



Haematology and Serum Biochemistry of Rabbit does Administered Methanolic Extracts of *Andrographis paniculata*

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Abstract

Thirty-six (36) nulliparous mixed-breed rabbit does, aged, 5-6 months were subjected to a scientific study to determine the effects of methanolic extracts of a medicinal plant, king of bitter (*Andrographis paniculata*) on the haematology and serum biochemical profile of rabbits does. The rabbits were randomly selected and assigned into four (4) treatment groups designated as T1, T2, T3 and T4 in a completely randomized design. Each treatment comprised nine (9) animals. The treatments were replicated 3 times with 3 animals per replicate. 1g of dried methanolic extract of *A. paniculata* was reconstituted in 100ml of distilled water to form a liquid suspension which was administered orally to the rabbit; early in the morning using a sterile syringe for a period of 8 weeks, at the rates of 0.0mg/kg, 125mg/kg, 250mg/kg and 500mg/kg respectively for T1, T2, T3 and T4, with T1 being the control experiment. Blood samples were collected from 6 animals per treatment group (a total of twenty-four rabbits) selected randomly across each replicate, at the onset of the experiment for basal studies. Subsequently, samples were collected fortnightly at week 4, 6, and 8, of administration of the extract for haematological and serum biochemical analysis. Data obtained were subjected to Analysis of variance; significant means were separated using Duncan's multiple range test. The results obtained at the end of the study showed significant differences ($P < 0.05$) in some of the blood parameters evaluated. RBC count increase significantly ($P < 0.05$), with increase in administration rate of the *A. paniculata* extract. There was however a decline in Hb, MCH and MCHC values. PCV, WBC, platelets and Lymphocyte counts were observed to be statistically similar. Serum glucose levels decreased significant with increase in consumption rate of the extract. An increase in Serum albumin, globulin and Total protein was recorded. Higher values were recorded in T2 and T3 for Alanine Transaminase while Aspartate Transaminase values tended to decline. It is concluded from this study that methanolic extracts of *A. paniculata* had significant influence on blood parameters of domestic rabbit.

Keywords: Extracts; Nulliparous; Rabbits; Does; Treatments; Haematology

Abbreviations

PCV: Packed Cell Volume; RBC: Red Blood Cell; Hb: Haemoglobin; WBC: White Blood Cell; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Introduction

The dire need for alternatives sources of good quality animal-based protein to meet the nutritional needs of our ever-growing

population has tremendously triggered interest in micro-livestock production. According to Food and Agriculture Organization, if the high growth rate in meat consumption in future years will have to be met, much of the increase in production would have to come from short-cycle animals such as rabbit [1]. Rabbits are prominent for their enormous potentials and good attributes which include: high prolificacy, short gestation period fast growth rate, high efficiency in converting forage to meat, high nutritional quality and

relatively low cost of production; Rabbit have the potential to bridge the gap in animal protein consumption [2]. The success of the rabbit enterprise like any other livestock venture, is a function of the general physiological state of the animals, which invariably reflects in their health status, growth rate, feed conversion, nutrient utilization and reproductive performance. Haematological values could serve as a baseline information for comparison in conditions of nutrient deficiency, physiology and health status of farm animals especially those kept under native husbandry system in Nigeria [3]. Several researches have been conducted in the recent past in an attempt to improve productivity and enhance the performance of farm animals in diverse areas of interest, using various feed additives, herbal derivatives and plant extracts to elicit various physiological responses. Some scientists investigated some plant parts and observed that the medicinal values of plants and their component phytochemicals such as alkaloids, tannins, flavonoids, phenolics and other compounds have been found to provoke certain physiological actions on the body [4]. *Andrographis paniculata*, commonly known as the "king of bitters," is an herbaceous plant belonging to the family, Acanthaceae and is found throughout tropical and subtropical Asia, Southeast Asia, and India. The leaves of the plant contain a bioactive constituent- andrographolide, which has been reported to exhibit a broad range of biological activities, such as anti-inflammatory, antibacterial, antitumor, antidiabetic, hypoglycemic and hepatoprotective [5]. In addition to Andrographolide, *A. paniculata* contains diterpene lactones, glycosides, flavonoids, alkaloids, phenols, catechine, saponins, and tannins. It has also shown immunity-boosting capabilities by stimulating the body's natural immune systems [6,7]. This study seeks to evaluate the influence of methanolic extracts of this medicinal plant on the haematology and serum biochemical properties of domestic rabbits.

Materials and Method

Experimental site

The experiment was conducted at the Rabbitry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The study area lies at an altitude of 122m and within the rainforest zone of south-eastern Nigeria it has binomial rainfall pattern and total annual rainfall range of 1700-2100mm [8]. The maximum ambient temperature of the area ranges from 27°C to 36°C during the hot dry season of the year (November-April) and minimum ambient temperature ranges from 20°C to 26°C during the cold rainy season (May-September). The relative humidity ranges 57 – 91 and is location in hot humid tropics [8].

Experimental animals and management

Thirty-six (36) nulliparous mixed-breed rabbit does, aged 6-7 months were used for this study. The animals were purchased from

reputable rabbit breeders and quarantined for a period of three (3) weeks before commencement of the experiment. The animals were housed in individual cells in wooden hutches and fed concentrate ration of alongside grass-legume forages. Fresh clean water was provided adlibitum.

Feedstuff	% composition
Maize	38
Maize offal	10
Soybean	14
Wheat offal	20
Palm Kernel Cake (PKC)	14
Bone meal	3.25
Salt	0.50
Vitamin Premix	0.25
Total (Kg)	100
Crude protein, CP (%)	16
Metabolizable Energy, ME Kcal/kg	2630

Table 1: Proximate composition of concentrate diet for experimental animals.

Experimental design and procedure

The thirty-six (36) nulliparous rabbit does were randomly selected and assigned into four (4) treatment groups designated as T1, T2, T3 and T4 in a completely randomized design. Each treatment comprised nine (9) animals. The treatments were replicated 3 times with 3 animals per replicate. Dried methanolic extract of *A. paniculata* was obtained from Natural Remedies Laboratories, India; a reputable human and animal herbal Pharmaceutical Laboratory. One gram (1g) of the dry powder of the *A. paniculata* extract was reconstituted in 100ml of distilled water to form a solution of 0.01g/ml concentration. The Concentration of the reconstituted extract was determined by dividing the mass of the dry *A. paniculata* extract by the volume of the solvent (distilled water) used. The extract was administered orally to the rabbit; early in the morning using a sterile syringe for a period of 8weeks, at varied doses as shown below

- **T₁**: (control): 0.0mg of extract per kg body weight per day.
- **T₂**: 125mg of extract per kg body weight per day.
- **T₃**: 250mg of extract per kg body weight per day.
- **T₄**: 500mg of extract per kg body weight per day.

The dose (ml) of the liquid suspension of the extract administered to the experimental rabbits, was determined using the formula: The average body weights of the experimental rabbit does ranged between 1568 – 1570kg.

$$\text{Dosage (ml)} = \frac{\text{Administration rate X concentration of solution}}{\text{Body weight of Animal}}$$

Data collection and analysis

Six (6) animals were randomly selected from each treatment group. Blood samples were collected early hours of the morning for haematological studies at the onset of the experiment for basal studies. Subsequently, samples were collected fortnightly, at week 4, 6, and 8, following administration of the herbal extract. 5ml of blood was extracted via the ear vein using a sterile hypodermic syringe. 2.5 ml was aspirated into a sample bottle containing an anticoagulant- ethylene diamine tetracetic acid (EDTA) for haematology. The remaining 2.5 ml was allowed to coagulate to produce serum for blood chemistry evaluation. The serum chemistry parameters of interest: urea, creatinine, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total protein, albumin, globulin, total bilirubin, conjugated bilirubin, cholesterol, glucose and serum electrolyte were prepared according to standard clinical chemistry procedure [9,10]. These serum components were deter-

mined using an automated biochemical analyzer (Eastman Kodak Co., Rochester, NY). The serum globulin values were determined by deducing the difference between serum total protein and serum albumin. The dressed weight of the rabbits was measured and then carcasses cut into individual parts and weighed. The data obtained were subjected to analysis of variance (ANOVA) as described by [11]. Significant means were separated using Duncan Multiple Range test [12].

The statistical models for the experiment is as shown below:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where: Y_{ij} = Single observation; μ = Overall mean; T_i = Treatment; ϵ_{ij} = Random error; ϵ_{ij} is independent, identical and normally distributed with zero mean and constant variance ($\epsilon_{ij} \sim iind 0, \sigma$).

Results and Discussion

Results obtained from the study on the effect of methanolic extracts of *A. paniculata* on the haematology and serum biochemistry of female rabbits are presented in tables 2 and 3 below.

Parameters	T ₁ (0.0mg/kg)	T ₂ (125mg/kg)	T ₃ (250mg/kg)	T ₄ (500ml/kg)	SEM
RBC (x10 ⁶ /mm ³)	3.95 ^c	4.58 ^{bc}	4.74 ^b	5.78 ^a	0.22
WBC (x10 ⁶ /mm ³)	55.67	61.67	58.00	60.33	4.73
PCV (%)	35.67	39.17	37.00	36.00	0.79
Hb (mg/dl)	12.33 ^a	11.07 ^{ab}	10.87 ^{ab}	10.47 ^b	0.31
MCV (fl)	75.70 ^{ab}	86.11 ^a	93.60 ^a	62.87 ^b	4.23
MCH (fl)	27.48 ^a	26.07 ^a	24.42 ^a	18.29 ^b	1.30
MCHC (mg/dl)	34.76 ^a	28.26 ^b	29.34 ^b	29.05 ^b	0.93
Platelets (x10 ⁹ /l)	292.33	290.33	289.67	298.00	3.28
Lymphocytes (%)	56.67	73.33	73.00	56.67	3.60
Neutrophils (%)	43.67 ^a	26.67 ^b	39.67 ^{ab}	34.33 ^{ab}	2.59
Monocytes	1.67	0.00	1.67	0.00	0.41
Eosinophils	0.00	0.00	0.00	0.33	0.08
Basophils	0.00	0.00	0.00	0.00	0.00

Table 2: Haematological profile of rabbit does administered *Andrographis paniculata* extracts.

^{ab,c,d} Means across rows with different superscripts differ significantly at P < 0.05; S.E.M: standard Error of the Mean; PCV: Packed Cell Volume; RBC: Red Blood Cell; Hb: Haemoglobin; WBC: White Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Parameters	T ₁ (0.0mg/kg)	T ₂ (125mg/kg)	T ₃ (250mg/kg)	T ₄ (500ml/kg)	SEM
Glucose (mg/dl)	78.00 ^a	73.00 ^a	54.67 ^b	46.33 ^c	4.06
Total Protein (mg/dl)	4.88 ^c	5.49 ^b	5.47 ^b	5.50 ^a	0.12
Albumin (g/dl)	3.17 ^b	3.18 ^b	3.27 ^b	3.51 ^a	0.05
Globulin (g/dl)	1.71 ^b	2.30 ^a	2.19 ^a	2.45 ^a	0.94
AST (IU/L)	71.23 ^b	71.15 ^b	69.10 ^b	61.92 ^a	1.28
ALT (IU/L)	38.96 ^b	48.07 ^{ab}	48.94 ^a	42.44 ^{ab}	1.74
Bilirubin (mg/dl)	0.55	0.38	0.42	0.37	0.03
Creatinine (mg/dl)	1.05 ^a	0.77 ^c	0.96 ^{ab}	0.82 ^{bc}	0.04
Urea (mg/dl)	22.96	21.47	23.53	19.86	0.94
Sodium [Na ⁺] (mEq/l)	136.32	138.88	139.27	144.11	1.80
Potassium [K ⁺] (mEq/l)	3.77 ^b	4.52 ^a	3.59 ^b	4.01 ^{ab}	0.13

Table 3: Serum biochemical profile of rabbit does administered *Andrographis paniculata* extracts.

^{a,b,c,d} Means across rows with different superscripts differ significantly at $P < 0.05$; S.E.M: Standard Error of the mean;

AST: Aspartate Transaminase; ALT: Alanine Transaminase

Values obtained for Red blood cell count range between $3.95-5.78 \times 10^6/\text{mm}^3$ and was found to be significantly higher ($P < 0.05$) in the treated group than in the control experiment. RBC count tended to increase with increase in the administration rate of the extract. The highest RBC value (5.78) was recorded in T₄ (500mg/kg). [13] and [14] in their study, reported RBC ranges of 4.10-5.60 and $3.60-4.20 \times 10^6/\text{mm}^3$ respectively for female rabbits. Similarly, [15] recorded RBC values ranging between 5.42-5.87 for Termond white rabbit does. There were no significant differences ($P > 0.05$) observed between the treatments, for WBC and PCV. Values recorded for PCV was higher than the range of 28.69-31.31% reported by [13]. Haemoglobin (Hb) value ranged from 10.47-12.33mg/dl and was found to be significantly lower in the treated groups than in the control. A lower range of 9.51-10.41mg/dl was recorded by [13]. [14] however, reported a haemoglobin range of 12.90-14.0g/dl in a similar study. MCV ranged between 62.87-93.60. Significantly higher ($P < 0.05$) values were obtained in T₂ (125mg/kg) and T₃ (250mg/kg) than in T₄ (500mg/kg) and the control T₁ (0.0mg/kg). A normal range of 78-95fl was indicated [16] for MCV in rabbits. A low MCV and MCH values is an indication of an anaemic conditions in the animal. MCH is the average mass of haemoglobin per red blood cell in a sample of blood [17]. MCH in this study was found to be significantly lower in T₄ (500mg/kg). Treatments T₂ (125mg/kg), T₃ (250mg/kg) and the control T₁ (0.0mg/kg), were statistically similar ($P > 0.05$). MCHC was with the range of 28.26-34.76. Significantly higher ($P < 0.05$) values were observed in the control, than in the treated group. MCHC ranges of 32.11-33.50 and 19.52-19.63 were reported by [13] and [15] respectively. Higher values ($P < 0.05$) were recorded for neutrophil count in the control experiment than in the treated groups. Values recorded for Platelets, lymphocyte and monocyte counts across the treatment groups were

statistically similar. [13] in a study, reported lymphocytes range of 58-73.0%, which is similar to 56.67-73.33% recorded in this study. However, lymphocyte counts of 61.67-69.67% was reported in another study [15]. Serum glucose ranged between 46.33-78.00mg/dl. serum glucose levels tended to decrease progressively with increase in the administration rate of the *A. paniculata* extract. Significantly lower ($P < 0.05$) values (54.67 and 46.33) were recorded for glucose in T₃ (250mg/kg) and T₄ (500mg/kg) respectively. The decline in serum glucose level is believed to be associated with the presence of andrographolide- the bioactive constituent of the extract used. Andrographolide has been reported to have antidiabetic, antihyperglycaemic and hypoglycemic properties [18,19]. A range of 18.0-75.80 was recorded in another study [12]. Total Protein increased significantly ($P < 0.05$) in the treated group, as the dose of the extract increased. A serum glucose range of 4.88-5.50 was recorded in this study. Serum glucose fell within the range of 3.00-6.90mg/dl recorded for rabbit does by [14] in a similar study. Serum albumin and globulin levels tended to improve progressively with increase in the administration rate of the extract. Significantly lower ($P < 0.05$) values were recorded for the liver enzyme, Aspartate transaminase (AST) in T₄ (500mg/kg). AST values obtained in T₂ (125mg/kg) and T₃ (250mg/kg) were similar to that of the control. ALT values significantly higher in the treated groups than in the control. Creatinine level was lower ($P < 0.05$) in the treated groups, as compared to the control. Creatinine values (0.77-1.05mg/dl) fell within a range of 0.70-1.20mg/dl recorded by [14]. There were no significant differences ($P > 0.05$) observed between the treatment for Bilirubin, urea and sodium concentration. Potassium concentration in the blood varied within a narrow range and was found to be relatively higher in T₂ (125mg/kg) than in the other treatment groups. Potassium concentration recorded

in this study (3.77-4.01) is within the range of 3.10-5.30 recorded by [13]. The results obtained from this study suggest that the *A. paniculata* extracts administered to the female rabbits in this study, had influenced of the blood profile of the animals. The significant reduction in blood glucose levels is suspected to be connected with andrographolide present in the *A. paniculata*. This is in consonance with earlier reports that andrographolide exhibit hypoglycemic and antidiabetic properties [18-20].

Conclusion

Andrographis paniculata has earlier been reported to elicit physiological responses in animals. Results obtained from this study reveals that the methanolic extracts of *A. paniculata* administered to the rabbit does, had significant influence on some blood parameters. The marked reduction in serum glucose level supports earlier assertions that the plant has hypoglycemic extracts. RBC and serum protein level were observed to improve. Higher protein levels in the treated group is suspected to be associated with enhanced liver function and nutrient metabolism brought about by andrographolide in the plant.

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