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Effects of *Vitellaria paradoxa* bark extracts on performance, histology and serum biochemistry of *Aspergillus*-challenged broiler chicks

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Abstract
A study was conducted on the antifungal properties of extracts of *Vitellaria paradoxa* bark on the performance of broiler chicks. The treatments were five levels of *V. paradoxa* bark extracts *viz*: non-challenged birds; birds challenged administered with antifungal agent; birds challenged without intervention; and challenged birds given 5, and 10mg/ml *V. paradoxa* bark extract. Feed intake was significantly ($p>0.05$) depressed due to infection by *A. flavus.* Birds given the positive control diet had the highest feed intake (510.0g/bird) while the birds on the negative control had the lowest feed intake. Feed intake for birds given *V. paradoxa* extract however, was dose related. At 10mg/ml, feed intake was similar to the values of birds fed the antifungal agent. Weight gain, protein and fat retention followed a similar trend as the feed intake. Broilers given positive control had significantly ($p>0.05$) highest weight gain (216g/bird). Relative weights of specific organs of broilers given the various treatments suggest that aflatoxicosis affects both the liver and the lungs. Birds given the negative controls had inflamed hepatocytes and deranged ileal mucosa. These histological effects were masked in birds given the synthetic antifungal agent as well as extracts of *V. paradoxa* bark (especially at 10mg/ml). The various treatments influenced only the serum protein. This study shows that bark extract of *V. paradoxa* exhibits potent antifungal properties comparable to the standard antifungal agent used. It is recommended that extract of *V. paradoxa* be applied to chicken production at 10mg/ml as an antifungal agent, especially against *A. flavus* infection.

Keywords: Aflatoxicosis; Antifungal; Broiler chicks; Histology; Performance

INTRODUCTION

Aspergillus flavus is a major food-borne pathogen that produces aflatoxin, a toxic and carcinogenic compound (Anath and Fayd, 2000). It is a leading cause of aflatoxicosis in poultry which results from ingestion of a contaminated feed. Aflatoxins, potent mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are a major concern in the poultry production. Aflatoxicosis in poultry production is characterized by restlessness, anorexia with lowered growth rate, poor utilization, decreased weight gain, decreased egg weight and production, increased susceptibility to environment and microbial stresses and mortality (Leeson *et al.*, 2005). The consumption of mycotoxin-contaminated diets by broilers have been reported to induce haematological, biochemical and liver physiological changes and growth depression, economic losses, increased mortality,

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decrease blood cell count, lower egg production, lower feed consumption, impairs resistance to infectious disease, reduces vaccination efficiency, gross and microscopic changes in the liver and other organs, such as hepatomegally, paleness, hydropic degeneration, fatty changes, bile duct, hyperplasia and periportal fibrosis (Espada *et al*., 1992; Fernandez *et al*., 1994; Ledoux *et al.*, 1999; Ortatatli and Oguz, 2001). Sur and Celik, (2003) reported that depletion of lymphoid organs such as the thymus and bursa of fabricius is a common manifestation of *A. flavus* infection, other symptoms include kidney and spleen lesion (Glahn *et al.*, 1991; Bilgic and Yesildere, 1992) unfavourable reproductive changes (Ortatatli *et al*., 2002). Impairment of the humoral and cellular immune response and infections agents (Ibrahim *et al*., 2000; Oguz *et al.*, 2003). Other concern is the possible transmission of fungal mycotoxin residues to meat and eggs from infected chicken which is potentially hazardous to human health. Heterocyclic metabolites of the genera *Aspergillus* are aflatoxin and ochratoxin. Celik *et al.*, (2000) reported that both lower and higher doses of AFB_1 affect the haematological parameters of the broiler chick resulting into a depressed cellular immunity due to suppression of the phagocytic activity of microphages and decrease in T-lymphocyte.

Aflatoxins has been known to be toxic and reported to cause immune suppression in birds (Pardue *et al*., 1985). These immune suppression effects of aflatoxins predispose the animal to many secondary infections due to other fungi bacteria and viruses (Javed *et al*., 2005). Sur and Celik (2003) reported that contamination of broiler feeds with aflatoxin resulted in a drastic reduction in performance both from growth and feed efficiency standpoint. The aflatoxin producing fungus, *Aspergillus flavus* is a causal agent of pre-harvested contamination of food commodities which can result in serious economic hardship to producers and adverse health impact on both humans and domestic animals. The liver is the primary target organs in animals and human where Aflatoxins B1 is metabolized to the toxic and carcinogenic Aflatoxins B1-epoxies form by cytochrome P450 enzyme (Harris *et al*., 1989). The disease produces hard nodular area in the lungs and an infection of the air sacs. Sometimes the air sac lesions are similar to that produced by infections sinusitic or CRD. In some birds, colonies of mold growth can be seen on the air sac membrane (Bolu *et al.*, 2011).

The shea tree or *Vitellaria paradoxa* is a member of the sapotaceae family and commonly called *igi emi* (Yoruba) *kwara kandaya* (Hausa) and *okwum* (Igbo). The plant is a small deciduous tree found commonly growing in savanna areas of the African continent (Lowe and Soladoye, 1999). *Vitelleria paradoxa* is a relatively versatile species, reflected in its large geographical distribution. The approximate chemical composition of the kernel per 100g dry matter is: fat 31-62g, protein 7-9g, carbohydrate 31-38g, unsaponifiable matter 2-12g. The fatty and composition of shea butter is approximately lauric acid trace, myristic acid trace, palmitic acid 4-8%, stearic acid 31-45% oleic acid 43-56%, linoleic acid %, linolenic acid trace and arachidic acid 1-2% (Aguzue, 2013). Roots have been used for medicinal purpose for horses (Ndukwe *et al*., 2007). Ahmed (2009) reported that the stem bark extraction has antifungal properties. This study investigated the effects of cold extract of the bark of *Vitellaria paradoxa* on the performance of broiler chicks challenged with *A. flavus*.

EXPERIMENTAL

Plant collection. The bark of Shea butter tree (V*itellaria paradoxa*) was collected from the permanent site of the University of Ilorin, Ilorin and environ. Ilorin is located on Latitude 08 29’N and longitude 004 35’E.

The elevation is 305m 1001'. Annual temperature range is 22-34C and annual precipitation is 80-12-mm (World Climate 2013). The plant was identified by experts in the Herbarium Unit of the University. The bark was collected daily at 08:00hrs and air-dried to a constant weight. The samples were pre-crushed in a mortar, and thereafter, it was blended in an electric blender (Moulinex, Philips) to a fine particle (0.5mm). 100g of the sample was soaked in 500ml of water for extraction. The mixture was fitted to a rotary shaker and agitated at 60rpm for 4hrs. The mixture was further filtered through a sterile 0.45um Millipore filter. The filtrates were evaporated to semi-solid mass and subsequently dried in a beaker on water bath to give a dark brown resinous mass. The dry extracts were later concentrated using the rotary evaporator (Model 349/2, Corning Limited) for the antimicrobial activity evaluation (Banso and Ayodele, 2001). The extract was reconstituted to 5mg/ml and 10mg/ml and administered through the drinking water in the treatments.

Source of *A. flavus*. *A. flavus* spore was collected from the Department of Microbiology, University of Ilorin and established by growing a plate of *Aspergillus flavus* on a culture of Potato Dextrose Agar (PDA) and incubated at 28°C for 5 to 7 days. The spores were later scraped off the surface of the culture plate for inoculation.

Experiment and management of birds. One hundred (100) day old Hubbard broilers of mixed sexes were purchased from a commercial hatchery in Ilorin, Nigeria. The birds were brooded in an electrically heated metabolic cage and thereafter allotted randomly into 5 different treatments (Table 2) and each treatment was replicated in four pens containing 5 birds per replicate. Birds were given a basal diet (Table 1) and water *ad libitum* during the trial period. Routine vaccinations and medications were administered.

Inoculation of chick feed with *A. flavus* spores. At the second week, bird feeds (except the positive control) were inoculated with the spores of *Aspergillus flavus.* The infected birds were placed under close observation for three to seven days within which they would have manifested infections. Confirmation of infection was established by caseous yellow deposits in the lung.

Data collection. The experiment was conducted for 6 weeks. Body weights of broilers were determined weekly. Feed consumption and weight gain were recorded and feed conversion ratio (feed intake/weight gain) was calculated. Mortality was recorded daily. During the third week of the 8-week study, protein and fat nutrient retention were carried out for 72 hours. Nutrient retention was calculated as follows:-

$$\text{Nutrient Retention} = \frac{(\text{Nutrient consumed} - \text{Nutrient voided in faeces}) \times 100.}{\text{Nutrient consumed}}$$

Collection of blood samples. At 42 days of age, two birds per replicate were randomly selected and slaughtered by cutting through the jugular vein. Blood samples were collected into vials containing EDTA to avoid blood clot formation. The red blood cell (RBC) and white blood cell (WBC) counts were determined by a haemocytometer method using Natt-Herrick solution; haematocrit (HCT) and haemoglobin (Hb) values were measured by microhematocrit and cyanomethemoglobin methods, respectively (Kececi *et al.,* 1998). Serum samples were collected by collecting blood samples into non-heparinised tubes and the blood was centrifuged to obtain serum. Individual serum samples were analyzed for total protein, total cholesterol and glucose using a kit (Pars Azmoon Company, Tehran, Iran). After slaughtering, the birds were dissected and the organs required for

histology (breast muscle, ileum and liver) were quickly dissected, and preserved in 10% formalin solution. The tissues were trimmed, fixed in Bouin fixation for 24 hrs, embedded as section at 5-6µ with a microton and stained with haematoxylin and eosin. The histological study was carried out according to the described method by King *et al.,* (1980).

Statistical analysis. Data were analyzed as a completely randomized design (CRD) by 1-way ANOVA procedure of SAS software (SAS Institute, 2003). Differences between treatment means were separated using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Feed intake was significantly ($p>0.05$) influenced by the treatments (Table 3). Feed intake depression was observed due to infection by *A. flavus*. Birds given the positive control diet had the highest feed intake (510.0g/bird) while the birds on the negative control had the lowest feed intake (321.8g/bird). Feed intake for the birds given *V. paradoxa* extract was directly related to the concentration. At 5 and 10mg/ml, *V. paradoxa* exhibited antifungal properties similar to the standard antifungal agent by masking feed intake depression associated with *A. flavus* infection. Weight gain followed a similar trend as the feed intake. Broiler chicks given the positive control had the highest weight gain (216g/bird). Feed conversion ratio was not influenced ($p<0.05$) by the treatments but broilers given the negative control had the least feed conversion. Protein and fat retention followed similar trends with the performance parameters. Leeson *et al*. (2005) reported that the symptoms of aflatoxicosis include anorexia, poor feed utilization and growth depression. In the same vein, Sur and Celik (2003) reported that contamination of broiler ration with aflatoxin resulted in a drastic reduction in performance both from growth and feed efficiency standpoint. Harris *et al*. (1989) reported that the liver is the primary target organs of Aflatoxins B1 in animals where it is metabolized to the toxic and carcinogenic Aflatoxins B1-epoxies form by cytochrome P450 enzyme. Feed intake and utilization was affected by *A. flavus* infection which resulted in the observed feed intake depression and poor growth of the birds fed the negative control treatment. However, birds given oral administration of *V. paradoxa* bark extract had better performance despite *A. flavus* challenge. This observation is consistent with the report of Ahmed (2009) that cold extracts of *V. paradoxa* exhibited antifungal properties against *A. fumigates*. In the same vein, Steven *et al*., (2003) reported that *V. paradoxa* contained eight catechin compounds, quercetin and trans-cinnamic acid with gallic acid as the major (27%) phenolic compound. Phytochemical screening of V. *paradoxa* has been reported to yields such substances as alkaloids, saponins, tannins and cardiac glycosides (El-Mahmood *et al*., 2008). Most of these compounds are known to inhibit microbial growths in a non-specific way. (Ayankunle *et al*., 2012).

Table 1. Composition of Experimental Diet (% DM)

Ingredients	% Inclusion
Maize	37.0
Corn Bran	6.0
Groundnut Cake	24.0
Soya bean Meal	24.0
Fishmeal	2.6
Bone Meal	2.5
Oyster Shell	1.5
Salt	0.2
*Vitamin/mineral Premix	0.2

Total	100.0

Nutrient composition. Protein: 23.5 %; Energy: 2700 kcal/kg; *Vitamin mineral premix contains antioxidant 125mg, Biotin 80mg, Choline chloride 500mg, cobalt 240g, copper 6mg, folic acid 1000mg, Iodine 1.4mg, selenium 240mg, Vitamin A 15,000IU, Vitamin B_1 200mg, Vitamin B_2 600mg, Vitamin B_6 400mg, Vitamin D_3 3000IU, Vitamin E 3000IU, Vitamin K 250mg, Zinc 60mg, Vitamin B_{12} 20mg.

Table 2. Composition of experimental treatments

Treatment	Infected with *A. flavus*	Supplemented with *V. paradoxa*	Supplemented with antifungal (furaprol)	Remark
1	-	-	-	Positive control
2	+	-	-	Negative control
3	+	-	+	Antifungal
4	+	+	-	5mg/ml
5	+	+	-	10mg/ml

Table 3. Effects of treatments on the performance of broiler chicks

Parameters	Treatment					SEM
	+ve control	Standard (antibiotic)	-ve control	5mg/ml extract	10mg/ml extract	
Total Feed intake (g/bird)	510.40^a	473.00^b	321.80^c	463.80^b	473.90^b	11.25
Total Weight gain (g/bird)	256.10^a	230.50^b	144.90^c	228.40^b	230.70^b	10.68
Feed:gain	2.0	2.0	2.3	2.0	2.0	1.58
Protein retention (%)	79.40^a	72.45^b	52.21^c	69.30^b	70.14^b	4.36
Fat retention (%)	80.40^a	58.21^c	35.25^d	67.43^b	78.27^a	4.85
Mortality (%)	0	0	4	1	0	5.26

a,b,c values having similar superscript within the row not significantly different (p>0.05)

Table 4. Effects of treatments on the relative weight of the specific organs of broilers (g/kg body weight)

ORGAN	TREATMENT					SEM
	+ve control (g)	Standard control (g)	-ve control [g]	5mg Extract (g)	10mg extract [g]	
Liver	16.43^a	12.94^b	9.0^c	12.19^b	14.01^b	2.05
Lung	4.60^a	2.40^b	1.50^c	3.50^a	3.80^a	1.10
Spleen	0.70	0.60	0.40	0.40	0.60	0.43
Heart	3.70	2.40	2.00	3.40	3.70	2.45

a,b,c values having similar superscript within the row not significantly different (p>0.05)

Table 5. Effects of treatments on the histology of specific organs

Treatment level (T)	Organs	Observation
Control	Breast muscle	Normal muscular tissue
	Ileum	Normal intestine tissue
	Liver	Normal liver tissue with evidence of regeneration (rapid mitotic division)
Standard control [Antifungal]	Breast muscle	Normal muscular tissue
	Ileum	Normal intestine tissue
	Liver	Mildly degenerated liver tissue
Negative control	Breast muscle	Stretched muscular tissue with mild nucleo-cytosis.
	Ileum	Mildly inflamed intestinal tissue
	Liver	Inflamed, hyper chronic liver tissue
5mg extract	Breast muscle	Normal muscular tissue
	Ileum	Mildly inflamed intestinal tissue
	Liver	Mildly inflamed liver tissue
10mg extract	Breast muscle	Normal muscular tissue
	Ileum	Normal intestine tissue
	Liver	Normal inflamed liver tissue

Table 6. Effects of treatments on the haematology and serum biochemistry of broilers

ORGAN	TREATMENT					SEM
	Control (g)	Standard control (g)	Negative control [g]	5mg extract (g)	10mg extract [g]	
PCV (%)	25	25	26	28	27	3.22
RBC (x 10^{12}/l)	10.44	11.12	10.25	11.21	10.94	2.46
WBC (x 10^{9}/l)	9.20	10.10	9.60	9.72	9.25	1.64
Neutrophils (%)	65.50	68.33	67.33	69.83	65.82	3.68
Lymphocytes (%)	28.67	29.67	27.67	29.73	29.55	2.92
Glucose (mmol/L)	3.20	2.70	2.65	2.70	2.90	0.96
Serum protein (mmol/l)	79.86[a]	76.45[a]	70.44[b]	75.48[a]	78.20[a]	3.96

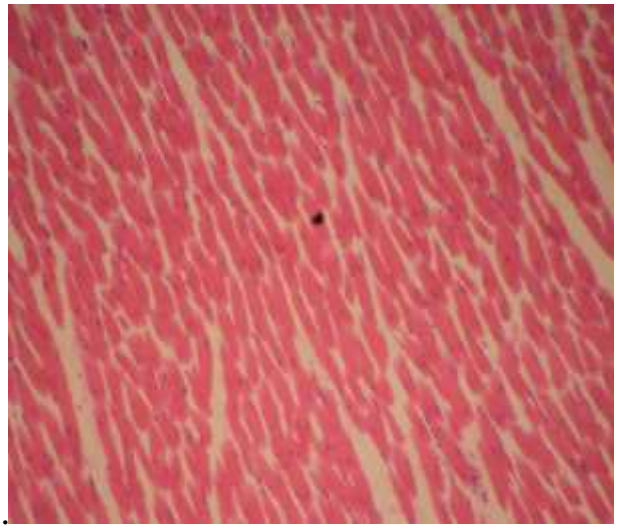

Fig. 1: Micrograph showing normal breast muscle of positive control birds x40

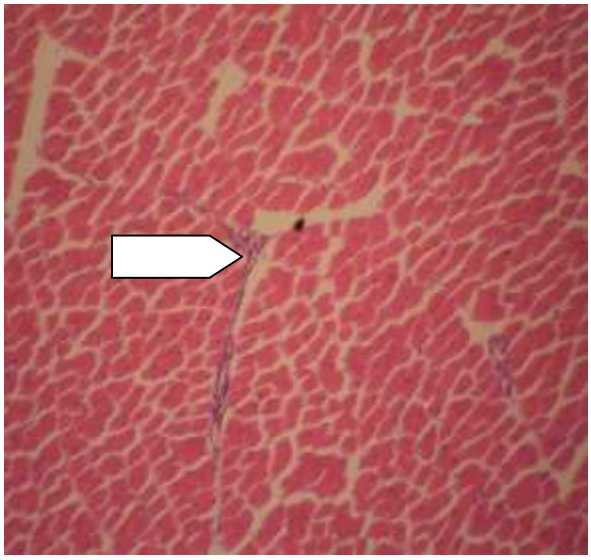

Fig 2: Micrograph showing lesions in the breast muscle of negative control birds x40

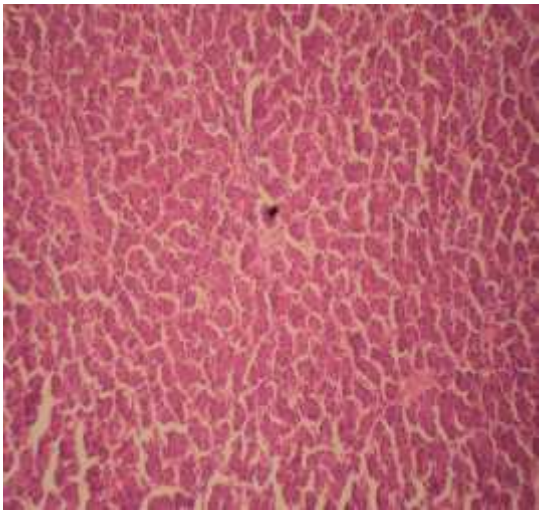

Fig 3: Micrograph showing normal liver of positive control birds x40

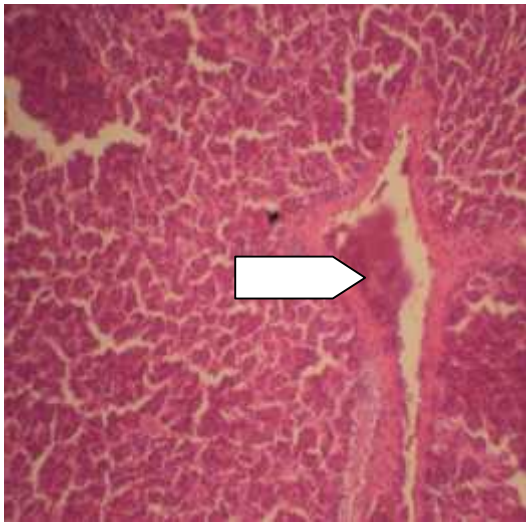

Fig 4: Micrograph showing inflamed liver architecture of negative control birds x40

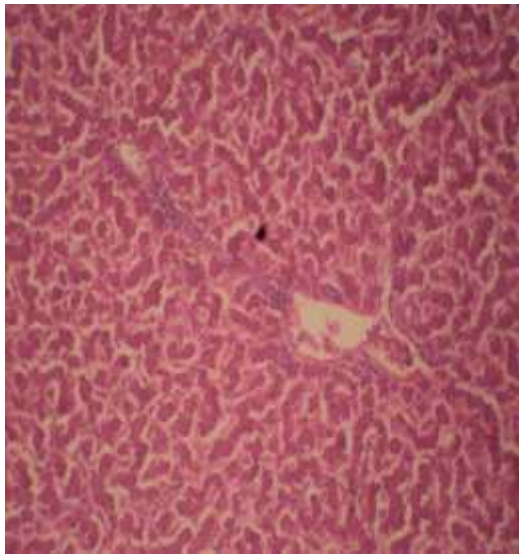

Fig 5: Micrograph showing normal liver structure at 10mg/ml extract x40

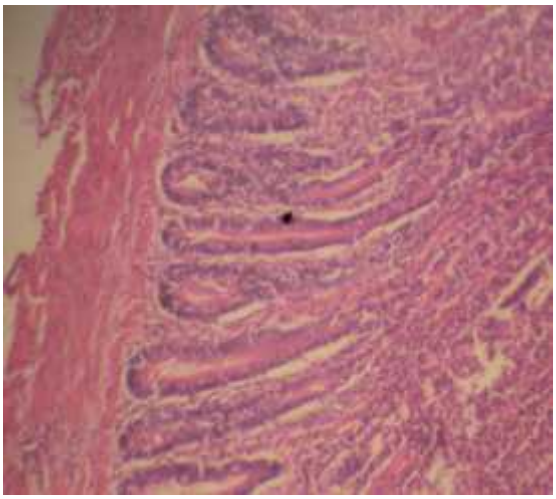

Fig 6: Micrograph showing normal ileal mucosa (villi) at 10mg/ml extract x40

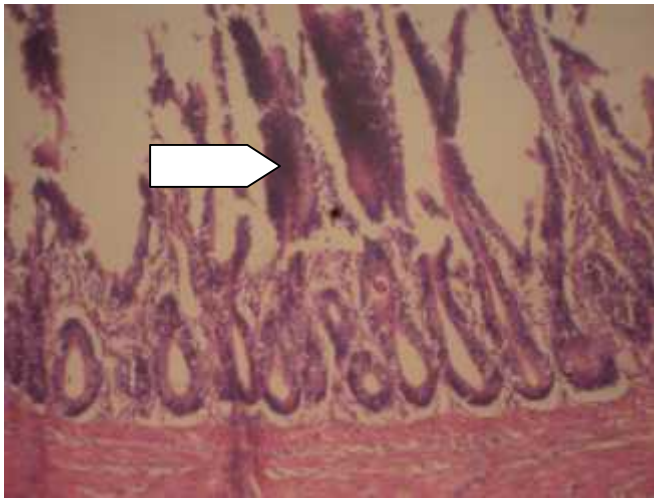

Fig 7: Micrograph showing abnormal ileal mucosa of negative control birds x40

Histology. The relative weight of specific organs of broilers given the various treatments suggests that aflatoxicosis affects both the liver and the lungs (Table 4). These organs were found to have reduced in relative weight in the negative control birds as compared with birds in the as compared to the other treatments. The spleen and heart were however found not to be significantly ($p<0.05$) affected by aflatoxicosis. The histological sections of the various tissues and organs are presented in micrographs (Figs 1-7). The bird given the negative controls had inflamed hepatocytes and deranged ileal mucosa (villi) (Table 5). These histological effects were masked in birds given the synthetic antifungal agent as well as cold extracts of *V. paradoxa* bark (especially at 10mg/ml). It has been reported that the liver is the primary target organs of Aflatoxins B1 (Harris *et al*., 1989; Che *et al*., 2011) while Bolu, *et al.* (2011) reported that *A. flavus* produces hard nodular area in the lungs and the air sacs.

Blood profile. The various treatments did not influence most of the blood profile parameters significantly ($p<0.05$) except for serum protein. Birds fed the negative control had the least value (70.44mmol/l). The results obtained in this study on haematology disagrees with earlier reports that

aflatoxicosis compromised haematological competences (Celik *et al*., 2000; Javed *et al*., 2005; An *et al*., 2008; Kamalavenkatesh *et al*., 2005 and Oguz *et al.,* 2000). The blood profile in this study is within normal range. However, the values obtained for serum protein tend to suggest that aflatoxicosis influences protein metabolism. Total serum protein is indicative of storage protein. The relatively low serum protein observed for negative control broilers was indicative of poor amino acid economy (Annongu, 1997; Bolu and Balogun, 2000).

Conclusion. From the findings of this study, cold bark extract of *V. paradoxa* exhibited potent antifungal properties comparable to the standard antifungal agent used. The antifungal potency is directly related to the concentration of the extract. At 10mg/ml, the effect of the extract was more pronounced. It is recommended that cold extract of *V. paradoxa* be applied to chicken production at 10mg/ml as an antifungal agent, especially against *A. flavus* infection. The trend observed in this study suggests that higher levels of the extract may give better potency.

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