Haematological and biochemical responses of rabbits to aqueous extracts of *Gmelina arborea* leaves

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Abstract. A total of twenty four crossbred growing rabbits (2-3 months old) were used in an experiment that lasted for 8 weeks. The animals were randomly grouped into four and assigned to four treatments of 0 (T1), 150 (T2), 300 (T3), and 450 mL/L (T4) of aqueous extracts of *Gmelina arborea*, prepared from freshly harvested *G. arborea* leaves and administered through drinking water. Red blood cells of the animals were significantly (P<0.05) increased at 150 mL/L (4.30 X 106/mm³) than those on 0 mL/L (3.33 X 106/mm³) which is the control (T1). Neutrophils progressively decreased from T1 to T4 which was significant (P<0.05) between T1 (43.00%) and T4 (35.00%). Lymphocyte counts recorded significant (P<0.05) difference between T1 (55.00%) and T4 (60.00%). Total protein and total globulin were significantly (P<0.05) depressed in rabbits receiving different concentrations of aqueous extracts of *G. arborea* leaves. Alkaline phosphatase, aspartate amino transaminase, urea and creatinine analyses of the animals on different groups, recorded significant (P<0.05) differences. Glucose analysis of the rabbits recorded a progressive significant (P<0.05) decrease as the concentrations of aqueous extracts of *G. arborea* leaves were increased (T1 = 4.50, T2 = 3.70, T3 = 3.55, T4 = 3.05 mmol/L). This study reveals the anti diabetic properties of aqueous extracts of *G. arborea* leaves and its ability to enhance renal function. However, it depressed total globulin which also affected the total serum protein levels of the animals.

Key Words: rabbits, aqueous extracts, *Gmelina arborea* leaves, haematological, biochemical.

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Introduction

In developing countries like Nigeria, scarcity and high prices of feedstuffs have led animal nutritionists and researchers to look for alternative, unconventional, and cheap sources of feeding materials (Adeniji 2000; Esonu *et al* 2005). This problem is probably due to the perpetual human population increase, resulting in great demand for food, particularly foods of animal origin and products (Smith 1998). In order to control these challenges militating against animal production in Nigeria, several attempts have been made with novel crops and shrubs to produce feed materials for livestock.

Gmelina arborea is one of the unconventional materials being explored for the production of feedstuff. Studies by Annongu & Folorunso (2003) recorded that *G. arborea* fruit meal when processed and at 30% dietary inclusion in pigs ration has no adverse effects on the haematology and serum biochemical analyses of the animals. The nuts of *G. arborea* when processed and incorporated into the diets of broiler chicks, did not produce deleterious effect on the performance and biochemical parameters investigated (Annongu *et al* 2004). Reports by Onabanjo & Onwuka (1998); Okagbare & Brathe (1999) indicated that goats fed *G. arborea* leaves alone constantly lost weight. This they attributed to inadequate energy intake by the goats to meet

their requirement for maintenance. Okagbare *et al* (2004) from their studies in feeding West African Dwarf goats with *G. arborea* leaves, supplemented with grasses (*Pannicum maximum* and *Pennisetum purpureum*), suggested the investigation of the antinutritional factors of *G. arborea* and determination of the optimum level of feeding.

Unfortunately, there is little or no information on the nutritional effects of *G. arborea* on the haematological and serum biochemical parameters of rabbits. Since food or feed components affect body constituents (Harper *et al* 1979), haematological and biochemical analyses become highly significant in the assessment of the nutritional effects of feedstuffs on the animals. As the quest for unconventional and cheap sources of feedstuffs for livestock continues, it becomes imperative to always investigate the health and physiological implications of such materials on the animals. This study, therefore, was designed to investigate the effect of aqueous extracts of *Gmelina arborea* leaves on the haematologiccal and biochemical parameters of domestic rabbits.

Materials and Methods

A total of twenty four crossbred growing rabbits (2-3 months old) were used for the experiment which lasted for eight weeks. The animals were randomly grouped into four, of six rabbits each, and further divided into three replicates of two rabbits each. Each rabbit was housed separately in two 3-tier hutches of twelve cages each. The groups were randomly assigned to four treatments; T1, T2, T3 and T4, containing 0, 150, 300 and 450 mL/L of aqueous extracts of G. arborea leaves, respectively. The experiment was carried out at the Teaching and Research Farm of Federal University of Technology, Owerri, Imo State, Nigeria. Fresh leaves of G. arborea were harvested daily, until termination of experiment, from Umuelem, Ihiagwa, Owerri West Local government area of Imo State, Nigeria; a nearby community to Federal University of Technology Owerri. The leaves were washed and allowed to dry at room temperature for 24 hours before being crushed with mortar and pestle until it turned into paste. Then 500 g of this paste was soaked in 1000 mL of water and left for 30 minutes. A clean silk cloth was then used to sieve out the liquid part of the G. arborea paste to get the aqueous extract of G. arborea leaves.

The aqueous extracts of G. *arborea* leaves was administered to the animals through clean drinking water at concentrations of 0, 150, 300 and 450 mL/L, for T1 (control), T2, T3 and T4, respectively for a period of eight weeks. The animals were given growers pellets of Top feed brand, ad-libitum.

Blood for haematological and serum biochemical analyses were collected from the auricular vein, fortnightly. The haematological analysis was done through standard procedures. Total white blood cell counts (WBC) were determined by the haemmocytometer method while the differential count smears were prepared and stained by the Leishman technique and enumerated by the longitudinal counting method (Schalm *et al* 1975). The packed cell volume (PCV) was determined by the microhaematocrit method and the haemoglobin (Hb) was determined by the cyanomethaemoglobin method (Schalm *et al* 1975; Thrall & Weiser 2002).

Determination of serum activity of alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (Alb) and globulins (Glb), urea, creatinine and cholesterol were carried out following standard procedures (Coles 1986; Meyer *et al* 1992; Evans 1996), using Quimica Clinica Aplicada (QCA) test kits (Quimica Clinica Aplicada, Spain) and a Spectrum lab 21A Spectrophotometer (Spectrum lab, England). Blood glucose level was determined using ACCU-CHEK Glucometer (Roche Diagnostics GmbH, Germany).

Data collected were subjected to analysis of variance (ANOVA) test to determine if there were significant differences among treatment means according to Steel & Torrie (1980). Where significant differences existed, means were separated using the Duncan's Multiple Range Test (DMRT).

Results

The haematological parameters of rabbits for this study is presented in Table 1. The haemoglobin (Hb) concentration, packed cell volume (PCV), total white blood count (WBC), eosinophils and monocyte counts of the rabbits were similar (P>0.05) between the groups. Significant (P<0.05) differences were recorded between the experimental groups for red blood cell counts (RBC), neutrophil and lymphocyte counts. No basophil was recorded.

Table 1. Haematological parameters of rabbits administered aqueous extracts of *G. arborea* leaves

Parameters	T ₁	T ₂	T ₃	T ₄	SEM
Hb (g/dl)	9.8	10.7	10.45	10.25	0.65
RBC (x 10 ⁶ /mm ³)	3.33 ^b	4.30ª	4.01 ^{ab}	4.15 ^{ab}	0.16
PCV (%)	32	35	34	33.5	2.13
WBC (x 10 ⁹ /L)	4	3.95	3.4	3.65	0.38
Neutrophils (%)	43.00ª	42.50 ^{ab}	40.00 ^{ab}	35.00 ^b	1.38
Eosinophils (%)	3	2.5	2.5	3.5	0.88
Basophils (%)	-	-	-	-	-
Lymphocytes (%)	55.00 ^b	57.00 ^{ab}	57.00 ^{ab}	60.00ª	0.75
Monocytes (%)	1	0	0.5	1.5	0.25

^{ab} means within a row with different superscripts are significantly different (P<0.05).

Animals on T2 (150 mL/L of aqueous extracts of G. arborea leaves) recorded the highest value for red blood cell counts (4.30 x 106/mm³), which were significantly (P>0.05) similar to the values for T3 (4.01 x 106/mm³) and T4 (4.15 x 106/mm³), but significantly (P<0.05) higher than T1 (3.33 x 106/mm³). The neutrophil value for T1 (43.00 %) was similar (P>0.05) to that of T2 (42.50%) and T3 (40.00%), but significantly (P<0.05) higher than T4 (35.00%). Animals on T4 recorded the highest lymphocyte counts of 60.00% which was similar to T2 (57.00%) and T3 (57.00%), but significantly (P<0.05) higher than T1 (55.00%). There were no basophils recorded for all the groups. Serum biochemical parameters of the rabbits as shown in Table 2 recorded significant (P<0.05) differences in the total protein (TP), globulin (Glb), alkaline phosphate (ALP), aspartate amino transaminase (AST), urea, creatinine and glucose. No significant (P>0.05) differences were recorded in albumin, alanine transaminase and cholesterol of the experimental animals. The rabbits on T1 (control) had the highest total protein value of 69.00 g/L, which was significantly (P<0.05) higher than the values for T3 (54.50 g/L) and T4 (54.00 g/L), but similar (P>0.05) to T2 (59.00 g/L) which was similar (P>0.05) to T1. The globulin values were 33.00, 24.00, 22.50 and 22.50 g/L for T1, T2, T3 and T4, respectively. The globulin value for T1 were significantly (P<0.05) higher than the other treatments which were similar (P>0.05). The alkaline phosphatase of the rabbits was significantly (P<0.05) higher in T2 (117.50 IU/L) than in T4 (59.50 IU/L), but similar (P>0.05) to T1 (98.00 IU/L) and T3 (93.50 IU/L) which were similar to T4. Aspartate amino transaminase of the rabbits in T1 (115.00 IU/L) was similar (P>0.05) to T2 and T3, but significantly (P<0.05) higher than T4 (51.50 IU/L) which was similar (P>0.05) to T2 and T3. The urea analysis recorded significantly (P<0.05) higher value in T3 (24.00 mmol/L) than in T1 (4.30 mmol/L) and T4 (21.00 mmol/L), but similar (P>0.05) to T2 (23.00 mmol/L) which was similar (P>0.05) to T4. Serum creatinine levels of the rabbits was significantly (P < 0.05) higher in T2 (61.00 mmol/L) than in T1 (20.00 mmol/L) and T4 (40.00 mmol/L) but similar (P>0.05) to T3 (50.00 mmol/L) which was similar (P>0.05) to T4. The rabbits in T1 recorded significantly (P<0.05) higher glucose level than the other treatments. The glucose value for T2 was significantly (P<0.05) higher than the value for T4 but similar (P>0.05) to T3 which was similar (P>0.05) to T4. The serum glucose values were 4.50, 3.70, 3.55 and 3.05 mmol/L for T1, T2, T3 and T4, respectively.

Table 2. Serum biochemical parameters of rabbits administered aqueous extracts of *G. arborea* leaves

Parameters	T ₁	T ₂	T ₃	T ₄	SEM
Total protein	69.00ª	59.00 ^{ab}	54.50 ^b	54.00 ^b	2.38
Albumin	36	35	32	31.5	0.88
Globulin	33.00 ^a	24.00 ^b	22.50 ^b	22.50 ^b	1.5
Alkaline phosphatase	98.00 ^{ab}	117.50ª	93.50 ^{ab}	59.50 ^b	11.13
Alanine amino transaminase	62	55	59	59	4.13
Aspartate amino transaminase	115.00ª	70.50 ^{ab}	51.50 ^{ab}	30.50 ^b	11.63
Urea	4.30°	23.00 ^{ab}	24.00ª	21.00 ^b	0.5
Creatinine	20.00°	61.00 ^a	50.50 ^{ab}	40.00 ^b	2.63
Cholesterol	2.27	2.27	1.96	1.76	0.26
Glucose	4.50ª	3.70 ^b	3.55 ^{bc}	3.05°	0.13

 abc means within a row with different superscripts are significantly different (P<0.05).

Discussion

The results of the haematological analyses of the rabbit, reveals that the haemoglobin (Hb) concentrations of rabbits administered different concentrations of aqueous extracts of G. arborea were higher than those of the control experiment. Although the Hb concentration values were not significantly (P>0.05) different among animals receiving aqueous extracts of G. arborea, there was an inverse decrease in Hb concentration of the animals as concentrations of G. arborea extracts increased. Annongu & Folorunso (2003) observed a non-significant (P>0.05) similar trend in their studies with G. arborea fruit meal on pigs. G. arborea leaves improved feed conversion ratio in West African Dwarf goats (Okagbare et al 2004), which implies better feed and nutrient utilization by the animals receiving the treatments, and may result in higher values of Hb concentrations than those animals not receiving the treatment. The decrease in Hb concentration with increasing concentration of aqueous extracts of G. arborea leaves may be as a result of increasing antinutritional factors in the extracts (Okagbare et al 2004) as the concentrations were increased. The significant (P<0.05) increase in red RBC of the rabbits receiving the treatments (which suggests better oxygen carrying capacity) may be an indication of the ability of G. arborea leaf extracts to enhance feed utilization. Packed cell volume (PCV), though not significantly (P>0.05) different among the experimental groups, increased in the rabbits receiving the treatments. The neutrophils and lymphocytes in rabbits administered aqueous extracts of G. arborea leaves differed significantly (P<0.05). At 450 mL/L administration of the aqueous extracts of G. arborea leaves, the neutrophil count of the rabbits became significantly (P<0.05) lower than the values for those on the control experiment (0.00 mL/L of aqueous extracts of G. arborea leaves). Annongu & Folorunso (2003) recorded similar decrease in neutrophil counts in their studies with pigs fed *G. arborea* fruit meal, which they attributed to one, or a combination of the phytotoxins, like tannins, tartaric acids, benzoic and butyric acids present in *G. arborea*. This may also be reason for the increased values of lymphocyte counts of the rabbits receiving the aqueous extracts of *G. arborea* leaves, which at 450 mL/L administration became significantly (P<0.05) higher than those of the animals on the control experiment.

The analyses of the serum biochemical characteristics of the rabbits recorded significant (P<0.05) decrease in total protein and total globulin values of the rabbits administered different concentrations of aqueous extracts of G. arborea leaves. This result is similar to the ones reported by Annongu & Folorunso (2003) in their studies on biochemical evaluation of G. arborea fruit meal as a swine feedstuff; and Annongu et al (2004) in their studies on the dietary effects of chemically treated G. arborea nuts on the performance and certain biochemical indices in broiler chicks. Although the decrease in total protein and total globulin reported by Annongu et al (2004) were not significant (P>0.05), a continuous progressive decrease was observed as the level of dietary inclusion of chemically treated G. arborea nuts increased from 5 to 25%. This non-significant (P>0.05) decrease may be due to their treatment of the test diets with Polyvinylpyrrolidone (PVP) for the detoxification of the phytotoxins present in G. arborea nuts. In this present study, the significant (P<0.05) decrease in total protein and total globulin may be attributed to the presence of phytotoxins present in the aqueous extracts of G. arborea leaves. This may suggest a compromised immune system of the animals since globulins are serum proteins involved in the immune system (Charles 2001). The non-uniform trend observed in the results of the alkaline phosphate (ALP) values of the experimental rabbits, which recorded significant (P<0.05) differences among the groups could be attributed to a number of factors including the homeostatic mechanisms of the animals, and the active ingredients in the aqueous extracts of G. arborea leaves being functionally relative to each other in respect of quantities available (Noboru 2001). Alkaline phosphatase is present in tissues throughout the entire body of the animal, but is particularly concentrated in the liver, bile duct, kidney, bone, and the placenta (Kim & Wycoff 1991). Futher research will be needed to determine the cause of the non-uniform trend and source of the elevated values in alkaline phosphatase of the animals receiving 150 mL/L of aqueous extracts of G. arborea leaves. Alanine amino transaminase (ALT) values of the experimental rabbits exhibited no significant (P>0.05) difference among the groups, but decreased in the animals receiving aqueous extracts of G. arborea leaves. Aspartate amino transaminase (AST) values recorded an inverse decrease with increasing concentrations of aqueous extracts of G. arborea leaves. AST occurs in a wide variety of tissues, but with high concentrations in muscular tissues and in liver (Kaneko 1980; Bush 1991; Dial 1995). The decrease in AST activity could therefore be a reflection of the absence of degenerative changes in the muscles and livers of the rabbits receiving the treatments. Urea and creatinine values of the experimental rabbits followed similar trends, with the rabbits receiving the treatments exhibiting significantly (P<0.05) higher values than those on the control group (T1). Similar results were obtained by Annongu & Folorunso (2003), and Annongu et al (2004) in their experiments with G. arborea on swine and broiler chicks,

respectively. Since the reference standard range of serum urea and creatinine levels for rabbits (Mitruka & Rawnley 1977) were maintained, and a recent research (Harita et al 2008) has associated lower serum creatinine level with increased risk of type 2 diabetes. The increased level of serum creatinine of the test animals relative to those on control group could suggest the anti diabetic property of aqueous extracts of G. arborea leaves. This is corroborated by the significant (P < 0.05) decrease in serum glucose of the test animals. The urea-creatinine ratio has been found to be an estimation factor of other metabolic problems besides those intrinsic to the kidney (Delanghe et al 1989); that a urea level raised out of proportion to creatinine level may indicate a metabolic disorder. The urea level of the animals in this study is in a similar trend and proportion to their creatinine levels, thereby suggesting absence of a metabolic disorder (especially renal function) as a result of the administration of aqueous extracts of G. arborea leaves.

Conclusion

This study reveals the ability of aqueous extracts of *G. arborea* leaves to increase oxygen carrying capacity of rabbits, by increasing their RBC counts, and the ability to enhance renal function in the rabbits. However, the depression in total protein and total globulin of the rabbits receiving the treatments is a source of concern and advocates for further research, especially with the elimination of the antinutritional factors present in *G. arborea* leaf extracts.

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