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HAEMATOLOGICAL AND WEIGHT CHANGES IN BROILERS DOSED WITH N-BUTANOL FRACTIONS OF *KHAYA SENEGALENSIS* EXTRACT

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ABSTRACT

A study was conducted to evaluate the weight changes of broiler chickens dosed with n-butanol fraction of *Khaya senegalensis* leaf extract. Sixty day-old broiler chicks were sourced from NAPRI, Zaria, brooded under standard management practice for 4 weeks and divided into 6 groups namely A, B, C, D, E and F. Graded doses of the n-butanol fraction of the leaf extracts of *Khaya senegalensis* of 11 mg/kg, 33 mg/kg, 99 mg/kg, 297 mg/kg and 891 mg/kg were administered orally to the birds in the respective groups listed above at four weeks old. Birds in Group F were given 0.2 ml of distilled water as control. The chickens were monitored for 14 days and those that survived were further observed for another two weeks after which the test was terminated. Two chickens per group were randomly selected, euthanized and post mortem examinations were conducted with samples taken for histopathology. The study confirmed the safety of the extract of *K senegalensis* even at high doses of 891 mg/ kg as regards to no clinical signs and mild gross lesions seen in this study as well as the high survival rate of 100% in the five medicated groups. Enlarged liver, heart and echymotic haemorrhages of the serosal surface of the duodenum characterized the gross lesions associated with the administration of the extracts in all doses. The histopathological lesions showed congestions of the intertubular spaces in the kidneys of the birds treated with 33 mg/kg and 297 mg/kg. A higher dose of 891 mg/kg had the liver of the treated birds with congested sinusoidal spaces. None of the haematological values were significantly changed when the extract was given even at 891mg/kg. Lymphocytosis was noticed across the treated groups. The progressive weight increase in both the medicated and un medicated group shows the ability of the extract to improve weight gain. Among the treatment groups, the differences in weight gain were recorded with highest in the 11mg/kg and the lowest in the 891mg/kg groups. Thus at lower doses (below 99mg/kg) *K senegalensis* can be used as growth promoter due to the increase in weight. This implies that though *Khaya senegalensis* causes weight gain as earlier established, it has some effect on vital organs and such can only be administered for a short duration. Due to paucity of information on the health benefits of the leaves of the plant *Khaya senegalensis* as they relate poultry production, it is recommended that more research in this area could be done.

Key words: *Khaya senegalensis,* extracts, haematology, weight, histopathology, broilers.

INTRODUCTION

Poultry keeping is the dominant form of livestock production in developing countries. In Nigeria, the rising cost of poultry feeds, problems of drug residues and resistance to diseases have become major problems militating against the poultry industry (Ogbe *et al*., 2008). Porter (1998) estimated that feed costs for poultry comprise roughly 66 % of the total production costs for eggs, broiler chickens or turkeys. In addition, severe enteric damage by bacterial infection will cause overt illness and high mortality in a poultry flock (Elmulsharaf *et al*., 2006). Consequently, due to the ban on antimicrobial growth promoters (AGP), the use of plant extracts has gained attention in animal feed strategies and formulations (Tipu *et al*., 2006).

Khaya senegalensis is a well-known medicinal plant (Sale *et al*., 2008; Ndjonka, *et al*., 2011; Kolawole, *et al*., 2012; Ibrahim *et al*., 2013) and the active compounds of *Khaya senegalensis* have been described by Yaun *et al*. (2013) and Nakatani *et al*. (2002). Proximate analysis of *K. senegalensis* leaves revealed that they are rich in dry matter, organic matter, minerals, gross energy and crude protein (Abdel-Wareth *et al*., 2014). In Nigeria, the bark of *Khaya senegalensis* is used to treat coccidiosis, helminthosis, amoebic dysentery and diarrhoea in poultry (Gefu *et al*., 2000). It has also been reported to have some therapeutic effects against *Trypanosoma* species (Otu *et al.*, 2009). Anti-oxidants and flavonoids which are occur naturally (Taheri *et al*., 2005) with many medicinal plants been good sources (Emami, 2007) are stored in different parts of some plants (Bhakuni *et al*., 2001; Brisibe *et al*., 2009). Phenolic compounds in *Khaya senegalensis* which are also antioxidants has been indicated to have anticoccidial activities (Naidoo *et al*., 2008; Meskerem and Boonkaewwan, 2013). Oxidants can be produced at elevated rates under patho-physiological conditions (Meskerem and Boonkaewwan, 2013). Phenolic compounds could be a major determinant of antioxidant potentials of foods and are therefore a natural source of antioxidants (Aberoumand and Deokule, 2008).The nutritional value KS leaves are 12% crude protein, 43% neutral detergent fibers and 17% ash in dry matter basis (Awohouedji *et al*., 2013). The leaves are given to cattle and camels as fodder. Branches of trees are lopped in Kaduna, Nigeria. The foliage is said to be not particularly palatable but to have high protein content 18.7% or 21.7% (Burkill, 1985). As expected several active compounds have been isolated from *Khaya Senegalensis* bark and leaves (Makut *et al*., 2008; Otu *et al*., 2009) including saponins, tannins, alkanoids, glycosides, steroids, calcedrin, terpenoids and flavonoids. Some limnoids have also been separated from *Khaya Senegalensis* bark and leaves (Nakatani *et al*., 2002; Yaun *et al*., 2013. This study was carried out to evaluate the effects of the n-butanol extracts of *Khaya senegalensis* on the haematological parameters and weight gain in experimental broiler chickens.

MATERIALS AND METHOD

Collection and Preparation of Plant materials for Extraction

Fresh leaves of *Khaya senegalensis* (KS) was collected from the environs of Ahmadu Bello University between the months of March and April due to their high bioactive content at these months (Ademola and Eloff, 2010) and dried under shade away from sunlight.Ten kilogramme (10kg) each of the dried leaves of *Khaya senegalensis* (KS) with voucher numbers 900181 (Otu *et al*., 2009) was ground to powder using mortar and pestle and sieved. Two kilogramme (2kg) of the powder for KS was defatted with 5 litres of Petroleum ether in a Soxhlet apparatus (Quick fit corning Ltd; Stafford, England) at 70^{o}C (Youn *et al*., 2003). The leaves were air dried at room temperature and then extracted by maceration method for 72 hours using 70% absolute methanol (Eloff, 1998). The crude extract was evaporated to

dryness on a water bath at a temperature of 90^0C for 6 hours.

Fractionation of Crude Methanol Extract

The method of Brain and Turner (1975) was adopted for the partial purification of the dried crude methanol extracts. Thirty grammes (30g) of the dried crude methanol extract for KS was suspended in 300ml of water and partitioned with three portions of 300ml petroleum ether respectively using separate funnels. The petroleum ether portions were each carefully separated into 3 labelled clean 1000ml beakers. Subsequently, the aqueous methanol portions were partitioned with three portions (each 300ml) of chloroform; followed by three equal volume of n-butanol. The portions were then referred to as petroleum ether, chloroform and n-butanol and aqueous portions respectively.The solvents were evaporated in a hot water bath 90^0C for 6 hours. The dried fractions were stored in a dessicator in well labelled containers until when needed.

Determination of maximum tolerated dose

The method described by Loomis (1978) was used to determine the maximum tolerated dose (MTD). Sixty (60) day-old chicks were sourced from NAPRI, Shika, Zaria, brooded under standard management practice for 4 weeks and divided into 6 groups namely A, B, C, D, E and F each containing 10 birds. Graded doses of the n-butanol fraction of the crude extracts 11mg/kg, 33mg/kg, 99mg/kg, 297mg/kg and 891mg/kg were administered orally to the birds in the respective groups mentioned above. Group F were given 0.2mls of distilled water as control. All the extracts were administered within the same period. The chickens were monitored for 14 days for adverse reactions such as changes in skin, feathers, ocular membrane, salivation, lacrimation, drowsiness, gait tremors, convulsion and death suggestive of toxicity within 72hours. The chickens that survived were monitored for another two weeks after which the test was terminated.The body weight gain with respect to age was determined on weekly basis from weeks 4 to 8 weeks. Two chickens per group were randomly selected, euthanized and post mortem examinations conducted. Organs examined for lesions included the lungs, heart, liver, spleen, kidneys, intestine and caeca.

RESULT

The birds that were given the 891mg/kg dosage of the extracts showed signs of respiratory difficulty but stabilized after 30minutes. However, all other groups of birds had no visible clinical sign seen.

Gross Lesions

Several macroscopic lesions were identified in the posted infected birds. These varied in the intensities and location with the concentration /doses of the extract administered. At 11mg/kg of the n-butanol fraction, the lesions were mainly those of enlarged proventriculus and echymotic haemorrhages of the serosal surface of the duodenum. Enlargement of the liver characterized the gross lesions associated with the administration of 33 mg/kg and 99mg/kg doses of the extract. Higher doses of 297 mg/kg and 891 mg/kg were associated with enlarged liver, congested spleen and enlarged heart (Plates 1 and 2). The organs of the control birds presented normal gross picture (Plate 3).

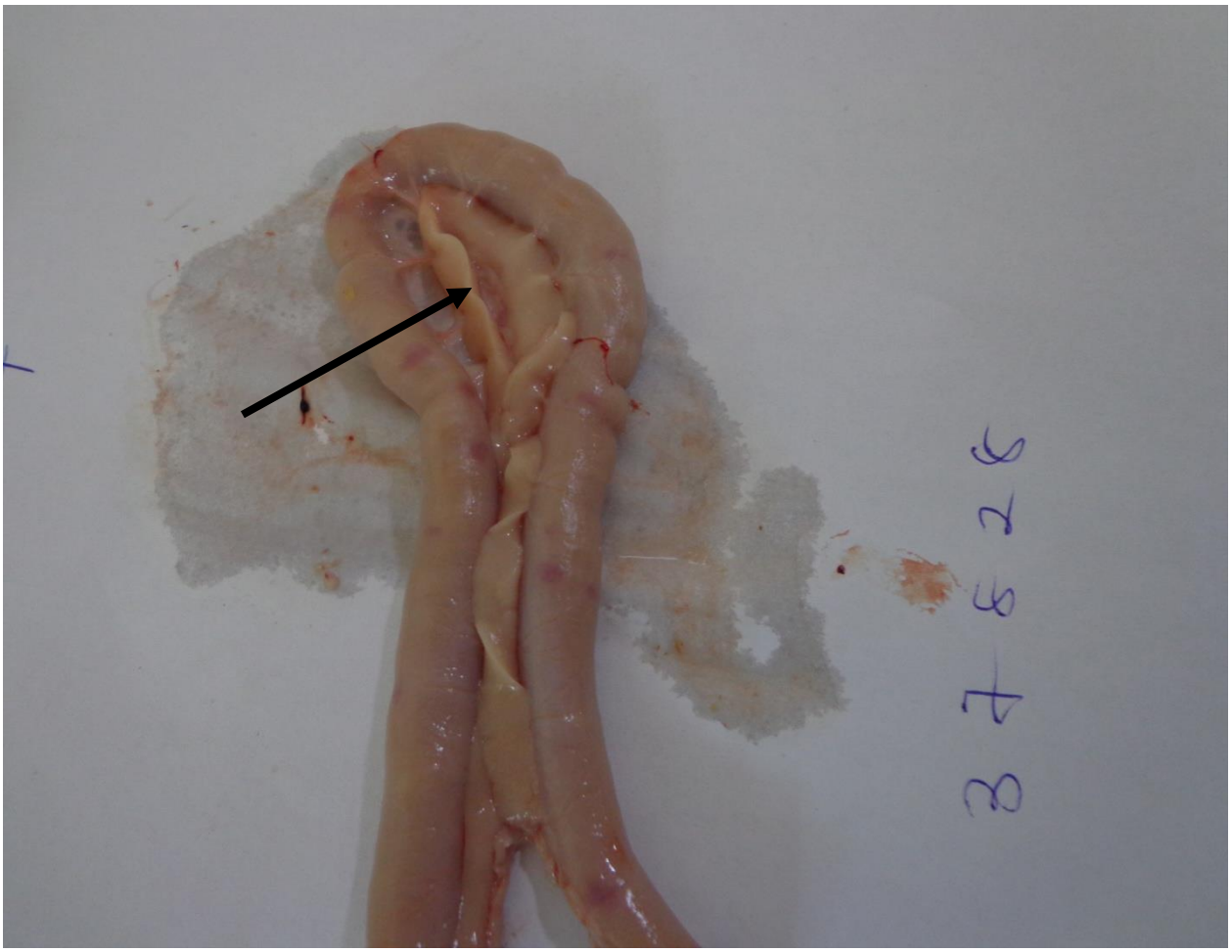


Plate 1: Small intestine of broiler chicken dosed with the n-butanol fraction of the extract (11mg/kg) *Khaya senegalensis* showing enlargement of the intestinal wall (arrow)

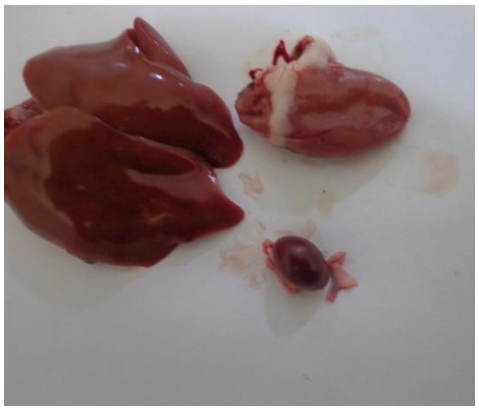


Plate 2: The liver (A), heart (B) and spleen (C) showing enlargements of the organs of birds dosed with 297mg/kg of *Khaya senegalensis*



Plate 3: Normal gross features of the liver (A), heart (B) and spleen (C) of the control broiler chickens given water (0.2ml/ bird)

Haematological values of broiler birds dosed given different doses of the n-butanol extract of *Khaya senegalensis* in the maximum tolerated dose study

There was statistically significant difference ($P < 0.05$) when the mean packed cell volume values for the different treatment groups (11, 33, 99,297,891mg/kg and control) were compared with the mean haemoglobin values. Also when the mean lymphocytic values were compared with the mean neutrophil values there was statistically significant difference ($P < 0.05$). Within the treatment groups, there was no statistically significant difference ($P>0.05$) when they were compared within each other though the PCV of the treated groups were higher than the control (28.14±3.96) with 33mg/kg treated group having the highest (29.15±3.80 vs28.14±3.96). Similarly, the Hb also had slightly higher values than the control with the 33mg/kg group having the highest (9.69±1.24 vs9.32±1.32). The total protein (TP) was slightly different with the overall values being lower than the control, though the 297mg/kg treated group had a higher value than the control (3.65±1.11 vs 3.60±0.59). The overall lymphocytic values were higher than the control though the 33mg/kg group had the highest value while the neutrophil had low values in the 11mg/kg, 33mg/kg and the 297mg/kg treated groups while the 99mg/kg and 891mg/kg groups had slightly higher values than the control (Table 1).

Table 1: The effect of the extracts of *Khaya senegalensis* on the haematological values of broilers treated with different doses of the extracts in the maximum tolerated dose study

Variable	Haematological Results (Mean ± SE) P<0.05					
	11mg/kg	**33mg/kg**	**99mg/kg**	**297mg/kg**	**891mg/kg**	**Control (water 0.2 ml/bird)**
PCV (%)	$_a$28.43±2.72	$_a$29.15±3.80	$_a$28.88±4.28	$_a$28.91±4.51	$_a$ 29.02±5.65	$_a$ 28.14±3.96
Hb (g/dl)	$_a$9.42±0.91	$_b$9.69±1.24	$_b$9.61±1.43	$_b$9.57±1.52	$_b$9.63±1.89	$_b$9.32±1.32
TP (g/dl)	3.42±0.55	3.47±0.62	3.41±0.62	3.65±1.11	3.38±0.55	3.60±0.59
Lymphocyte (%)	$_c$89.73±5.08	$_c$91.00±3.46	$_c$89.14±4.56	$_c$90.96±3.69	$_c$89.83±3.78	$_c$89.56±3.78
Neutrophil (%)	$_d$9.32±5.08	$_d$8.24±3.57	$_d$9.90±4.24	$_d$8.53±3.39	$_d$9.70±4.09	$_d$9.63±3.93

Keys
SE- Standard Error
P<0.05- Statistically significant
PCV- Packed Cell Volume
Hb- Haemoglobin
TP- Total Protein
a- Means with similar letters are not statistically significant
b-Means with different letters are statistically significant

Weight

The baseline weight of the birds is as shown in Table 2. One week after the administration of the extract, the mean weight of the control treatment group when it was compared with other treatment groups (11mg/kg and 33mg/kg) was statistically significant (P<0.05). There was statistical significant differences between the mean weights of birds treated with 11mg/kg of the n-butanol fractions of crude methanol extract of*Khaya senegalensis* compared with 99mg/kg, 297mg/kg, 891mg/kg treated groups and the control (P<0.05)two weeks post treatment. However, there were no significant differences in the mean weights between 11mg/kg and 33mg/kg (P>0.05). When the 33mg/kg treatment group was compared with 891mg/kg and 297mg/kg groups, there were significant differences in their means (P<0.05). Three weeks post-treatment showed significant differences in the comparison of the mean weights between 11mg/kg , 297mg/kg, 891mg/kg and the control (P<0.05) and four weeks post-treatment, there were significant differences (P<0.05) in the mean weights of 11mg/kg , 297mg/kg, 891mg/kg treatment groups and the control (Tables 3). Among the treated groups, the highest weight gain was recorded in the 11mg/kg treatment group while the lowest was recorded in the 891 mg/kg group (Table 4).

Table 2: Pre-treatment mean weight (grammes) of the experimental birds

Treatment	Weight (grammes) (Mean ± SE) P<0.05
Extract 11mg/kg	449.44± 17.11
Extract 33mg/kg	338.80±16.23
Extract 99mg/kg	303.33±17.11
Extract 99mg/kg	260.40±16.23
Extract 99mg/kg	204.60±16.23
Control	205.86±19.40

Keys:

SE- Standard Error

P<0.05- Statistically significant

Table 3: Mean weights (grammes) of birds one to four weeks post treatment with *Khaya senegalensis*

Treatment	Weight (grammes) (Mean ± SE) P<0.05			
	Weeks Post treatment			
	1	2	3	4
Extract 11mg/kg	$_{a}$643.00±24.92	$_{a}$927.00±38.33	$_{a}$1176.00±56.57	$_{a}$ 1450.00±74.46
Extract 33mg/kg	$_{a}$555.00±24.92	$_{a}$804.00±38.33	$_{a}$1060.00±56.57	$_{a}$ 1300.00±74.46
Extract 99/mg/kg	$_{a}$510.00±24.92	$_{b}$693.00±38.33	$_{a}$1048.00±56.57	$_{a}$ 1195.00±74.46
Extract 297/mg/kg	$_{a}$449.00±24.92	$_{b}$600.00±38.33	$_{b}$872.00±56.57	$_{b}$1110.00±74.46
Extract 891/mg/kg	$_{b}$368.00±24.92	$_{b}$619.00±38.33	$_{b}$835.00±56.57	$_{b}$1067.00±74.46
Control	$_{b}$383.00±24.92	$_{b}$671.00±40.40	$_{b}$901.00±63.25	$_{b}$1031.25±83.25

Keys:

SE- Standard Error
P<0.05- Statistically significant
a-Means with similar letters are not statistically significant
b-Means with different letters are statistically significant

Table 4: Mean weight gain (grammes) of the experimental birds treated with *Khaya senegalensis* four weeks post treatment

Variable	Weight (grammes) (Mean ± SE) P<0.05		
	Final Weight	Initial Weight	Weight Gain
Extract 11mg/kg	1450.00±74.46	449.44± 17.11	*$_{a}$1000.56±57.35
Extract 33mg/kg	1300.00±74.46	338.80±16.23	$_{b}$961.20±58.23
Extract 99/mg/kg	1195.00±74.46	303.33±17.11	$_{b}$891.67±57.35
Extract 297/mg/kg	1110.00±74.46	260.40±16.23	$_{b}$849.6±58.23
Extract 891/mg/kg	1067.00±74.46	204.60±16.23	$_{b}$862.4±58.23
Control	1031.25±83.25	205.86±19.40	$_{b}$825.39±63.85

Keys:

SE- Standard Error
P<0.05- Statistically significant
a-Means with similar letters are not statistically significant
b-Means with different letters are statistically significant
*- Highest weight gain

Survival Rate

The survival rate of birds treated with the n-butanol fraction of the crude methanol extract of *Khaya senegalensis* was 100% even with the highest concentration of 891mg/kg as no mortality was recorded in the treatment groups (Table 5).

Table 5: Survival Rates of birds treated with different doses the extract *Khaya senegalensis*

Group	No of Birds	Dose (mg/kg)	Pre treatment mortality	Post treatment mortality	Total mortality	Survived Birds	Percentage (%) Survival
A	10	11mg/kg	0	0	0	10	100
B	10	33mg/kg	0	0	0	10	100
C	10	99mg/kg	0	0	0	10	100
D	10	297mg/kg	0	0	0	10	100
E	10	891mg/kg	0	0	0	10	100
F	10	0.2ml(water)	0	2	2	8	80

Histopathological Findings

The histopathological lesions showed congestions of the intertubular spaces in the kidneys of the birds treated with the n-butanol fractions of the crude methanol extracts of *Khaya senegalensis* at the doses of 33mg/kg and the 297 mg/kg (Plates 4.11 and 4.12). The liver of the birds treated with the dose of 891 mg/kg had congestion of the sinusoidal spaces (Plates 4.13). However, no significant findings were

seen in the small intestine of the 99 mg/kg group and that of the control group which given water at the dose of 0.2 ml/ bird.

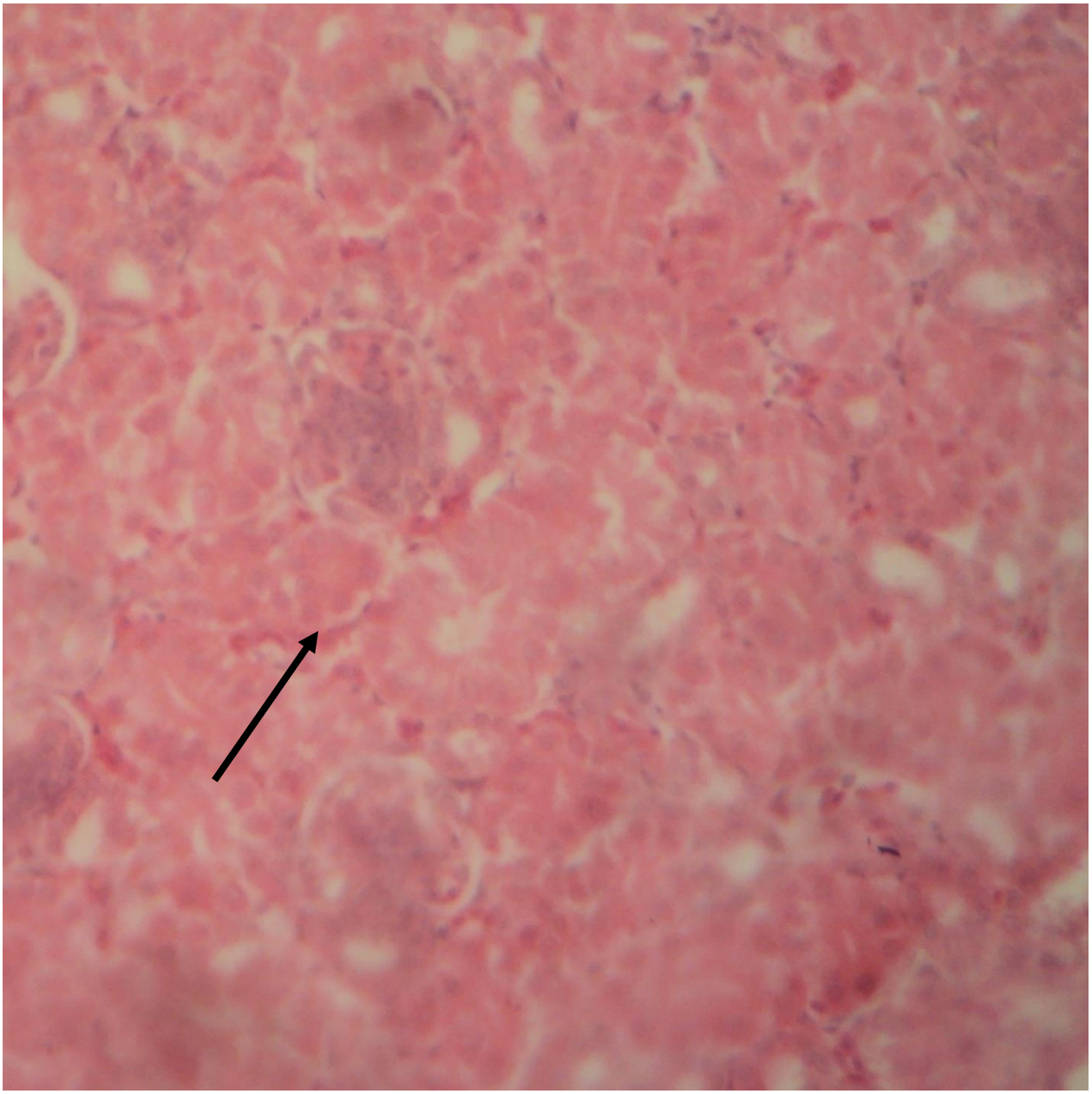


Plate 4: Congested intertubular spaces of the kidney of a bird dosed with 33mg/kg of *Khaya senegalensis* extract (arrow). (H and E) × 40 magnification

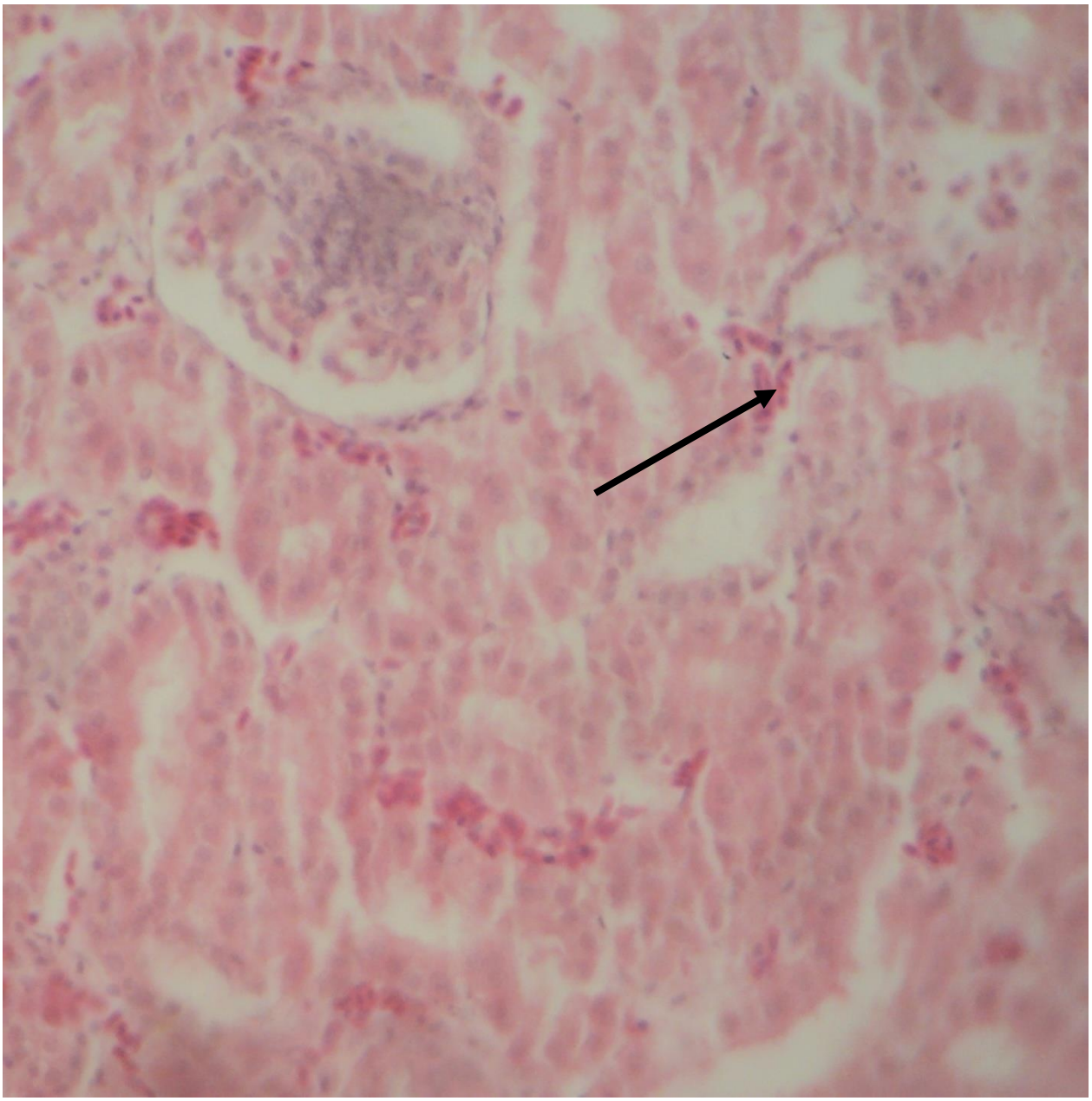


Plate 5: Congested intertubular spaces in the kidney of a bird treated with extracts of *Khaya senegalensis* at a dose of 297 mg/kg (arrow). (H and E) × 40 magnification

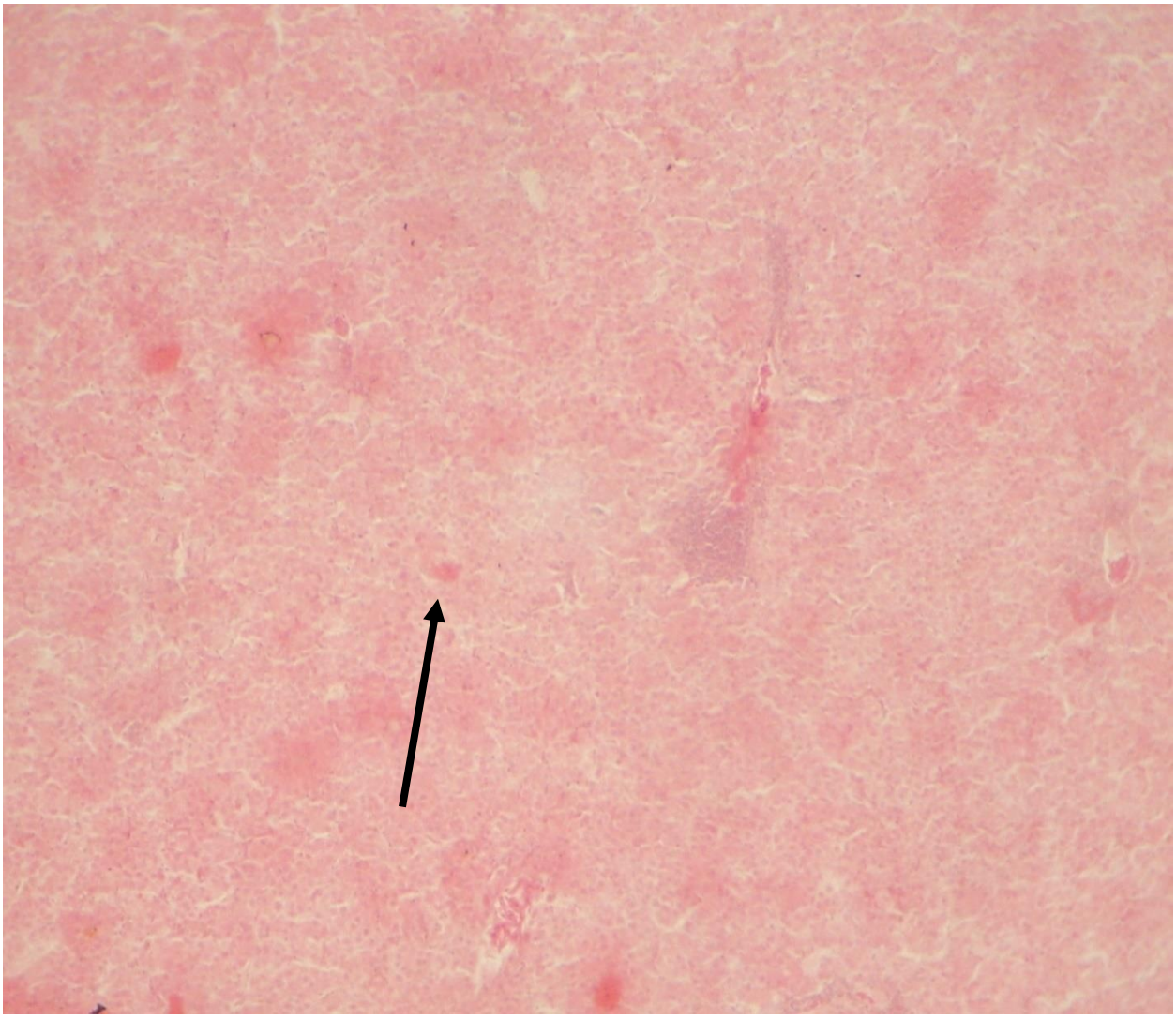


Plate 6: Congested sinusoidal spaces in the liverof a bird treated with extracts of *Khaya senegalensis* at the dose of 891mg/kg (arrow). (H and E) × 40 magnification

Selection of the maximum dose tolerated by the birds

Results obtained from the study revealed that although all the birds were active when they were given the extracts of *Khaya senegalensis*, the 891 mg/kg treated group had respiratory distress but they stabilized after 30 minutes. Also at 4 weeks post administration of the extracts of *Khaya senegalensis*, the 297mg/kg and 891mg/kg treated groups had lower weight gains. The 99mg/kg dose was selected as the maximum tolerated dose. However, three dose levels were selected from the 99 mg/kg (which are as follows; 11mg/kg, 33mg/kg and the 99mg/kg) for the evaluation of the anticoccidial effects of the extracts *in vivo*(Table 6).

Table 6: Maximum tolerated dose/toxicity of n-butanol fractions of crude methanol leaf extract of *Khaya senegalensis*

Dose(mg/kg)	11	33	99	297	891	Control
Initial no. of birds	10	10	10	10	10	10
Mortality	0	0	0	0	0	2
Observation	a	A	a	Ac	abc	a
Inference	-	-	-	+	+	-

Keys:
a= active 6hours to 4weeks post treatment, b=showed initial respiratory difficulty for 30minutes after treatment, c= lower weight gain 4 weeks post treatment, - = not toxic, + = mildly toxic

DISCUSSION

The study confirmed the safety of the extract of *K senegalensis* even at high doses of 891 mg/ kg as regards to no clinical signs and mild gross lesions seen in this study. Nwosu *et al*. (2011) reported that even when *K senegalensis* was administered at a dose of 2000mg/kg only mild clinical signs were seen. The high survival rate of 100% in the five medicated groups indicated the high safety margin of the plant. This is in agreement with Usman *et al*. (2011) who recorded no mortality in a study using *K senegalensis*. Abdel-Wareth *et al*. (2014) also reported similar findings when experimental rabbits were fed with *K senegalensis* leaves and no mortality was recorded.

None of the haematological values were significantly changed when the extract was given even at 891mg/kg. The increase in the packed cell volume and the haemoglobin count could be related to the active ingredients present in the extract. The packed cell volume and the haemoglobin count been higher than the control are in agreement with Ali *et al*. (2011) and Usman *et al*. (2011) who also had a similar finding when aqueous extract of *K senegalensis* was given to rats though at higher doses it was said to have a destructive effect on the bone marrow. Lymphocytosis was noticed across the treated groups. This could be as a result of the effect of the extract on the internal organs. Dkhil *et al*. (2012) reported that lymphocyte increase could be a prominent response of body tissues facing injurious impact. The increase in the lymphocytic values of the treated groups confirm the reports of Brisibe *et al*. (2008) and Sani *et al*. (2013) on the positive effects of natural flavonoids on the immune system of different species which focus on mainly the T lymphocyte stimulation and increasing blood lymphocytes and the lymphocytosis is indicative of inflammation (Abdel Wareth *et al*., 2014).

The progressive weight increase in both the medicated and un medicated group shows the ability of the extract to improve weight gain as reported by Nwosu *et al*. (2011). Among the treatment groups the differences in weight gain was recorded with highest in the 11mg/kg and the lowest in the 891mg/kg groups may be associated with the hepatotoxic and nephrotoxic effect at higher doses. The hepatoprotective, hepatotoxic and nephrotoxic effects of *K senegalensis* leaves have been reported by Adebayo *et al*. (2003), Abubakar *et al*. (2010) and Ali *et al*. (2011). Thus at lower doses *K senegalensis* can be used as growth promoter due to the increase in weight. Abdel-Wareth *et al*. (2014) also reported similar findings in an experiment when rabbits were fed with a diet containing 35% *K senegalensis* leaves, the test

animals were said to have increased weight gain and carcass traits, however the reverse was the case when a diet containing 65% of the leaves was used and the rabbits had lower weights than the former group. The weight gain in the 11mg/kg group might also be associated with the antioxidant compounds present in the extract (Ali *et al*.,2011) and the nutrient values of the leaves of *K senegalensis* which are said to be rich in gross energy and crude protein (Abdel-Wareth *et al*., 2014). This implies that though *Khaya senegalensis* causes weight gain as earlier established, it has some effect on vital organs and such can only be administered for a short duration.

The histopathological lesions seen are in agreement with Abdel-Wareth *et al.* (2014) who reported some degree of nephrotoxicity among the experimental rabbits fed diets with *K senegalensis* leaves. He also discovered that the leaves had some effects on vital organs which are dose-dependent. This was noticed at high doses where there was lymphocytosis which was indicative of inflammation. This may mean that although *K senegalensis* may be tolerated physiologically, there may be some injurious effects associated with the administration of higher doses. This is in agreement with Onu *et al.* (2013) who reported similar findings in a study on rats that the severity of lesions seen was concentration dependent being more severe in those rats given the highest concentration of the extract.

CONCLUSION AND RECOMMENDATION

The ability of extracts of the n-butanol fraction of *Khaya senegalensis* to cause weight gain was demonstrated in this study. Also at higher dosages (891 and 297 mg/kg) due to signs associated with their administration, the 99 mg/kg dose was selected as the maximum tolerated dose. Thus *Khaya senegalensis* could be used at a lower concentration below 99mg/kg and should be given for a short duration. Due to paucity of information on the health benefits of the leaves of the plant *Khaya senegalensis* as they relate poultry production, it is recommended that more research in this area could be done.

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CONFLICT OF INTEREST

The authors' wishes to state that there are no conflicts of interests.

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