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Haematological parameters and serum testosterone of West African dwarf rams treated with aqueous extract of *Cnidoscolus aconitifolius* (Chaya)

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The effect of *Cnidoscolus aconitifolius* (Chaya) plant extract on the haematological and serum testosterone of West African dwarf (WAD) breeding rams was carried out. The plant has been used traditionally for both medicinal and non-medicinal purposes. Eight West African dwarf rams were used in this study. The experiment spanned through 14 weeks. The weight of the animals was between 20 to 25 kg and the age between 18 to 24 months at the completion of the experiment. These animals were divided into groups A and B, there were 4 animals in each group. The animals were fed on concentrates and Chaya leaf extract was administered for 4 weeks. This study concludes that the use of 20 or 30% aqueous extract of *C. aconitifolius* for a period of four weeks led to a significant reduction in testosterone concentration. Therefore, the prolonged feeding of *C. aconitifolius* may precipitate decreased sexual function, a reduction in libido and erectile dysfunction because of low testosterone concentration and also a decrease value on haemoglobin which can precipitate anaemia and finally infertility in the male animals.

Key words: *Cnidoscolus aconitifolius,* heamatological parameters, serum testosterone, ram.

INTRODUCTION

Cnidoscolus aconitifolius (Chaya) is a perennial shrub of the Family Euphorbiaceae, commonly found in the tropics. It is commonly eaten as vegetable in soup condiment in South Western Nigeria where it is called “Iyanalpaja”. High fiber content and antibacterial activities of this plant have been reported (Oyagbemi et al., 2008). Apart from the antibacterial activities (Lenzen, 2008), the ameliorative effect of *C. aconitifolius* on anaemia and increased erythrocyte osmotic fragility induced by protein energy malnutrition (PEM) has been reported while its antidiabetic property has also been elucidated (Oyagbemi et al., 2008).

Chaya is being fed to animals as a laxative, diuretic, circulation stimulant and to improve digestion (Rowe, 1994), but there is paucity of information on its effects on reproduction in the sheep. Thus, the need to carry out this study to determine the effects of Chaya plant on hea-matological parameters and serum testosterone assay of

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the ram.

MATERIALS AND METHODS

Animals used for experiment

The study was conducted on eight healthy West African dwarf rams kept at the Small Ruminant Unit of Department of the Veterinary Surgery and Reproduction, University of Ibadan. The experiment spanned for 14 weeks. The weight of the animals ranged between 20 to 25 kg and they were aged between 18 to 24 months. They were divided into two groups A and B, with 4 animals per group.

Management of experimental animals

The sheep were housed in a concrete and well ventilated pen with four animals per pen. They were permanently in the pen and were fed with concentrates throughout the period of experiment. The size of the pen was 4.7 m × 3.14 m. The pen was regularly cleaned and the sheep were stabilized or allowed to acclimatize with the new environment for 2 weeks before they were fed with Chaya leaf extract. Animals were de-wormed with albendazole bolus after weighing. They were vaccinated against peste de petit ruminatum (PPR) using PPR vaccine (NVRI, VOM) and veterinary attentions were provided when required. The animals were divided into 2 groups and fed on sheep concentrates containing crude protein 19.16%, crude fibre 6.63%, fat 3.67%, energy 2,372 Kcal, calcium 0.88%, lysine 0.60% and different volume of Chaya leaf extract which contains varying level of Chaya leaf extract.

Location of study

This study was carried out at the Small Ruminant Unit and The riogenology laboratory of the Department of Surgery and Reproduction, University of Ibadan. University of Ibadan is about 6 km to the North of Ibadan city, at latitude 2nd longitude 3° 54' East at mean altitude of 277 m above Sea level. The annual rainfall is 1,200 mm, most of which fall between April and November, and a dry season from December to March. This study was carried out between April and June, 2012 under the same ambient temperature (27 to 31°C) and relative humidity of about 80%.

Plant material and treatment groups

C. aconitifolius leaves were harvested within the Campus of the University of Ibadan, Oyo State, Nigeria and was identified at the herbarium of the Department of botany, University of Ibadan. The leaves were picked, cleaned, weighed and macerated. Daily 100 ml of distilled water was added to 20 and 30 g of the *C. aconitifolius* leaf.

Group A was fed with 20% Chaya leaf extract.
Group B was fed with 30% Chaya leaf extract.

This was based on the recommended percentage value reported by Oyagbemi et al. (2008). Each ram in the Group A and B was given 5 ml of the Chaya leaf extract orally and once daily for a period of 4 weeks.

Blood analysis

Determination of packed cell volume (PCV) by microhaematocrit method

Plain capillary tubes were filled with the blood samples up to 2/3 of the whole length. The vacant end of each tubes were placed in the haematocrit and centrifuged for five minutes at a speed of 3000 revolutions per minute and the percentage of the packed cell volume were read from the graphic reader (Schalm et al., 1975).

Erythrocyte (RBC) count

The RBC count was made using haemocytometer. Erythrocyte diluting pipette was used to draw the blood samples to a point marked 0.5 on the pipette. The tip of the pipette was wiped free of blood and filled with blood cell diluting fluid drawn to a point marked 101 on the pipette.
This was then mixed together. About one third of the content of the pipette was discarded and the counting chamber filled. The cells were then observed under the microscope. Erythrocytes in 5 of the 25 squares in the central area of each chamber of the haemocytometer were counted, taking the 4 corner squares and central one (Schalm et al., 1975).

Leucocyte count

Total white blood cell count was made in a haemocytometer using the white blood cell diluting fluid. Leucocyte diluting pipette was used to draw blood sample to a point marked 0.5 and filing up to the 11 mark using the leucocyte diluting fluid. The white blood cells in the 4 large corner square of the haemocytometer chamber were then counted and the total multiplied by 50 (Schalm et al., 1975).

Differential white blood cell count

Thoroughly cleaned smooth slides were used. A clean slide with a small drop of blood was placed on a flat surface. Another slide was then used to make a thin smear. The slide was then allowed to air-dry and then fixed in absolute methanol for about 5 min. Giemsa stained slides were used for leucocyte count. They were examined for different leucocyte types under oil immersion of a microscope. The different leucocyte types were then expressed as percentage of the total (Reece, 1997).

Quantitative determination of testosterone in serum

Testosterone was quantitated by the enzyme immunoassay (EIA) Method (Bosch, 1978b).

Data analysis

The data generated was analyzed using the test of homogeneity of variance, multiple comparisons and analysis of variance (one-way ANOVA). SPSS version 15 for Windows (SPSS Inc, 2006) and Microsoft Excel Professional Plus (Microsoft Corporation, 2010) were used to carry out all procedures.

Table 1. Haematological results of WAD rams fed 20% Chaya extract during pre-treatment, 2nd week and 4th week post-treatment.

Parameter	Pre-treatment	2nd Week	4th Week
PCV (%)	36.67±2.40[a]	28.67±1.20[a]	29.33±4.18[a]
Hb(%)	12.27±0.70[b]	9.43±0.39[a]	10.93±0.43[ab]
RBC ($\times10^{12/7}$)	15.27±2.18[a]	12.56±1.77[a]	16.11±2.32[a]
WBC ($\times10^{9}$/L)	8.87±2.03[a]	19.53±6.00[a]	9.73±0.47[a]
Platelets ($\times10^{9}$/L)	10.67±0.67[a]	8.67±0.67[a]	9.33±0.67[a]
MCV (Fl)	24.00±2.00[a]	24.00±5.03[a]	18.67±1.86[a]
MCH (Pg)	7.33±0.67[a]	7.67±1.67[a]	6.00±0.58[a]
MCHC (%)	33.00±0.00[a]	33.00±0.00[a]	33.00±0.00[a]
Lym (%)	32.33±5.04[a]	30.00±5.03[a]	28.33±4.91[a]
NEUT (%)	67.00±4.93[a]	69.00±4.58[a]	71.33±4.70[a]
MONO (%)	2.00±0.00[a]	1.50±0.50[a]	1.00±0.00[a]
ESR	1.67±0.67[a]	2.67±0.67[a]	4.67±0.88[a]

[a,b,c]Means with same superscripts are not significantly different at 0.05 level along the row.

RESULTS

Haematological results

Table 1 shows the haematological results of West African dwarf (WAD) rams fed 20% Chaya extracts during the three periods of the experiment. Packed cell volume (PCV) decreased from (36.67 ± 2.40%) pre-treatment to (28.67 ± 1.20%) at 2nd week post-treatment and then slightly increased to (29.33 ± 4.18%) at 4th week post-treatment. There were no significant differences ($P > 0.05$) between PCV values for the three periods. Haemoglobin values significantly ($P < 0.05$) decreased from (12.27 ± 0.70%) pre-treatment to (9.43 ± 0.39%) at 2nd week and then increased, though not significant ($P > 0.05$), to (10.93 ± 0.43%) at 4th week post-treatment. Haemoglobin during the 4th week post-treatment was not significantly different ($P > 0.05$) from haemoglobin values during pre-treatment period of the experiment. Red blood cell (RBC) count decreased from ($15.27 \pm 2.18 \times 10^{12/7}$) pre-treatment to ($12.56 \pm 1.77 \times 10^{12/7}$) at 2nd week post-treatment and then increased to ($16.10 \pm 2.32 \times 10^{12/7}$) at 4th week post-treatment. The changes in RBC count during the three periods were not significant ($P > 0.05$).

Total white blood cell (WBC) count increased from ($8.87 \pm 2.03 \times 10^{9}$/L) at pre-treatment to ($19.53 \pm 6.00 \times 10^{9}$/L) at 2nd week post-treatment and then decreased to ($9.73 \pm 0.47 \times 10^{9}$/L) at 4th week post-treatment. There were no significant changes ($P > 0.05$) in total WBC throughout the periods of the experiment. Platelets count decreased from ($10.67 \pm 0.67 \times 10^{9}$/L) at pre-treatment to ($8.67 \pm 0.67 \times 10^{9}$/L) at 2nd week post-treatment and then increased slightly to ($9.33 \pm 0.67 \times 10^{9}$/L) at 4th week post-treatment. There were no significant changes ($P > 0.05$) in the platelets count throughout the period of the experiment.

Mean corpuscular volume (MCV) was relatively constant between pre-treatment (24.00 ± 2.00 Fl) and 2nd week post-treatment (24.00 ± 5.03 Fl) and then decreased at 4th week post-treatment to (18.67 ± 1.86 Fl) but the decrease in MCV at the 4th week post-treatment was not significant ($P > 0.05$). Mean corpuscular haemoglobin (MCH) values increased from (7.33 ± 0.67 Pg) at pre-treatment to (7.67 ± 1.67 Pg) at 2nd week post-treatment and then decreased to (6.00 ± 0.58 Pg) at 4th week post-treatment. There was no significant ($P > 0.05$) change in MCH values throughout the periods of the experiment. MCHC values were same (33.00 ± 0.00%), throughout the three periods of the experiment.

Lymphocyte value decreased as the week post treatment increased. The values decreased from (32.33 ± 5.04%) pre-treatment to (30.00 ± 5.03%) and then decreased further to (28.33 ± 4.91%) at 4th week post-treatment. The decrease in lymphocyte values was not significant ($P > 0.05$) throughout the period of the experiment. Neutrophils increased throughout the period of the experiment from (67.00 ± 4.93%) at pre-treatment to (69.00 ± 4.58%) at 2nd week post-treatment and further increased to 71.33 ± 4.70% at 4th week post-treatment. The increase in neutrophils was not significant ($P > 0.05$) throughout the period of the experiment.

Monocytes, just like lymphocytes, kept decreasing throughout the period of the experiment. Monocyte values decreased from (2.00 ± 0.00%) at pre-treatment to (1.50 ± 0.50%) at 2nd week post-treatment and further decreased to (1.00 ± 0.00) at 4th week post-treatment. Decrease in monocyte values was not significant ($P > 0.05$) throughout the period of the experiment.

Table 2. Haematological results of WAD ram fed 30% *Chaya* extract during pre-treatment, 2nd week and 4th week post-treatment.

Parameter	Pre-treatment	2nd Week	4th Week
PCV (%)	35.5±5.95	27.75±2.25	31.25±1.65
Hb(%)	11.75±1.99	9.13±0.75	9.93±0.65
RBC ($\times10^{12/7}$)	14.11±2.50	10.82±1.18	14.82±2.38
WBC ($\times10^{9}$/L)	11.63±1.96	15.60±2.35	10.00±0.96
Platelets ($\times10^{9}$/L)	10.50±2.06	7.50±0.96	9.00±0.58
MCV (Fl)	24.75±1.65	28.00±3.19	20.75±2.46
MCH (Pg)	6.50±1.89	8.75±1.03	6.75±0.85
MCHC (%)	33.00±0.00	33.00±0.00	33.00±0.00
Lym(%)	31.25±1.80	27.75±3.40	29.00±3.70
NEUT (%)	68.50±1.94	71.75±3.42	70.75±3.50
MONO (%)	1.00±0.00	1.00±0.00	1.00±0.00
ESR	2.25±0.63	3.25±0.63	4.25±0.63

Means with same superscripts are not significantly different at 0.05 level along the row.

Table 2 shows the haematological results of WAD rams fed 30% Chaya extracts during the three periods of the experiment. PCV decreased from (35.50 ± 5.59%) pre-treatment to (27.75 ± 2.25%) at 2nd week post-treatment and then increased to (31.25 ± 1.65%) at 4th week post-treatment. There were no significant differences ($P > 0.05$) between PCV values for the three periods. Haemoglobin values decreased from (11.75 ± 1.99%) pre-treatment to (9.13 ± 0.75%) at 2nd week and then increased, though not significant ($P > 0.05$), to (9.93 ± 0.65%) at 4th week post-treatment. There were no significant difference ($P > 0.05$) between haemoglobin values during three periods of the experiment. Red blood cell (RBC) count decreased from ($14.11 \pm 2.50 \times 10^{12/7}$) pre-treatment to ($10.82 \pm 1.18 \times 10^{12/7}$) at 2nd week post-treatment and then increased to ($14.82 \pm 2.38 \times 10^{12/7}$) at 4th week post-treatment. The changes in RBC count during the three periods were not significant ($P > 0.05$).

Total white blood cell (WBC) count increased from ($11.63 \pm 1.96 \times 10^{9}$/L) at pre-treatment to ($15.60 \pm 2.35.00 \times 10^{9}$/L) at 2nd week post-treatment and then decreased to ($10.00 \pm 0.96 \times 10^{9}$/L) at 4th week post-treatment. There were observed no significant changes ($P > 0.05$) in total WBC throughout the periods of the experiment. Platelets count decreased from ($10.50 \pm 2.06 \times 10^{9}$/L) at pre-treatment to ($7.50 \pm 0.96 \times 10^{9}$/L) at 2nd week post-treatment and then increased to ($9.00 \pm 0.58 \times 10^{9}$/L) at 4th week post-treatment. There were no significant changes ($P > 0.05$) in the platelets count throughout the period of the experiment.

MCV increased from pre-treatment (24.75 ± 1.65 Fl) to (24.00 ± 5.03 Fl) at 2nd week post-treatment and then decreased at 4th week post-treatment to (20.75 ± 2.46 Fl) but there were no significant changes ($P > 0.05$) in MCV throughout the three periods. MCH values increased from (6.50 ± 1.89 Pg) at pre-treatment to (8.75 ± 1.03 Pg) at 2nd week post-treatment and then decreased to (6.75 ± 0.58Pg) at 4th week post-treatment. There was no significant ($P > 0.05$) change in MCH values throughout the periods of the experiment.

MCHC values were the same (33.00 ± 0.00%), throughout the three period of the experiment. Lymphocyte value decreased from (31.25 ± 1.80%) at pre-treatment to (27.75 ± 3.40%) and then increased to (29.00 ± 3.70%) at 4th week post-treatment. The changes in lymphocyte values were not significant ($P > 0.05$) throughout the period of the experiment. Neutrophils increased from (68.50 ± 1.94%) at pre-treatment to (71.75 ± 3.42%) at 2nd week post-treatment and then slightly decreased to (70.75 ± 3.50%) at 4th week post-treatment. The changes in neutrophils were not significant ($P > 0.05$) throughout the period of the experiment. Monocytes values were the same, (1.00 ± 0.00%), throughout the three periods of the experiment. ESR values were increasing as the period post treatment increases. ESR increased from (2.25 ± 0.63) at pre-treatment to (3.25 ± 0.63) at 2nd week post-treatment and further increased to (4.25 ± 0.63) at 4th week post-treatment. The increase in ESR from control throughout the three periods was not significant ($P > 0.05$).

Hormonay assay

Serum testosterone

Table 3 shows the effect of Chaya extract on serum

Table 3. Serum testosterone of WAD rams fed 20 and 30% *Chaya* extract during pre-treatment, 2nd week and 4th week post-treatment.

Extract (%)	Mean ± SD (nml/L)		
	Pre-treatment	2nd Week	4th Week
20	16.5±14.62*	16.6±22.43	4.6±3.05*
30	7.48±9.48*	6.9±10.34	1.33±0.44*

*Mean values are significantly different at (P>0.05) level along rows.

testosterone at the three periods of the experiment for rams fed 20 and 30% Chaya extracts. There was a progressive decrease in the testosterone values of WAD rams fed 30% as the week post treatment increased. The decrease was significant ($P < 0.05$) at the 4th week post treatment. The same trend was noticed at the 4th week with the rams treated with 20% extract and the decrease was significant ($P<0.05$).

DISCUSSION

The blood analysis result obtained for the haemogram were within the normal range and there was no significant difference ($P < 0.05$) between the blood parameters except for the heamoglobin value which shows a significant decrease ($P < 0.05$) from (12.27 ± 0.70%) control to (9.43 ± 0.39%) at 2nd week post treatment with 20% Chaya extract. This indicates that the administration of Chaya extract will reduce the oxygen carrying capacity of the blood which may result in ischaemia, tissue anoxia and death. There was a progressive increase in the erythrocyte sedimentation rate (ESR) as the week post treatment increased in both groups treated with 20 and 30%, although the increase was not significant ($P > 0.05$). It can therefore be said that Chaya extract may cause a slight increase in ESR which indicate that there may be damage to reproductive tissues, resulting in inflammation.

Hormonal assay result reveals that there was a progressive significant decrease ($P < 0.05$) in testosterone values of WAD rams fed 30% as the week post treatment increased. Testosterone value decreased from control (7.48 ± 9.48) to (1.33 ± 0.44) at 4th week post treatment. The same trend was noticed at the 4th week with the rams treated with 20% extract and the decrease was significant ($P < 0.05$). This result agrees with the report of Adeniji et al. (2008) on the effect of 20% Chaya extract on the serum testosterone of WAD breeding bucks. It can therefore be said that 20 or 30% extract of *C. aconitifolius* will cause a significant reduction in the testosterone value of WAD rams. This suggests that continuous use of Chaya extract may result in decreased sexual function, a reduction in libido and erectile dysfunction.

Conclusion

This study concludes that the use of 20 or 30% aqueous extract of *C. aconitifolius* for a period of four weeks led to a significant reduction in testosterone concentration. Therefore, the prolonged feeding of *C. aconitifolius* may precipitate decreased sexual function, a reduction in libido and erectile dysfunction because of low testosterone concentration and also a decrease value of haemoglobin which can precipitate anaemia and finally infertility in the male animals.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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