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Effects of *Aspilia africana* on Oestrogen and Testosterone Concentrations in West African Dwarf Rams

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Abstract

Oestrogen and testosterone concentrations in West African Dwarf rams administered aqueous *Aspilia africana* extract were examined in the study using twenty-four rams. The experiment was in Completely Randomized Design with 4 treatment groups and 6 rams per treatment group. Rams in treatment 1 (control) received 10ml of distilled water, while those in T_2, T_3 and T_4 received 1000mg/kg Body Weight (BW), 2000mg/kg BW and 3000mg/kg BW of aqueous *Aspilia africana* extract, respectively. The extract was administered orally using 10ml syringe for for 64 days. Rams in all the treatment groups were fed 2kg of same forages and 500g of the same concentrate diet, daily. Blood samples were collected from 4 rams per treatment group, pre, during and post-experiment and used to determine sera concentrations of testosterone and oestradiol. Result of the study revealed no significant difference ($P>0.05$) in oestradiol and testosterone concentrations of the rams pre-experiment, whereas significant differences ($P>0.05$) were observed among the various treatment groups during and post-experiment. While oestradiol level was observed to decrease from 112.50pg/ml–80.00pg/ml in T_1 (control), the treated groups (T_2, T_3 and T_4), were observed to follow a reverse trend with oestradiol increasing from 117.50–122.50, 175.00–187.50, 230.00–235.00 (pg/ml) in T_2, T_3 and T_4, respectively. Whereas, testosterone levels (ng/ml) was observed to increase from 14.56 to 15.00 and thereafter to 15.72 (ng/ml) in the control group (T_1) before, during and post-experiments, respectively. T_2, T_3 and T_4 recorded decrease in testosterone level during and post-experiment. It was observed that administering more than 2000mg/kg BW of aqueous *Aspilia africana* could have significant effect on oestradiol and testosterone levels. This may have deleterious effects on libido and fertility of the rams. Therefore, *Aspilia africana* should not be fed to rams meant for breeding until an anti-dote which can suppress its anti-fertility effect is discovered.

Keywords

Aqueous, Oestrogen, Hormone, Testosterone, Ram

1. Introduction

In sub-Saharan Africa, sheep provide almost 30% of the meat consumed and about 16% of the milk. Sheep contributes about 50% of the total domestically produced meat in Nigeria. Sheep requires less capital as they are exclusively herbivorous animals and most breeds prefer to graze on grasses and other short forages [1].

Aspilia africana, commonly called African marigold plant is a weed grazed by cattle and sheep and is mostly used in Western States of Nigeria as feed for livestock. The herb is a good source of minerals such as Ca, P, Mg, Na, Fe, and Zn. Its phytochemical analysis revealed that it is rich in alkaloids, flavonoids, glycosides, phenols, phytosterol, saponins, tannins and tarpenes, some of which have phytoestrogenic activities [2-7]. It was also established that the leaves enclose carbohydrate, especially, monosaccharides [8]. In Africa use,

this plant for therapeutic reasons to cure a number of ailment such as sciatica, lumbago, malaria, scurvy, tuberculosis, rheumatism and even sexually transmitted disease (STD) like gonorrhea [8]. *A. africana* is a medicinal plants which contains a wide range of biological activity including antiviral, fungicide, anti-inflammatory and wound healing agent and antibacterial due to the presence of thiarubines, a derivative of 1, 2-dithiocyclohexane 3, 5-diene. It has also been reported to stimulate lactation [9].

As reported by Ogbuewu [10] the plane of nutrition given to animals can affect libido, fertility and reproduction. The process of reproduction in farm animals is initiated and regulated by hormones at the level of the brain [11]. According to Wei *et al.* [12], hormones are the most important environmental factors that affect germ cell development. All causes that have an impact on or can change hormonal conditions may affect sperm production. Courot and Ortavant [13] also stated that spermatogenesis in the ram is sensitive to variations in the level of circulating hormones, it could be perturbed by phytoestrogens [14]. O'Donnel *et al*. [15] also posited that the administration of oestrogenic substances may cause adverse effect on spermatogenesis and male fertility. Furthermore, libido is regulated by the release of testosterone, produced by specialized cells in the testes [16]. Spermatogenesis also depends on the action of testosterone [17].

However, *Aspilia africana* has been reported to be a growth promoter [18] with many medicinal values but little research has been conducted to validate the safety of the plant, with regards to reproduction.

Therefore, this study was conducted to examine the effect of administration of aqueous *Aspilia africana* extract on oestrogen and testosterone concentrations in West African Dwarf rams.

Figure 1. *African marigold (Aspilia africana) plant.*

2. Materials and Methods

2.1. Location and Site of Experiment

The research was conducted in Teaching and Research Farm of Department of Animal Science, Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus, Oruk Anam L. G. A., Akwa Ibom State Nigeria.

Obio Akpa is located between latitudes 5°17'N and 5°27'N and between longitudes 7°27'E and 7°58'E. It has an annual rainfall ranging from 3500mm–5000mm and average monthly temperature of 25°C. Akwa Ibom State is a coastal State lying between latitudes 4°28'N and 5°3'N and between longitudes 7°27'E and 8°20'E, with a relative humidity between 60–90%. It is in the tropical rainforest zone of Nigeria.

2.2. Collection, Preparation and Administration of Extract

Fresh leaves of *A. africana* were collected from Nung Uyo Idoro village in Uyo Local Government Area of Akwa Ibom State, Nigeria. The leaves were sorted to remove contaminants, dead matter and sand particles. They were prepared fresh to prevent loss of bioactive ingredients which can take place during drying. The leaves were chopped into tiny pieces with chopping stick and sharp knife and ground using hand blender to produce *A. africana* leaf meal. One thousand grams (1000g) of the leaf meal was measured into conical flasks and extracted with 600ml distilled water. The mixture was filtered into 250ml conical flasks with Whatman paper no. 1. The solution was filtered while the filtrate was concentrated to a semi-solid form using a rotary evaporator at 40°C to produce gel-like aqueous *A. africana* extract. This was weighed and the solution prepared as 100mg/ml, 200mg/ml and 300mg/ml respectively.

2.3. Experimental Animals and Management

Twenty-four (24) pubertal West African Dwarf rams of average weight of 4.65kg, aged 6–9 months from farm record, also confirmed by dentition, were sourced from 4 Local Government Areas (Uyo, Abak, Oruk Anam and Etim Ekpo) of Akwa Ibom State and used for the study. The flock was managed intensively. The sheep were quarantined for 2 weeks before the commencement of the experiment. Routine medications against endo and ectoparasites as well as suitable vaccination, together with fumigation were performed during the pre-experimental period. The animals were randomly assigned to 4 treatment groups, with one 1 ram per pen. The pens were constructed with concrete halved walls and iron doors. The research farm was well ventilated. The sheep were properly identified using plastic neck-tags.

The health of the animals was properly monitored and adequate treatment was given to unhealthy animals. Routine inspection and regular cleaning were carried out.

2.4. Experimental Diet

The rams were fed 2kg of forages daily. The forages consisted of: *Panicum maximum* (guinea grass), *Pennisetum purpureum* (elephant grass) and *Cynodon nlemfuensis* (star grass). Each animal also received 0.5kg

(500g) of concentrate daily. Water was provided ad-libitum throughout the study. The quantity of forage and concentrate diet offered to the animals were weighed daily and the left-over feeds were weighed every morning using a sensitive electronic balance. Tables 1 and 2 show the composition of the concentrate diet given to the experimental animals.

Table 1. *Gross composition of concentrate.*

Ingredients	%
Maize	40.01
Soybean meal	4.31
Rice bran	41.30
Palm kernel cake	11.38
Bone meal	2.00
*Vitamin/mineral premixes	0.50
Salt	0.50
Total	100

Vitamin/mineral premixes (Growers) produced by Animal Care Product/Care Services Konsult (Nig) Ltd, Iperu Road-Ibadan Express way, Ogera Remo, Ogun State. *Vitamin Premix: Vit. A=8,000,000 I. U, Vit D_3=1,700,000 I. U, Vit. E=5,000mg, Vit K_3=150mg, Folic acid=200mg, niacin=15,000mg, Vit. B_2=3,000mg, Vit. B_{12}=5mg, Vit. B_1=1000mg, Vit. B_6=1000mg, biotin=20mg. antioxidant=125,000mg. Mineral Premix: Cobalt=100mg, Selenium=100mg, iodine=100mg, Iron=25,000mg, Manganese=45,000mg, Copper=3,000mg Zinc=35, 000mg, Choline/chloride=100,000mg.

Table 2. *Proximate Composition of Formulated Concentrate Diet.*

Parameters	Percentages
Drymatter	86.26
Crude protein	12.71
Ether Extract	7.59
Crude fibre	7.6
Ash	5.46
Nitrogen free extract	52.9
Metabolizable energy (Kcal/kg)	2529.57

2.5. Experimental Design

The experiment was in completely randomized design. The treatment consisted of oral administration of aqueous *A. africana* extract at 0mg/kg body weight (T_1; control), 1000mg/kg weight (T_2), 2000mg/kg body weight (T_3), 3000mg/kg (T_4). Six rams were randomly assigned to each treatment and balanced for weights. Each treatment was replicated 3 times with two (2) rams per replicate. The experimental model was as follows:

$$Yij=\mu + T_1 + Eij$$

Where:
Yij=Individual observation
μ=Overall mean
Ti=Treatment effect
eij=Random errors, which is assumed to be independently, identically and normally distributed with zero mean and constant variance (iind) (P=0.05).

2.6. Administration of Aqueous Extract to Experimental Animals

After 2 weeks of quarantine and acclimatization, the aqueous extract of *Aspilia africana* was administered once a day orally, for 64 days. Ten milliliters (10mls) syringes were used for the administration of the extract. The control group (T_1) received 10mls of distilled water, orall, while treatments 2, 3 and 4 also orally received 10mls of each of the following 100mg/kg, 200mg/kg and 300mg/kg body weight of aqueous extract of *Aspilia africana*, respectively.

2.7. Blood Collection for Analysis

At day 1 (i.e., a day after the two weeks quarantine and acclimatization), day 28 of the experiment (period of administration of extract) and day 71 (a week after administration of the extract) of the experiment, blood samples (5ml) each were collected from 4 rams per treatment group and used to determine the sera concentrations of testosterone and oestradiol.

2.8. Data Analysis

Data obtained were subjected to Analysis of Variance (ANOVA) [19] Statistically significant difference between treatment mean values was determined using Fisher's Least Significant Difference as described by Akindele [20].

3. Results

3.1. Oestradiol Concentrations in West African Dwarf Rams Administered Aqueous *Aspilia africana* Extract

Figure 2 presents the results of oestradiol concentrations in rams administered aqueous *Aspilia africana* extract.

The result for oestradiol assay pre-experiment revealed no significant differences (P>0.05) among the various treatment groups as shown in Figure 2. However, during and after the experimental period, significant differences (P<0.05) were observed between the mean values for oestradiol (pg/ml) concentrations among the various experimental groups. While oestradiol level was observed to decrease from 112.50pg/ml–80.00pg/ml in T_1 (control), the treated groups (T_2, T_3 and T_4), were observed to follow a reverse trend with oestradiol increasing from 117.50–122.50, 175.00–187.50, 230.00–235.00 (pg/ml) in T_2, T_3 and T_4, respectively.

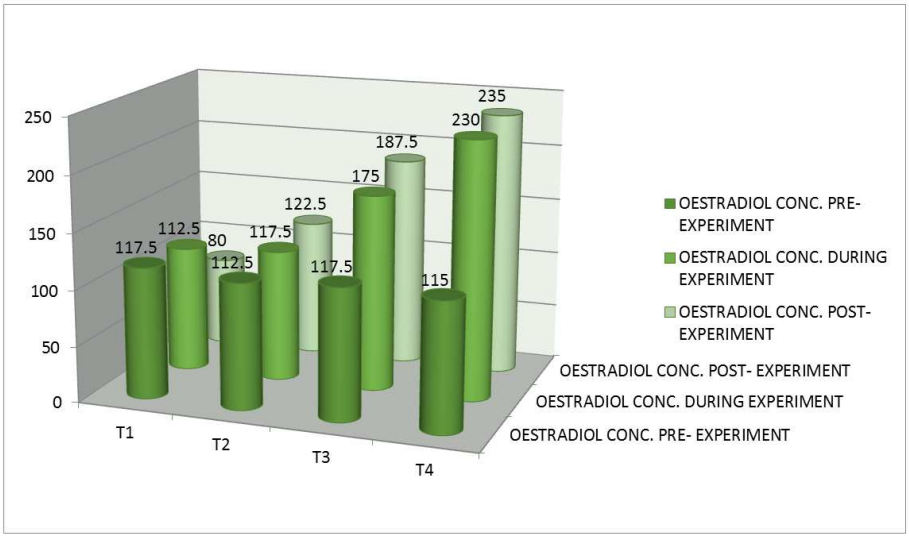

Figure 2. *Oestradiol concentration in rams administered aqueous Aspilia africana extract.*

3.2. Testosterone Concentrations in West African Dwarf Rams Administered Aqueous *Aspilia africana* Extract

Figure 3 presents the results of testosterone concentrations in rams administered aqueous *Aspilia africana* extract.

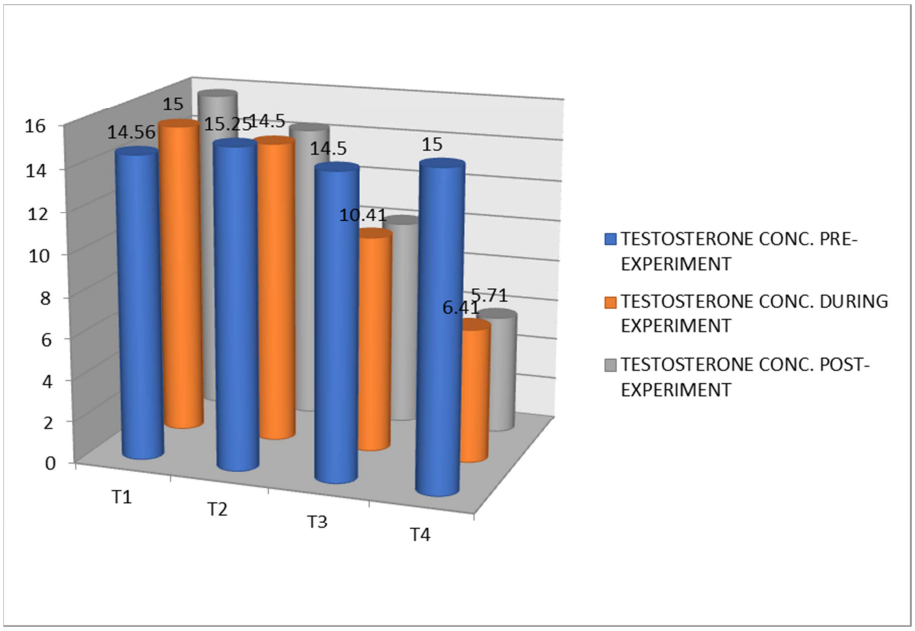

Figure 3. *Testosterone concentration in rams administered aqueous Aspilia africana extract.*

Testosterone levels were not significantly different ($P>0.05$) among the various treatment groups before the period of administration of the extract as shown in figure 3. During and after the experimental period, significant differences ($P<0.05$) were observed between the mean values for testosterone (ng/ml) concentrations among the various experimental groups. While, testosterone level (ng/ml) was observed to increase from 14.56 to 15.00 and thereafter to 15.72 (ng/ml) in the control group (T_1) before, during and post-experiments, respectively, T_2, T_3 and T_4 recorded decrease in testosterone level during and post-experiment.

4. Discussion

4.1. Oestradiol Concentration in Rams Administered Aqueous *Aspilia africana* Extract

The observed dose dependent increase and significant differences in oestradiol level in animals in T_2, T_3 and T_4 could be associated with the aqueous *A. africana* which was administered at different doses to rams in these treatment groups. The significantly ($P<0.05$) higher Oestradiol

concentration in rams in T_2, T_3 and T_4 could be an indication that *Aspilia africana* possesses phytochemicals with estrogenic properties/activities (phytoestrogens) and may negatively affect fertility. This is in line with the reports by Andersen [2], Okwu and Josiah [3], Okwuonu *et al.* [4], Abii [5], Ilondu [6] and Asumeng [7], that phytochemical screening of *A. africana* revealed that it contains alkaloids, flavonoids, glycosides, phenols, phytosterol, saponins, tannins and tarpenes, some of which have phytoestrogenic activities. These phytoestrogens could perturb spermatogenesis [14], decrease sperm concentration [21] resulting in deleterious effects on the rams' fertility. The inhibitory effect of steroidal saponin has been documented by Tamura *et al.* [22]. Phytoestrogen might as well cause underdevelopment and malformation of the male reproductive tract. As was reported by O'Donnel *et al*. [15], the administration of oestrogenic substances may cause adverse effect on spermatogenesis and male fertility. The findings of this study agree with the report by Jefferson [23] that phytoestrogen exposure can affect testes function and can have significant impact on reproductive health. The results obtained in this experiment is consistent with the report by Rochira *et al.* [24] that excess oestrogen influence testis function, spermatogenesis and ultimately fertility.

4.2. Testosterone Concentration in Rams Administered Aqueous *Aspilia africana* Extract

The reduction and significant differences ($P<0.05$) among the treated groups (T_2, T_3 and T_4) in results obtained for testosterone level may be due to either the 'agonistic' or 'antagonistic' effects of the pytoestrogen in the aqueous *A. africana* extract administered to these groups of rams. This is in line with the report by Carreau and Hess [14] that dietary pytoestrogens may be 'agonistic' in the absence of endogenous oestrogen but 'antagonistic' when present with endogenous oestrogen. The excess oestrogen reported for the treated rams in the present study could have increased the production of sex-hormone-binding globulin (SHBG) which is the component of blood that renders free testosterone inactive as was observed by Carreau and Hess [14]. The experimental extracts could have also affected the rams' ability to produce testosterone as a result of its phytoestrogen content. Excess estrogen in the plant extract might have influenced testosterone secretion in an indirect way by altering Leutinizing Hormone (LH) pulsatility as was observed by Schanbacher [25]. This finding agrees with the report by Life Extension [26] that excess oestrogen is taken up by testosterone receptor site in cells throughout the body.

When an oestrogen molecule occupies a testosterone receptor site on a cell membrane, it blocks the ability of serum testosterone to induce a healthy hormonal signal. According to Life Extension [26], it does not matter how much free testosterone is available if excess oestrogen is competing for the same cellular receptor sites. The findings of this experiment is also in accordance with the report by Zhang *et al.* [27] that seminal testosterone levels of infertile individuals in his study were lower than in normospermic individuals whereas seminal oestradiol level in azoospermic were significantly higher than in normospermic and non-obstructive azoospermia (NOA).

5. Conclusion

From the study, it was observed that administration of *Aspilia africana* could increase oestradiol concentrations while lowering testosterone concentrations in rams. Administering more than 2000mg/kg BW of aqueous *Aspilia africana* was observed to have significant effects on oestradiol and testosterone concentrations. This may have deleterious effects on libido, fertility and reproduction and may negatively affect animal production, Therefore, *Aspilia africana* should not be fed to rams meant for breeding until an anti-dote which can suppress its anti-fertility effect is discovered.

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