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Research Article

Serum Biochemical Effects of *Alstonia boonei* Leaf Extract Administered against Coccidiosis in Broilers

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Abstract

A cheap cost-effective herbal alternative to coccidiostats was prepared by dissolving pre-weighed blended leaves of Alstonia boonei in ethanol-water (70:30) to extract the bioactive components. The efficacy/potency of the hydro-alcoholic extract against coccidiosis were then determined in 100 broiler chicks, in a completely randomized design (CRD)! The leaf extract was administered twice daily, to groups of birds at different concentrations (0ml/4L, 5mL/4L and 10mL/4L) and their biochemical effects were examined at 8th weeks old. The concentrations of serum enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin (CBIL) were not significantly different ($P > 0.05$) between test groups and the minor control (non-treated birds) indicating healthy liver and kidney functions. There were no significant treatment related effects ($P > 0.05$) on blood creatinine, urea, bicarbonate, K+ and Cl-, whereas Na+ concentration was affected significantly ($p < 0.05$). Except with the main control (embazin treated birds), the mean levels of alkaline phosphatase were non-significantly different ($P > 0.05$) between the herbal test groups. There were variations in serum albumin levels between the different experimental groups but each value fell within the normal range (3.0 - 5.5g/dL). The mean concentrations (mg/dL) of Total bilirubin ranged from 0.12 in embarzin treated birds, to 0.20 in non-treated birds. There were no symptoms of coccidiosis in the different groups of alstonia extract and embarzin treated birds indicating efficacy of the extract against the disease. The concentrations of blood electrolytes and serum enzymes were not adversely affected by the extract! And there were no mortalities during the trial period.

Keywords: Broiler Chicks; Coccidiostat; Embarzin; Alstonia Leaf Extract; Serum Chemistry

Introduction

The use of chemical feed additives and pharmaceutical products in the health management of birds has been a long-time practice in the poultry industry! For example, prophylactic and anaphylactic drugs such as coccidiostats are useful in protecting and promoting the health and growth of birds and hence also the growth of the industry [1,2,10,17,24,25]! The benefits include good health care facilitates, higher production levels, good quality poultry meat and meat products which are thus made readily available and affordable. However, the complications related to antibiotics use in poultry are obvious! For example, the effects of drug residues in poultry meat on the consumers of broilers, and the development of resistance to antibiotics by the pathogens over time, have presented challenges over the past decade. Hence the need for alternative therapies in poultry health management has been suggested [14,17,18,24]. In this regard many plant species have been found useful either directly or indirectly for disease control in animals and humans [9,10,17,25]! Amongst them is *Alstonia boonei,* which belong to family Apocynaceae; and is known by different local names in Nigeria and elsewhere in the tropics. The plant grows abundantly in Nigerian rain forest belt! There are indications that the Alstonia plant parts contain chemical constituents which can be exploited therapeutically in the management of diseases such as dysentery, typhoid, gonorrhea and asthma and ailments such as ulcers, toothache, snakebites, rheumatic pain and sores. In fact extracts of the bark/leaves of the plant have been reported to possess a wide range of pharmacological properties including anti-rheumatic, anti-inflammatory, analgesic, anti-malaria, antipyretic, anti-diabetic, anti-helminthic, antimicrobial and antibiotic properties [3,14,15,17,18,21,23]. This study was conducted on the prem-

ise that the bioactive constituents of *Alstonia boonei* could also have anti-coccidal properties useful in broiler health management. The objectives were to undertake hydro-alcoholic extraction of the leaves and examine the efficacy of the extract administered orally against coccidiosis in broilers, and the effects of this on the serum chemical profile of the birds.

Materials and Methods

Site location

The experiment was conducted at the University Teaching and Research Farm (UTRF), Benin City, Nigeria. The university is located between latitude 6° and 63°N and longitude 5° 40 and 6^{L}'E. The location records a mean annual temperature range 24.5 - 32.7°C, annual rainfall 1498 - 3574mm with a mean value of 2162 mm, and relative humidity of 63.5 - 81.7% with a mean value of 72.5%. The daily sunshine range is between 5.86 and 7.5 hours with a mean value of 6.68 hours.

Site preparation

Routine maintenance, cleaning and disinfection of facilities in the poultry house was undertaken in preparation for the arrival of the chicks. Equipment and materials (drinkers, feeders, pots lightening) required for the rearing of chicks were fixed.

Experimental materials, treatments and design

Fresh leaves of *Alstonia boonei* were harvested from Alstonia trees which grow abundantly and luxuriantly within the University campus in Benin City. Leaves were dried for 2 weeks and milled into fine powder using British milling machine. Then a portion (3kg) of leaf powder was weighed out using a top loading balance and placed in a 2 liter flat bottom flask containing the extracting solvent. The solvent was a large volume of hydro-alcohol made up of ethanol and water in the ratio of 70:30. The solvent-solvent mix was shaken and then left to stand for about 72 hours, Thereafter the suspension was stirred vigorously and then filtered through a muslin cloth. Then the solution was evaporated in a water bath at 75.5°c to obtain the extract. The extract was discharged into a glass bottle, labeled and kept in cool storage (refrigerator) till when required.

The extract was administered to the growers through their drinking water twice daily (morning and evening) at specific concentration (5mL/4L or 10mL/4L) according to treatment groups. A total of 5 liters was given to the experimental unit per day. The conventional coccidiostat (Embarzin 4g/4L) was administered to the main control group (Tr-4), while a minor control/non-treated group (Tr-1) received neither Embarzin nor Alstonia extract for therapy. Thus there were four experimental treatments.

Management of experimental birds

A total of 100 birds (broiler chicks) were purchased from a commercial Hatchery in Oyo. The chicks were transported in cages to the UTRF Farm project in Benin-City, where the field experiment was conducted. All 100 chicks were brooded manually and electrically for 2 weeks! They were fed ad-lib with commercial Broiler starter (24% CP). Thereafter birds were randomly selected and distributed into four experimental units/pens, each made up of 25 birds, in completely randomized design (CRD) according to the treatments. The birds were fed starter diet until 4 weeks of age! Then diet was changed to broiler finisher, which was given daily until the end of trials. At 6 weeks, the birds were infected by inoculating with the protozoan *(Eimeria necatrix)* and then treatment of the diseases started four days after inoculation and continued till week 8th when the experiment was terminated. The birds were observed for symptoms of toxicity or ill-health throughout the trial period wherein, along with embarzin or alstonia extract, the birds were also administered the following medications

- Intraocular vaccination against Newcastle disease at *day* one.
- Ganadexil (broad spectrum antibiotic) for a period of 4 days from 3 days of age.
- First Gumboro vaccine at 9 days of age; and second Gumboro vaccine at 16 days of age.
- Lasota vaccine (New castle booster) was administered at 23 days (3 weeks) of age.

Blood collection

At the end of the drug trials, blood samples were collected from five (5) birds, selected randomly, per treatment group. The birds were fasted overnight before the blood collection. Early in the morning, blood was collected from the birds by venipuncture using needles and syringes! 2ml of blood was drawn from each bird and then gently discharged into a clean heparin bottle to prevent coagulation. Contamination of blood was prevented by using a different set of needle and syringe for each bird. Thereafter the serum biochemical index values were measured in the Clinical Biochemistry laboratory, using standard analytical techniques.

Statistical analysis

Analysis of variance (ANOVA) was performed for the data generated from the investigation using the GenStat 12th edition at 5% probability level. The significant means were separated using Duncan Multiple Range Test (DMRT) of the same statistical software.

Results

Liver and kidney function test of broilers at 8th weeks

Serum biochemical index values measured in the birds showed that TR2-Birds administered 4g embazin forte per 4L of water recorded the highest value of alkaline phosphatase (ALP) - (102.33g/dL) which was significantly higher ($P < 0.05$) than the levels measured in other treatment groups of birds. The mean ALP level in Tr3-birds which were administered 5mL/4L concentration of the leaf extract was 82.00g/dL. Tr4-Birds, administered 10mL/4L concentration of the leaf extract, measured 79.67g/dL while the Control-birds (Tr1) given fresh water without Embazin forte or Alstonia extract recorded 90.00g/dL (Table 1). The mean values of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Conjugated bilirubin (CBIL) were not significantly different ($P > 0.05$) between the treatment groups. Significant differences ($p < 0.05$) were recorded for Total bilirubin (TB) levels (mg/dL) between Tr3-Birds (0.12mg/dL) and other treatment groups which measured similar and non-significantly different ($P > 0.05$) concentrations (Tr-1 0.20, Tr-2 0.173, Tr-4 0.20 mg/dL) - Table 1.

Blood urea, creatinine and electrolytes levels in Broilers at 8th weeks

There were no significant differences ($P > 0.05$) in blood urea or creatinine levels between the treatments groups. Urea levels (mg/dL) ranged from 10.67 in Tr-1 birds to 12.33 in Tr-3 birds. Also there were no significant treatment effects ($P > 0.05$) on blood electrolytes (K^+ and CL^-); while there was variation in the concentrations (mEq/L) of sodium ions (Na^+) wherein Tr-3-birds measured significantly ($P < 0.05$) higher values (159.0) than Tr-4 (146.7) and Tr-2 (149.0) birds (Table 1). No significant differences ($P > 0.05$) were recorded for serum bicarbonate ion between the test groups (Table 2).

Serum protein and lipid profile of Broilers at 8th weeks

The lowest level of Albumin (g/dL) was recorded for Tr-2-birds (1.47) while the highest level was found in the Tr-3-birds (1.80g/dL)! And the differences between them were significant ($p < 0.05$); whereas albumin levels measured in non-treated Tr-1 (1.63) and embarzin treated Tr-4 (1.73) groups of the birds were not significantly ($P > 0.05$) different (Table 2). There were no significant differences in globulin concentrations between different groups. The highest value of Globulin was measured in the non-treated Tr-1 group (1.93g/dL) and the lowest was found in embarzin treated Tr-4 group (1.27g/dL). Total cholesterol levels ranged in values from Tr-2 (135.30g/dL) to Tr-3 (150.70g/dL)! The differences were non-significant ($p > 0.05$). Also differences in triglyceride levels were not significant, with values which range between 48.00g/dL in Tr-4-birds and 56.00g/dL in Tr3-birds. The lowest value of HDL cholesterol was measured in Tr-3-birds (38.67g/dL)! That was significantly ($P < 0.05$) lower than the concentration in each of other treatment groups! Between those HDL cholesterol levels were not significantly different ($P > 0.05$). The variation in LDL cholesterol levels between different treatment groups was not statistically significant ($P > 0.05$)! The values ranged from 55.67g/L in Tr-2-birds to 67.33g/dL in Tr-3-birds (Table 2).

Parameters	Tr-1	Tr-2	Tr-3	Tr-4	SEM
Alkaline phosphatase (IU/L)	90.00[a]	102.3	82.02[a]	79.67[a]	7.73 bacde
Alanine transferase (IU/L)	12.7[a]	8.04[a]	8.33[a]	8.01[a]	5.34 bacde
Aspartate transferase (IU/L)	111.0 bacde bacde bacde bacde bacde bacde bacde bacde a	110.30	110.7 bacde	108.055	3.82
Conjugated Bilurubin (mg/dL)	0.06 bacde	0.06	0.06	0.06	0.00
Urea (mg/dL)	10.67	12.00	12.33	11.32 bacde	0.46
Na+(mEq/L)	150.70	149.2	150.03	146.7	2.51
K+(mEq/L)	3.50 bacde	3.38	3.80	3.67	0.20
Chloride (mEq/L)	114.7	115.3	116.3	112.0	1.35

Table 1: Indices of Liver and kidney functions in broilers treated with embarzin and alstonia.

Key: TR1: Birds given fresh water (control) without Embazin forte or Alstonia extract; TR2: Birds administered 4g embazin forte per 4L of water. TR3: Birds administered 5mL/4L concentration of the leaf extract. TR4: Birds administered 10mL/4L concentration of the leaf extract.

**Values along the same row with different superscripts are significantly different ($p < 0.05$).

Discussion

Liver and kidney functions in matured broilers

Serum index values are the clinical indicators of the health status of humans and animals. Their measurements are considered as necessary confirmatory tests in addition to morphological and physiological manifestations in the form of symptoms, which may not always be evident! Thus in toxicological trials of new products, liver and kidney function tests are performed by examining the parameters of serum chemistry and peripheral blood [4,5,7,11]. There are normal range values for each blood index in a healthy animal! Thus the results of toxicological evaluations should indicate if

Parameters	Tr-1	Tr-2	Tr-3	Tr-4	SEM
Bicarbonate (mEq/L)	21.67[a]	21.0[a]	20.67[a]	19.33[a]	1.24
Total protein (g/Dl)	3.58[a]	3.03[a]	3.03[a]	3.00[a]	0.21
Albumin (g/dL)	1.63[ab]	1.47[b]	1.80[a]	1.75[ab]	0.08
Globulin (g/dL)	1.93[a]	1.57[a]	1.33[a]	1.27[a]	0.20
Creatinine (mg/dL)	0.63[a]	0.53[a]	0.67[a]	0.67[a]	0.04
Total Cholesterol (mg/dl)	137.70[a]	135.32[b]	150.70[a]	144.29[a]	6.40
Triglycerides (mg/dL)	49.67[a]	48.35[a]	56.05[a]	38.12[a]	2.87
HDL cholesterol (mg/dL)	61.28[ab]	69.67[a]	38.69[b]	68.30[a]	7.54
LDL cholesterol (mg/dL)	64.66[a]	55.71[a]	67.13[a]	66.32[a]	4.99

Table 2: Serum protein and lipid profile of broilers treated with embarzin and alstonia extract.

Key: TR1: Birds given fresh water (control) without Embazin forte or Alstonia extract; TR2: Birds administered 4g embazin forte per 4L of water. TR3: Birds administered 5mL/4L concentration of the leaf extract. TR4: Birds administered 10mL/4L concentration of the leaf extract.

*Values along the same row with different superscripts are significantly different ($p < 0.05$).

a measured value falls within or outside the normal range [4,7,11]. For example, the bicarbonate ion acts as a buffer to maintain the normal levels of acidity (pH) in blood and other fluids in the body! In the domestic bird, the normal values of bicarbonate ions in the blood and body fluids range between 13.0 and 25.0 mmol/L [7]. In this study the concentration of bicarbonate ion for each treatment group fell within that normal range! Also of interest, no significant differences ($P > 0.05$) were observed between the test groups. This implies that Alstonia leaf extract and Embarzin had no adverse effect on bicarbonate levels in broilers.The treatments were effective in the reduction of serum electrolytes (Na^+ and Cl^-) which measured values (3.0 - 5.5 meq/L) that fell within normal range for birds [5,14].

The normal levels of blood urea in broilers range (2.0-10.0mg/dL). There were no treatment effects; i.e. no deviation from normal was found in any treatment group; and there were no significant differences ($p > 0.05$) between the embarzin and alstonia treated birds. However we observed an insignificant increase in urea levels in the alstonia extract treated birds (Tr-3 and Tr-4) in comparison with the main control/embarzin-treated birds (Tr-1) and the minor control/non-treated birds (TR-2). Urea levels were higher in the test group (Tr-3) that were administered the high dose (10mL/4L of water) of alstonia leaf extract. A question arising from this is what would have been the result had higher doses of alstonia extract been administered? Perhaps elevated urea levels above the normal limit? That question suggests a hypothesis, that alstonia leaf extract could present variable levels of avian and possibly mammalian toxicity, affecting liver and renal functions, when ingested above critical levels! And the answer to this hypothesis can be sought through further research to confirm and establish critical levels of use of the leaf extract in the health management of birds and other species. In addition, the bioactive substances in the leaves would require chemical identification and characterization through instrumental techniques.

The main control group (birds treated with embarzin, Tr-2) measured significantly lower creatinine levels ($p < 0.05$) in their blood compared to the minor control group (birds administered neither embarzin nor alstonia extract, Tr-1) and the two test groups of alstonia treated birds (test groups Tr-3 and Tr-4).

Notwithstanding the aforementioned, it is noteworthy that elevated urea and creatinine levels in the blood of animals has been attributed to a number of factors, which include increased protein metabolism due to starvation, stress, drugs or damage to renal tissue [5,7,16,19-21]. Therefore there could be factors likely inherent in the alstonia leaves which may be implicated as responsible for inducing increased protein metabolism, leading to higher urea and creatinine levels, as we found in the birds (Table 2). This can only be ascertained through further research.

There were no indications of treatment related effects on the concentrations of Alkaline phosphatae (ALP) in the broilers. Birds treated with 5ml of extract (Tr-2) showed significantly higher levels ($P < 0.05$) than other treatment groups; and this cannot be attributed to dose response. Previous studies indicate that elevations of ALP are most common with liver disease and enteritis; as well as in hyper-parathyroidism-induced stimulation of osteoplastic activities [7,8]. The alanine aminotransferase (ALT) and aspartate aminotransferase (ASP) levels in all treatment groups fell within reference range values and were not significantly different ($P > 0.05$) in their concentrations indicating healthy liver function.

Plasma protein profile of matured Broilers

In this study all the groups of experimental birds - the embarzin-treated group (main control), the test groups, treated with alstonia leaf extract, and the non-treated group, given water only (minor control), measured plasma protein concentrations that fell within normal range values; although albumin and globulin levels fell below the normal range of 1.9 - 3.2g/dL and 2.0 - 4.0g/dL respectively! The alstonia extract caused a significant decrease ($p <$

0.05) in the concentrations of total plasma protein, albumin and globulin in the different test groups, in comparison with the the embarzin-treated birds and the non-treated birds-table 2.

Reported studies have shown that increases in plasma protein are related to dietary and sometimes experimental factors, such as increased dietary protein level or protein content of treatment, and state of hydration. Health factors such as hemorrhage or inflammation of tissue or organ or other abnormal physiological or biochemical interactions have also been implicated [5,7,12,16,19,20,21]. In this study we found a decrease rather than an increase in plasma protein levels! This implies that none of the aforementioned conditions can be imputed! However, the variations observed could not be considered as related to treatment effects! Firstly, there was a significant reduction in the levels of the protein fractions in birds treated with low dose (5mL) alstonia extract in comparison with birds given water only (non-treated group)! Whereas the embarzin-treated group, and the group treated with high dose (10mL) of alstonia extract, as well as the non-treated group (0mL) recorded similar levels of the different protein fractions (Table 2). Thus we deduce that other factors of variability in experimental measurements, rather than treatment effects, could have taken a toll in laboratory analysis. This axiom may hold through when it is considered that there have been reports indicating that birds with liver complications tend to show loss of albumin and measure extremely low plasma protein concentrations in combination with decreases in albumin-globulin ratio [5,7,8]. In this study, we recorded low albumin concentrations (below the reference value (1.9 - 3.2g/dL); and low globulin concentrations, also below reference range (2.0 - 4.0g/dL)! However we found no indications of liver or renal dysfunction, or of anaemia as evidenced by the indices of liver and kidney functions which showed normal values (Table 1).

Plasma lipid profile

Cholesterol is an important biomarker in toxicological and clinical investigations. It is a precursor of cholesterol esters, bile acids and steroid hormones. Serum cholesterol levels may be affected by type of feed, such as oat bran diet having a lowering effect or wheat bran diet having an elevating effect on serum cholesterol [5,8,11,16]. However, physiologically the concentration of serum cholesterol is thyroid dependent [4,7,8,11] implying that the thyroid hormone controls both the rate of synthesis and the rate of catabolism of this metabolite. Therefore abnormal serum cholesterol levels would result from thyroid dysfunction! Hypothyroidism and other vascular diseases are the main pathological conditions leading to abnormal increases in serum cholesterol. Other degenerative conditions variously reported in this regard include hyper-adrenocorticism, diabetes mellites, acute pancreatitis and biliary obstruction [4,7,8,11]. In addition, hyper-cholesterolemia, manisfested as abnormal increases in serum cholesterol levels, has been associated with hepatic disease, cholestasis protein losing enteropathy and nephrotic syndrome. On the other hand abnormal decreases in serum cholesterol has been reported under conditions of inherited protein deficiencies abnormalities in intestinal digestive processes, mal-absorption and in hepatic disease [4,7,8,11]. Thus abnormal concentrations of cholesterol are evidently pointers to pathological conditions in mammals.

In this study, cholesterol levels in the broilers were neither elevated nor reduced beyond normal range values which is 50.0 - 350.0 mg/dL [12,16,19,20]! Although the administration of the alstonia leaf extract or of the coccidiostat resulted in elevated cholesterol levels, there were no significant differences ($P > 0.05$) between the control and test groups! Hence hyper-cholesterolemia was not manifested in the birds.

Also we found a concentration of high density lipids (HDL) that was inversely proportional to the low density lipids (LDL) and Triacylglycerides (TAG). HDL protects the body from accumulation of fats as it captures LDL and TAG to the liver for storage or excretion. In the group of broilers given low dose of alstonia extract, we found a low level of HDL and increased levels of TAG and LDL which deviated from normal, although not significantly different ($p > 0.05$) from the TAG and LDL in control birds (Table 2).

Such deviation could result from renal dysfunction due to infection! Reports on previous studies with ducks indicated that healthy birds had a low concentration of bilirubin in their sera, while infected birds (particularly hepatitis viral infection) showed high bilirubin concentration [7]. In this study the broilers showed healthy physical conditions throughout the trial period! This indicates that the dose levels of the ALSTONIA leaf extract given to the birds were not critical to elicit adverse effects! However deviation from normal levels of TAG, LDL and bilirubin could be pointers to the patho-physiological conditions that may result when the birds are predisposed to infection when under higher doses of the leaf extract, or when the extract are administered above critical levels.

Potential of ALSTONIA in ethno-medicine

The therapeutic value of many plant species, including the Alstonia, has been demonstrated in many studies [3,6,14,17,18,20,22,24]. Thus, as with every synthetic drug like embarzin, the biological properties of the extracts of natural drug plants, like Alstonia, can be exploited in ethno-medicine! However this is possible only at doses that would not alter adversely the biological steady state of the animal subject, including the organ functions, serum chemis-

try and the peripheral blood [3,9,14,23,25]. Previous reports indicate that the phyto-constituents of extracts of *Alstonia boonei* plant include saponins, glycosides, flavonoids, terpenes, steroids, carotenoids, coumarins, alkaloids, anthraquinones, glycosides, cyanogenic glycosides [8,15]. In this study we found that the hydro-alcoholic extract of *Alstonia boonei* leaf was as effective as the coccidiostat (embarzin) in the control and treatment of coccidiosis at the dose levels (5.0 - 10.0ml/L water) given to birds between the 4th and 8th weeks. This implies that effective managerial skills are also essential for plant product to be gainfully exploited in the health management of broilers.

Conclusion and Recommendation

This study was conducted on the premise that the bioactive components in Alstonia leaves could have anti-coccidal properties. The hydro-alcoholic extract of the leaves was thus administered orally to examine the efficacy against coccidiosis and determine the effects on the serum chemical profile of the birds. There were no adverse responses, organ or tissue damaged nor occurrence of mortalities in the birds due to the extract. Coccidiosis was effectively controlled by the anticoccidial drug (embarzin) used as well as by alstonia leaf extract. However, higher doses of the extract may elicit adverse responses. Thus effective management is recommended in order to get maximum benefit from the use of alstonia. In order to establish the economic benefit of this plant, further research is recommended on the characterization of the bioactive components of alstonia leaves, their critical levels of toxicity, and their biological properties, including indications and contra-indications in humans and in various species of animals.

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