization of mesangial/endothelial cells **(Fig. 15)** or coagulative necrosis with retention of tuft architecture, hypereosinophilic cytoplasm and pyknosis **(Fig. 16).** Multifocally, there was vacuolar and hydropic degeneration of the lining epithelial cells of some proximal and distal convoluted tubules characterized by swollen pale vacuolated cytoplasm **(Fig. 17).** Coagulative necrosis of tubular epithelial cells characterized by hypereosinophilic cytoplasm and pyknotic nuclei were also detected **(Fig. 18).** The lumina of some renal convoluted tubules contained homogenous, brightly eosinophilic material (hyaline casts) **(Fig. 19).** Moreover, remaining tubules at all levels were moderate to markedly ectatic and lined by attenuated epithelium.

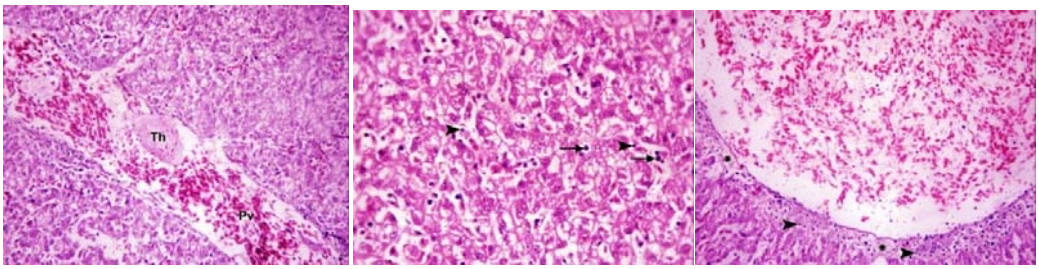


Fig. (6): Liver of duckling treated with aflatoxin, Group 5, showing marked dilatation and congestion of portal vein (PV) with fibrin thrombus (Th) in the lumen. H&E stain x 200.

Fig. (7): Liver of duckling treated with aflatoxin, Group 5, showing small numbers of macrophages (arrow) and lymphocytes (arrow head) in the dilated sinusoids. H&E stain x 400.

Fig. (8): Liver of duckling treated with aflatoxin, Group 5, showing perivascular oedema (asterisk) mixed with lymphocytic cellular infiltration (arrowhead). H&E stain x 200.

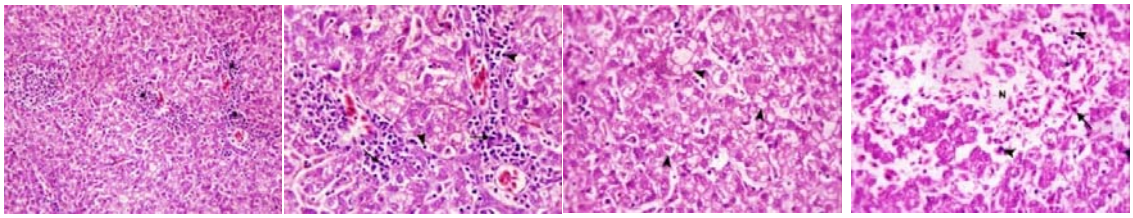


Fig. 9 Liver of duckling treated with aflatoxin, Group 5, showing perivascular mononuclear cellular aggregates (asterisk). H&E stain x 200.

Fig. 10 Hight power of the previous figure showing perivascular mononuclear cellular aggregates mainly macrophages (arrow) and lymphocytes (arrow head). H&E stain x 400.

Fig. 11 Liver of duckling treated with aflatoxin, Group 5, showing marked vacuolar and hydropic degeneration of the hepatocytes characterized by swollen, pale, vacuolated cytoplasm (arrowhead). H&E stain x 400.

Fig. 12 Liver of duckling treated with aflatoxin, Group 5, showing lytic necrosis (N) of the hepatocytes characterized by loss of hepatic cord architecture and replaced by erythrocytes (arrow), fibrin and small numbers of inflammatory cells (arrowhead). H&E stain x 400.

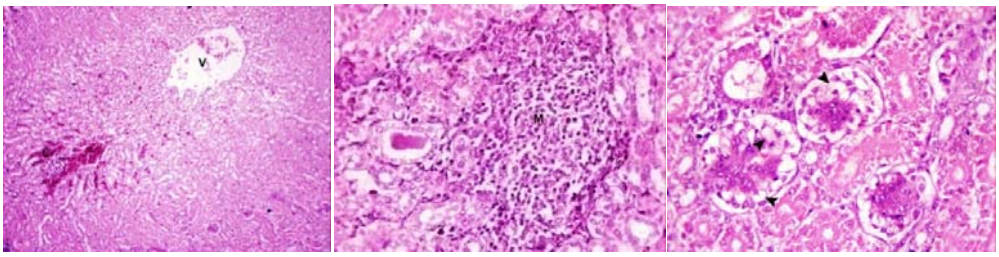


Fig. 13 Kidney of duckling treated with aflatoxin, Group 5, showing congestion of the cortical blood vessels (V) and intertubular capillaries (C). H&E stain x 100.

Fig. 14 Kidney of duckling treated with aflatoxin, Group 5, showing aggregates of mononuclear inflammatory cells (M), separating, surrounding and effacing variable areas of renal architecture in the cortex. H&E stain x 400.

Fig. 15 Kidney of duckling treated with aflatoxin, Group 5, showing enlarged glomeruli with degeneration of glomerular tufts characterized by vacuolization (arrowhead) of mesangial/endothelial cells. H&E stain x 400.

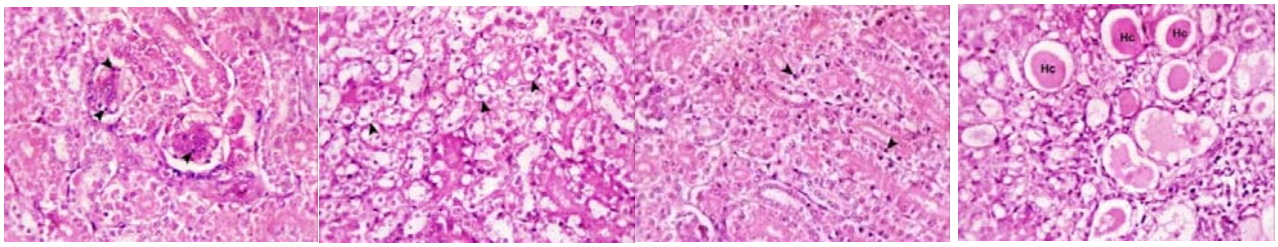


Fig. 16 Kidney of duckling treated with aflatoxin, Group 5, showing coagulative necrosis of glomerular tuft cells with retention of tuft architecture, hypereosinophilic cytoplasm and pyknosis(arrowhead). H&E stain x 400.

Fig. 17 Kidney of duckling treated with aflatoxin, Group 5, showing marked vacuolar and hydropic degeneration of the lining epithelial cells of some proximal and distal convoluted tubules characterized by swollen pale vacuolated cytoplasm(arrowhead). H&E stain x 400.

Fig. 18 Kidney of duckling treated with aflatoxin, Group 5, showing coagulative necrosis of tubular epithelial cells characterized by hypereosinophilic cytoplasm and pyknotic nuclei (arrowhead). H&E stain x 400.

Fig. 19 Kidney of duckling treated with aflatoxin, Group 5, showing hyaline casts (Hc) in the lumen of some renal convoluted tubules. H&E stain x 400.

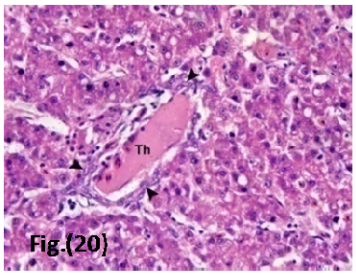
7. Improve liver and kidney tissues by using propolis extract

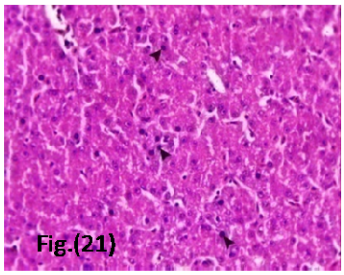
7.1 Ducklings received Aflatoxin B_1 plus propolis 2% (G 1)

The livers of the treated ducklings showed congestion of the central veins, portal blood vessels. Perivascular edemas infiltrated with mononuclear inflammatory cells were noticed in few cases. There were fibrin thrombi partially or completely occluded the lumen of the portal veins; the portal areas revealed a mild hyperplastic

proliferation of the bile duct epithelium **(Fig. 20).** Multifocally, the surrounded hepatocytes exhibited mild to moderate degeneration characterized by discrete pale variable sizes cytoplasmic vacuoles **(Fig. 21).** Occasionally, individual coagulative necrosis of the hepatocytes hypereosinophilic cytoplasm and pyknosis shrunken hepatic cells with hypereosinophilic cytoplasm and pyknotic nuclei were also noticed **(Fig. 22).**

The examined kidneys revealed congestion of the renal blood vessels and intertubular capillaries. The renal cortex revealed perivascular oedema mixed with few numbers of lymphocytes. Rarely, glomerular tufts exhibited necrosis of mesangial/endothelial cells characterized by hypereosinophilic cytoplasm and pyknosis Multifocally, the cortical renal tubules were lined by swollen, and vacuolated epithelial cells (degeneration) **(Fig. 23)** or occasionally by epithelial cells with hypereosinophilic cytoplasm and pyknotic nuclei (necrosis) **(Fig. 24).**





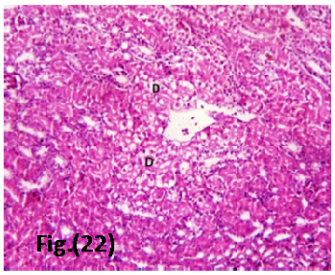
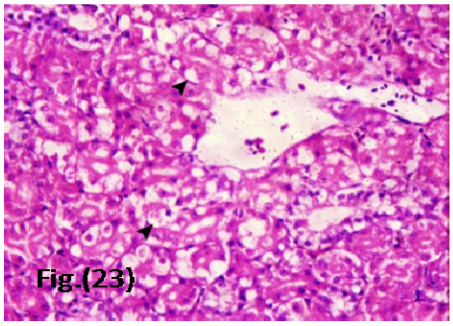


Fig. 20 Liver of duckling treated with aflatoxin and propolis at 2%, Group 1, showing fibrin thrombus (Th) in the lumen of the portal vein and the mild hyperplastic proliferation of the bile duct epithelium (arrowhead). Note also cytoplasmic vacuoles in the hepatocytes. H&E stain x 400.

Fig. 21 Liver of duckling treated with aflatoxin plus propolis extract at 2%, Group 1, showing individual coagulative necrosis of the hepatocytes with hypereosinophilic cytoplasm and pyknotic nuclei (arrowhead). H&E stain x 400.

Fig. 22 Kidney of duckling treated with aflatoxin plus propolis extract at 2%, Group 1, showing vacuolar and hydropic degeneration (D) of renal tubules. H&E stain x 200.



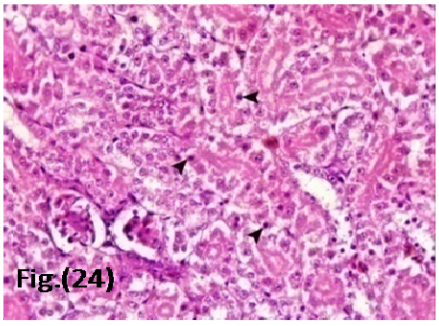


Fig. 23 High power of the previous figure showing degenerated renal tubules lined by swollen, vacuolated epithelial cells (arrowhead). H&E stain x 400.

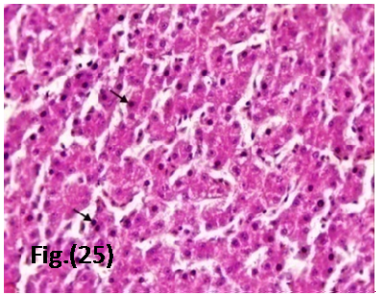
Fig. 24 Kidney of duckling treated with aflatoxin plus propolis extract at 2%, Group 1, showing necrotic renal tubules lined by epithelial cells with hypereosinophilic cytoplasm and pyknotic nuclei (arrowhead). H&E stain x 400.

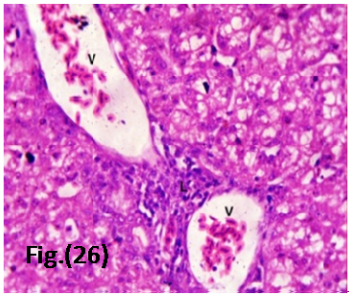
7.2 Ducklings received Aflatoxin B_1 plus propolis 5% (G 2)

The livers showed congestion of the portal blood vessels and leucocytic cellular infiltration in the portal areas particularly lymphocytes **(Fig. 25).** Fibrin thrombi partially or completely occluded the lumen of the portal veins were attached to the blood vessel wall **(Fig. 26).** Multifocally, degenerative changes of the hepatocytes in the form of vacuolar and hydropic degeneration were commonly observed around central veins **(Fig. 27).**

Occasionally, individual to random areas of coagulative necrosis characterized by retention of hepatic cord architecture and shrunken hepatocytes with hypereosinophilic cytoplasm and pyknotic nuclei were noticed **(Fig. 28).** The portal areas revealed mild hyperplasia of the biliary epithelium. Furthermore, periductal infiltrate by moderate numbers of lymphocytes was also detected**(Fig. 29).**

The examined kidneys revealed congestion of the cortical blood vessels and intertubular capillaries with focal areas of intertubular haemorrhages **(Fig. 30).** Rarely, the blood vessels revealed hypertrophy and vacuolar degeneration of the wall. Multifocally, the proximal and distal convoluted tubules in the renal cortex showed either coagulative necrosis or degenerative changes characterized by cloudy swelling, vacuolar and hydropic degeneration of their lining epithelium **(Fig. 31).** Necrotic tubular epithelial cells were hypereosinophilic with shrunken, pyknotic nuclei, loss of cellular detail, and karyolysis **(Fig. 32).** Degenerated tubular epithelial cells were occasionally swollen with pale vacuolated cytoplasm **(Fig.33).**





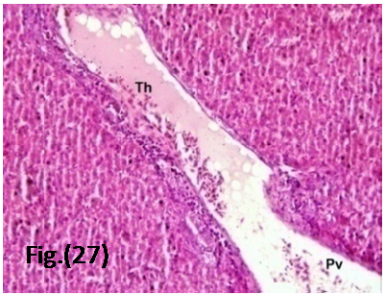
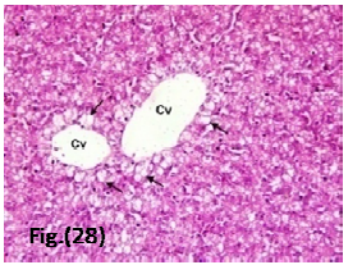
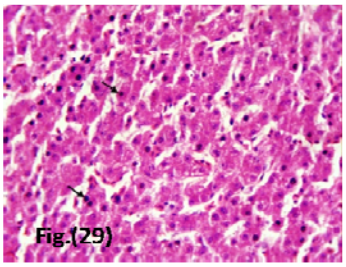


Fig. 25 Liver of duckling treated with aflatoxin and propolis 5%, Group 2, showing individual coagulative necrosis of the hepatocytes with hypereosinophilic cytoplasm and pyknoticnuclei(arrow). H&E stain x 400.

Fig. 26 Liver of duckling treated with aflatoxin plus propolis extract at 5%, Group 2, showing congestion of the portal blood vessels (V) and lymphocytic cellular infiltration (L) in the portal areas. H&E stain x 400.

Fig. 27 Liver of duckling treated with aflatoxin plus propolis extract at 5%, Group 2, showing fibrin thrombus (Th) were attached to the wall of the portal vein (Pv). H&E stain x 100.





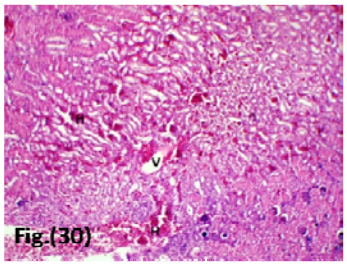
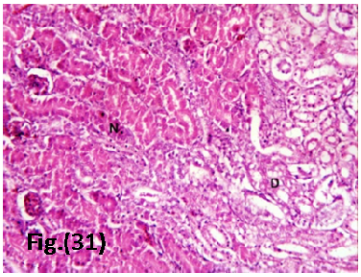
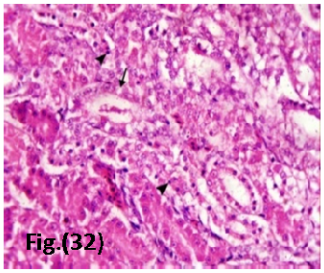


Fig. 28 Liver of duckling treated with aflatoxin plus propolis extract at 5%, Group 1, showing vacuolar and hydropic degeneration (arrow) of the hepatocytes around central veins (CV). H&E stain x 200.

Fig. 29 Liver of duckling treated with aflatoxin extract plus propolis at 5%, Group 2, showing individual coagulative necrosis of the hepatocytes with hypereosinophilic cytoplasm and pyknoticnuclei(arrow). H&E stain x 400.

Fig. 30 Kidney of duckling treated with aflatoxin plus propolis extract at 5%, Group 2, showing congestion of the cortical blood vessels (V) and intertubular capillaries with focal areas of intertubular haemorrhages (H). H&E stain x 100.





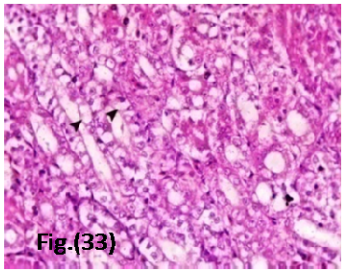


Fig. 31 Kidney of duckling treated with aflatoxin plus propolis extract at 5%, Group 2, showing coagulative necrosis (N) and degenerative changes (D) of the lining epithelium of proximal and distal convoluted tubules in the renal cortex. H&E stain x 200.

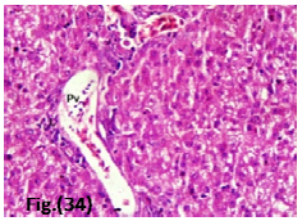
Fig. 32 Kidney of duckling treated with aflatoxin plus propolis extract at 5%, Group 2, showing necrotic tubular epithelial cells with shrunken, pyknotic nuclei(arrowhead), loss of cellular detail, and karyolysis(arrow). H&E stain x 400.

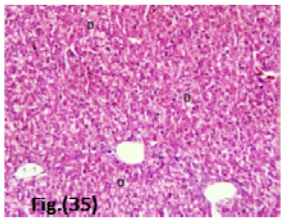
Fig. 33 Kidney of duckling treated with aflatoxin plus propolis extract at 5%, Group 2, showing degenerated tubular epithelial cells with swollen pale vacuolated cytoplasm (arrowhead). H&E stain x 400.cytoplasm.

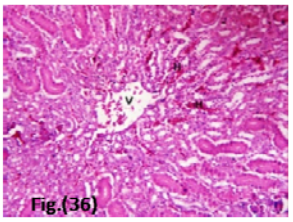
7.3 Ducklings received Aflatoxin B_1 plus propolis 10% (G 3)

The livers of the treated ducklings showed congestion of the central veins and portal blood vessels, with mild perivascular inflammatory cellular infiltration mainly lymphocytes **(Fig. 34).** Multifocally, there were random areas of vacuolar and hydropic degeneration of the hepatocytes characterized by swollen pale vacuolated cytoplasm **(Fig. 35).** Occasionally, individual coagulative necrosis of the hepatocytes characterized by retention of hepatic cord architecture and shrunken hepatocytes with hypereosinophilic cytoplasm and pyknotic nuclei were noticed.

The examined kidneys revealed congestion of the renal blood vessels and intertubular capillaries with focal areas of intertubular haemorrhages **(Fig. 36).** The glomeruli and renal convoluted tubules in both cortex and medulla revealed normal histological appearance similar to the control group. It was an enhancement as shown in **(Fig. 37).** Rarely, few tubules were lined by swollen, vacuolated epithelial cells (degeneration) or by epithelial cells with hypereosinophilic cytoplasm and pyknotic nuclei (necrosis). It was the best concentration used.







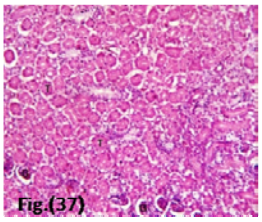


Fig. 34 Liver of duckling treated with aflatoxin plus propolis extract at 10%, Group 3, showing congestion of the portal blood vessels (Pv), with mild perivascular inflammatory cellular infiltration, mainly lymphocytes (L). H&E stain x 400.

Fig. 35 Liver of duckling treated with aflatoxin plus propolis extract at 10%, Group 3, showing random areas of vacuolar and hydropic degeneration (D) of the hepatocytes characterized by swollen pale vacuolated cytoplasm. H&E stain x 200.

Fig. 36 Kidney of duckling treated with aflatoxin plus propolis extract at 10%, Group 3, showing congestion of the renal blood vessels (V) and intertubular capillaries with focal areas of intertubular haemorrhages (H). H&E stain x 200.

Fig. 37 Kidney of duckling treated with aflatoxin plus propolis extract at 10%, Group 3, showing the normal histological appearance of the glomeruli (G) and renal convoluted tubules (T) in the cortex. H&E stain x 200.

7.4 Ducklings received Aflatoxin B_1 plus propolis 20% (G 4)

The microscopical examination of livers showed congestion of the central veins and portal blood vessels. Multifocally, mild vacuolar and hydropic degeneration of the hepatocytes characterized by swollen pale vacuolated cytoplasm were seen **(Fig. 38).** However, variable areas of hepatic parenchyma preserve normal histological criteria as a result of liver improvement.

The examined kidneys revealed congestion of the renal blood vessels and intertubular capillaries. The renal cortex revealed massive areas of haemorrhages around the congested blood vessel **(Fig. 39)** and in between the convoluted tubules **(Fig. 40).** Moreover, the glomeruli and renal convoluted tubules in both cortex and medulla revealed normal histological appearance similar to the control group **(Fig. 41).** It was improved exhibit the renal cortex revealed massive areas of haemorrhages around the congested blood vessel.

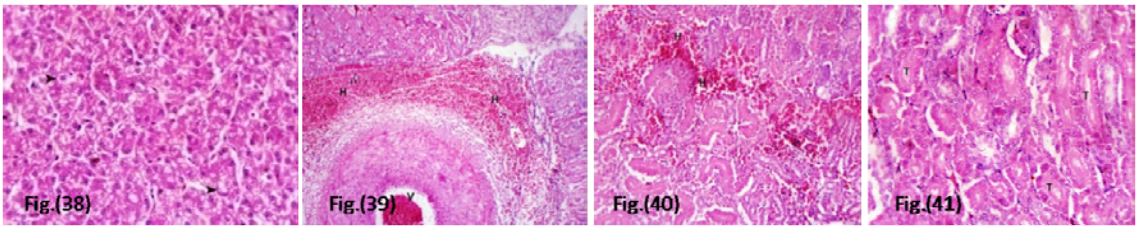


Fig. 38)Liver of duckling treated with aflatoxin plus propolis extract at 20%, Group 4, showing mild vacuolar and hydropic degeneration of the hepatocytes characterized by swollen pale vacuolated cytoplasm (arrow head). H&E stain x 200

Fig. 39 Liver of duckling treated with aflatoxin plus propolis extract at 20%, Group 4, showing massive areas of haemorrhages (H) around the congested blood vessel (V). H&E stain x 100.

Fig. 40 Liver of duckling treated with aflatoxin plus propolis extract at 20%, Group 4, showing massive areas of haemorrhages (H) in between the convoluted tubules. H&E stain x 200.

Fig. 41 Kidney of duckling treated with aflatoxin plus propolis extract at 20%, Group 4, showing the normal histological appearance of renal convoluted tubules (T) in the cortex. H&E stain x 400.

Similar results were obtained by many investigators. [28,50] reported that one to seven day old duckling given a feed with a concentration of 300 to 600 ppb of AFB_1 for 7 to 14 days had serious hepatic lesions, as well as significant death rate. [51] concluded that, the toxicity of aflatoxins, in general, may be categorized as acute or chronic. Acute aflatoxicosis causes marked signs of disease or death. The liver is usually pale, atrophied or necrotic. Symptoms include loss of appetite and lethargy leading to death. Chronic aflatoxicosis are not readily discernible in affected animals. Visible symptoms may include reduced growth, appetite, and feed efficiency. The liver may appear normal but histological examination will likely reveal abnormalities. Aflatoxins had detrimental effects, which increased with level, on phagocytotic ability and tumour necrosis factor-like substance secretion [51] and caused liver damage [52] in ducks. AF acts as an inhibitor of protein synthesis and, subsequently, dividing cells and tissues with a high protein turnover such as that found in the liver, immune system or gut epithelium, which is most susceptible to the toxic effects of AF. In this respect, exposure to AF has

been demonstrated to suppress the immune response in poultry. AF can repress the development of the thymus gland or influence the relative weight of the bursa of Fabricius, which may result in serious deficiencies in both cellular and antibody responsiveness of the chicken immune system [52].

Conclusion: This study is one of the first where an antimicrobial property of propolis against *A. flavus* fungus which contaminant chicken and fish feeds. These results suggest that the propolis extract is excellent antifungal activities. This approach is considered as an environmental friendly approach in contrast to physical and biological techniques of detoxification. Various methods of decontamination are found to vary in their efficacy in toxin removal from feed products. The cost of the decontamination process is very important in choosing the cheapest and the most effective method in aflatoxin removal from the contaminated feed products.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

Consent: NA

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