

Crassocephalum crepidioides improves semen quality and gonadometric indices in the West African dwarf bucks

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Abstract

The medicinal properties of Crassocephalum crepidioides against certain disease conditions have been studied. However, its effects on the male animal fertility is unknown. In the study, the effect of the aqueous leaf extract of Crassocephalum crepidioides on semen quality and gonadometric indices in the West African Dwarf (WAD) bucks was investigated. A total of 20 WAD bucks were assigned into two groups: G1 and G2 (n=10). Group G1 was supplied a daily oral dose of 250mg/kg aqueous leaf extract of Crassocephalum crepidioides for 14 days, while G2 served as the control. Semen and organ samples were harvested from the bucks at days 7 and 14 post-treatment. The treatment caused an increase in sperm motility, sperm livability, semen volume and sperm count in the treated group at day 7 post-treatment compared to the control. The sperm motility increased progressively till day 14 post-treatment. The total number of spermatozoa with morphological abnormalities also decreased progressively from days 7 to 14 post-treatment when compared to the control. The body weight, scrotal circumference, testicular and epididymal lengths and diameters as well as testicular germinal height, testicular tubular diameter and epididymal luminal diameter increased significantly at day 14 post-treatment when compared to the control. Treatment of WAD bucks with oral 250mg/kg aqueous leaf extract of Crassocephalum crepidioides for 14 days slightly improved the sperm quality and gonadometric indices. The plant possesses a fertility-enhancing potential, therefore, feeding of breeding bucks with Crassocephalum crepidioides leaves should be encouraged.

Keywords: Epididymis, fertility, goats, semen, testes

Crassocephalum crepidioides améliore la qualité de la semence et les indices gonadométriques chez les boucs nains d'Afrique de l'Ouest



Résumé

Les propriétés médicinales de Crassocephalum crepidioides contre certaines maladies ont été étudiées. Cependant, ses effets sur la fertilité des animaux mâles sont inconnus. Dans l'étude, l'effet de l'extrait aqueux de feuilles de Crassocephalum crepidioides sur la qualité du sperme et les indices gonadométriques chez les boucs nains d'Afrique de l'Ouest (NAO) a été étudié. Un total de 20 boucs NAO ont été répartis en deux groupes : G1 et G2 (n = 10). Le groupe G1 a reçu une dose orale quotidienne de 250 mg/kg d'extrait aqueux de feuilles de Crassocephalum crepidioides pendant 14 jours, tandis que G2 a servi de témoin. Des échantillons de sperme et d'organes ont été prélevés sur les boucs aux jours 7 et 14 après le traitement. Le traitement a entraîné une augmentation de la motilité des spermatozoïdes, de la vivabilité des spermatozoïdes, du volume de sperme et du nombre de spermatozoïdes dans le groupe traité au jour 7 après le traitement par rapport au groupe témoin. La motilité des spermatozoïdes a augmenté progressivement jusqu'au jour 14 après le traitement. Le nombre total de spermatozoïdes présentant des anomalies morphologiques a également diminué progressivement du jour 7 au jour 14 après le traitement par rapport au témoin. Le

poids corporel, la circonférence scrotale, les longueurs et diamètres testiculaires et épидидymaires ainsi que la hauteur germinale testiculaire, le diamètre tubulaire testiculaire et le diamètre luminal épидидymaire ont augmenté de manière significative au jour 14 après le traitement par rapport au témoin. Le traitement de boucs NAO avec un extrait aqueux de feuilles de *Crassocephalum crepidioides* à 250 mg/kg par voie orale pendant 14 jours a légèrement amélioré la qualité du sperme et les indices gonadométriques. La plante possède un potentiel d'amélioration de la fertilité, par conséquent, l'alimentation des mâles reproducteurs avec des feuilles de *Crassocephalum crepidioides* doit être encouragée.

Mots-clés: Épидidyme, fertilité, caprins, sperme, testicules

Introduction

The use of plants for healing purposes is very common in developing countries especially in the rural areas; this is probably due to the perceived benefits and lower side effect profile of natural products that are extracted from plants (Leonardo *et al.*, 2000; Leroy *et al.*, 2015). *Crassocephalum crepidioides* is an invasive herb included in the Global Compendium of Weeds and classified as one of the most aggressive weeds commonly found in tropical and subtropical regions (Randall, 2012). It is a popular plant and a common food supplement in Nigeria that belongs to the genus *Crassocephalum* and the family *Asteraceae* (Agbogidi, 2010). The leaves are used for soup and sauces in the Southern part of Nigeria and other humid zones of West and Central Africa (Adjatin *et al.*, 2013). The mucilage leaves are slightly laxative and are given to women after childbirth for treating stomach ache and liver complaints. An infusion of the leaf is taken against colds, burns, earache, sore (leaf sap) and filarial parasite-infected eyes (Gbadamosi, 2012, Adjatin *et al.*, 2013b). The leaves are also used in the treatment of indigestion and headache in Southern Nigeria, also the leaf sap is given to treat stomach upset in Congo while in Uganda, it is used for the treatment of fresh wounds (Denton, 2004). The medicinal potentials of *Crassocephalum crepidioides* against certain disease conditions have been studied. However, its effect on male animal fertility is unknown, therefore our study investigated the effect of aqueous leaf

extract of *Crassocephalum crepidioides* leaf on semen quality and gonadometric indices in the West African Dwarf breeding bucks.

Materials and methods

Experimental animals

The study was carried out at the Animal Research Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. Twenty (20) West African Dwarf (WAD) bucks were used for this study. The animals were fed twice daily with cassava peels, Dussa and freshwater were daily served *ad libitum*. The animal weighed between 15-17kg at the commencement of the study.

Plant material

Fresh leaves of *Crassocephalum crepidioides* were harvested from the Botanical Garden of the University of Ibadan, Nigeria, and were identified at the herbarium of the Department of Botany, University of Ibadan, with a voucher number (UIH-22688). The aqueous extract of *Crassocephalum crepidioides* leaves was prepared as described (Omotayo *et al.*, 2015).

Study design

Twenty goats were randomly assigned into two groups: G1 and G2 (n=10). Group G1 received a daily oral dose of 250mg/kg aqueous leaf extract of *Crassocephalum crepidioides* for 14 days, while G2 served as the control. Semen and organ samples were collected from the bucks at days 7 and 14 post-treatment.

Semen collection and analysis

Semen collection was done by the

electroejaculation method (Oyeyemi *et al.*, 1996). Sperm motility, liveability, concentration and Morphological abnormalities were evaluated using a modified method described by Ajani and Oyeyemi (2014).

Organs harvest and gonadometrics

Orchidectomy was performed by the open castration method as described by Raji and Ajala, (2015). The testis and epididymis were weighed using an electronic scale. Also, the testicular length and diameter were measured as described by Oyeyemi *et al.* (2012)

Histomorphometry of the testes and epididymides

Histological sections of the testes and epididymis were done as described by Olukole and Obayemi, (2010). Photomicrographs were taken at x40, x100 and x400 magnifications with a Digital Microscope, (VJ-2005 DN MODEL BIO-MICROSCOPE®). The morphometrical analyses were done using TS View CX Image® Software, file version 6.2.4.3 and Motic Image 2000 (China). The slides were examined under the microscope and the following measurements were taken using a calibrated eye-piece micrometer (Graticules Ltd. Toubridge Kent): the seminiferous tubular diameter, epididymal luminal diameter, germinal epithelium and epididymal epithelial height (Olukole and Obayemi, 2010).

Data analysis

Data generated were analysed using the Test of Homogeneity of variance, multiple comparisons and analysis of variance (One-Way ANOVA). The SPSS version 15 for windows (SPSS Inc, 2006) and Microsoft excel Professional Plus (Microsoft Corporation, 2010) were used to carry out all procedures.

Results

Spermogram

The results of the spermogram revealed an increase ($P>0.05$) in percentage (%) sperm

motility, sperm livability, semen volume and sperm count in the treated group at day 7 post-treatment. The increase ($P>0.05$) in % sperm motility was progressive till Day 14 post-treatment. However, there was a decrease ($P>0.05$) in the semen volume and sperm count at Day 14 post-treatment except for the % sperm liveability (Table 1). Also, from days 7 to 14 post-treatment, there was a progressive decrease ($P>0.05$) in the number of spermatozoa with tailless head, headless tail, rudimentary tail, bent tail, bent mid-piece, looped tail when compared with the control. A similar trend of decrease was observed for the total abnormal cell count except for the curved tail and curved mid-piece (Table 2).

Gonadometric indices

Biometrical assessment of the testes and epididymis

The results of the testicular and epididymal biometry showed a progressive increase ($P>0.05$) in the mean body and testicular weights of the treated bucks from days 7 to 14 post-treatment when compared with the control. At day 14 post-treatment, the mean values of left testis length (LTL), left testis diameter (LTD), right epididymal weight (REW) and left epididymal weight (LEW) were higher than the control and the values at day 7 post-treatment ($P>0.05$). There was a significant ($P<0.05$) increase in scrotal circumference at day 14 post-treatment when compared with the control (Table 3).

Histomorphometric assessment of the testes and epididymis

The treatment caused a significant decrease ($P<0.05$) in the mean testicular germinal height, testicular tubular diameter and epididymal luminal diameter at day 7 post-treatment compared to the control. However, their values at day 14 post-treatment were significantly ($P<0.05$) higher than values at day 7 post-treatment and the control. Epididymal germinal height decreased significantly across days 7 to 14 post-treatment (Table 4).

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Table 1: Spermogram of WAD bucks treated with aqueous extract of *Crassocephalum crepidioides* at days 7 and 14 post-treatment

Group	Control	Day 7	Day 14
Sperm Motility (%)	87.00±4.06 ^a	95.00±0.00 ^a	96.50±1.50 ^a
Sperm Livability (%)	96.40±1.60 ^a	98.00±0.00 ^a	98.00±0.00 ^a
Semen Volume (mls)	0.36±0.14 ^a	0.50±0.00 ^a	0.25±0.25 ^a
Sperm Count (x10 ⁶)	214.00±55.14 ^a	316.00±18.00 ^a	198.00±0.00 ^a

Values are reported as mean±SEM

^{abc}: Means in the same row with different superscript differ significantly (P<0.05).

Table 2: Sperm morphology of WAD Bucks treated with aqueous extract of *Crassocephalum crepidioides* at days 7 and 14 post-treatment

Parameters (%)	Control	Day 7	Day 14
Tailless Head	2.38±0.00 ^a	0.40±0.40 ^a	1.81±0.69 ^a
Headless tail	1.50±0.00 ^a	0.54±0.54 ^a	0.81±0.40 ^a
Rudimentary tail	0.43±0.00 ^a	0.00±0.00 ^a	0.37±0.08 ^a
Bent Tail	2.38±0.00 ^a	1.50±1.06 ^a	1.73±0.42 ^a
Curved tail	2.59±0.00 ^a	4.62±0.32 ^a	5.39±1.63 ^a
Curved mid-piece	3.03±0.00 ^a	3.39±1.42 ^a	4.40±1.29 ^a
Bent mid-piece	1.73±0.00 ^a	1.00±0.80 ^a	0.88±0.33 ^a
Looped tail	0.65±0.00	-	1.91±1.04
Total abnormal cells	14.70±0.00 ^a	10.45±0.35 ^a	9.35±2.65 ^a
Total normal cells	85.30±0.00 ^a	89.55±0.35 ^a	80.65±2.65 ^a
Total cells	462.00±0.00 ^a	484.00±23.00 ^a	482.33±28.52 ^a

Values are reported as mean±SEM

^{abc}: Means in the same row with different superscript differ significantly (P<0.05)

Table 3: Biometrical assessment of the testes and epididymis of WAD Bucks treated with aqueous extract of *Crassocephalum crepidioides* at days 7 and 14

Group	Control	Day 7	Day 14
Body weight (Kg)	8.00±0.00 ^a	13.00±4.00 ^a	17.00±0.00 ^a
Left testis weight (g)	27.50±0.00 ^a	31.00±0.00 ^a	32.75±3.85 ^a
Right testis weight (g)	31.50±0.00 ^a	22.50±9.50 ^b	32.85±4.35 ^c
Left testis length (mm)	14.77±0.00 ^a	4.58±1.01 ^b	28.70±3.90 ^c
Right testis length (mm)	34.85±0.00 ^a	4.77±0.59 ^a	25.90±5.50 ^a
Left testis diameter (mm)	4.00±0.00 ^a	2.79±0.39 ^a	9.68±2.72 ^a
Right testis diameter (mm)	15.30±0.00 ^a	2.54±0.13 ^b	8.60±0.10 ^c
Right epididymal weight (g)	6.62±0.00 ^a	5.00±3.00 ^a	7.12±0.28 ^a
Left epididymal weight (g)	5.60±0.00 ^a	4.50±2.50 ^a	7.34±0.16 ^a
Scrotal circumference (cm)	18.00±0.00 ^a	5.75±0.75 ^b	18.85±0.15 ^c

Values are reported as mean±SEM

^{abc}: Means in the same row with different superscript differ significantly (P<0.05).

Table 4: Histomorphometrical assessment of the testes and epididymis of WAD bucks treated with aqueous extract of *Crassocephalum crepidioides* at days 7 and 14 post-treatment

Parameters	Control	Day 7	Day 14
Testicular Germinal height (µm)	542.64±14.71 ^a	376.85±17.99 ^b	604.28±27.46 ^c
Testicular tubular diameter (µm)	2639.37±103.67 ^a	2169.02±67.69 ^b	4031.91±196.73 ^c
Epididymal luminal diameter (µm)	3395.11±113.57 ^a	2707.95±190.09 ^b	5956.32±287.71 ^c
Epididymal germinal height(µm)	560.37±24.05 ^a	510.87±45.42 ^b	350.20±22.01 ^c

Values are reported as mean±SEM

^{abc}: Means in the same row with different superscript differ significantly (P<0.05).

Discussion

The study investigated the effect of aqueous leaf extract of *Crassocephalum crepidioides* on semen quality and gonadometric indices of the West African Dwarf (WAD) breeding bucks. Findings from this study showed that the treatment of WAD bucks with the aqueous leaf extract of *C. crepidioides* for 14 days caused a slight increase in sperm motility, liveability, semen volume and sperm count after 7 days of exposure. This observation suggests that the plant had no negative effect on the fertility of the bucks. Some authors have also reported such an increase in sperm motility, livability, semen volume and sperm count, following the administration of certain plant extracts in WAD goats (Garner and Hafez, 1993; Oyeyemi *et al.*, 2009). The progressive increase in the sperm motility from days 7 to 14 post-treatment suggests that prolonged use of the extract might offer some benefits of enhancing the fertilization potentials of spermatozoa of bucks. Sperm motility, livability and count are key indices of potential fertility in male animals (Garner and Hafez, 1993). Therefore, an increase in the number of actively motile sperm cells in the semen will enhance mating outcome in breeding flocks. Also, the progressive decrease in the total number of sperm cells with tail abnormalities between Days 7 to 14 explains the high motility scores observed in this study. Reduction in the number of morphologically abnormal spermatozoa normally improve semen fertility. (Noakes *et al.*, 2001; Oyeyemi *et al.*, 2009). Our findings in this study therefore imply that the aqueous leaf extract of *C. crepidioides* possesses fertility-enhancing property by improving the semen quality of WAD bucks. Observations from the biometrical assessment of the testes and epididymis of WAD bucks in the current study revealed a transient decrease in the scrotal circumference, left testis

length, left testis diameter, right epididymal weight and the left epididymal weight within after 7 days of treatment. This might be attributed to stress on the animals due to handling, change in feeding pattern and confinement during the study. However, these values slightly increased at day 14 post-treatment suggesting that the plant is not toxic to the animals. The assertion is further strengthened by the progressive increase in testicular weights of the rats from days 7 to 14 post-treatment. Authors have shown that scrotal circumference, testicular and epididymal diameters in goats are direct correlates of semen volume and sperm count (Raji *et al.*, 2015). This suggests that feeding of bucks with the aqueous leaf extract of *C. crepidioides* could boost spermatogenesis, thereby enhancing the fertility of breeding bucks. Histomorphometric observation of the testes and epididymis in our current study also followed a similar pattern with the biometrical assessment. The marked increase in testicular germinal height, testicular tubular diameter and epididymal luminal diameter at day 14 post-treatment further affirmed that *Crassocephalum crepidioides* may enhance the fertility potentials of bucks,

Conclusion

We concluded that oral administration of 250mg/kg aqueous leaf extract of *Crassocephalum crepidioides* for 14 days could enhance the sperm quality and gonadometric indices in the West African Dwarf breeding bucks by slightly increasing the sperm motility, liveability, count as well as testicular and epididymal sizes. Therefore, the feeding of breeding bucks with *Crassocephalum crepidioides* leaves should be encouraged.

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