Effects of *Ficus syncomorus* and *Datura metel* stem-bark extracts on the sperm characteristics of Yankasa rams

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Abstract

Interest in medicinal plants for the management of myriad of conditions including reproductive disorders refractory to orthodox medicinal care is on the increase. *Ficus syncomorus* and *Datura metel* are two of such plants with folkloric evidence of aiding fertility in human. This study investigated the effect of aqueous stem-bark extracts (200 mg/kg) of *F. syncomorus* and *D. metel* respectively on the sperm characteristics of Yankasa rams. Twelve (12) matured (15 – 16 months) old rams were used in this study and randomly assigned into three (I, II, III) groups of four (4) animals each. Group I served as the control while II and III served as the treatment groups and received daily oral doses (200 mg/kg) of *F. syncomorus* and *D. metel* extracts respectively for 7 consecutive days. Semen was collected from all the groups at the end of the treatments using Electro-ejaculation method and evaluated by light microscopy. The mean semen volume (68.70 ± 4.2 to 65.62 ± 2.00) and percentage progressive motile cells significantly ($p < 0.05$) reduced 7 days post treatment in group III (84.05 ± 1.3) compared to the control (85.20 ± 1.32) and the group II (86.56 ± 0.40) animals. The mean sperm count, the percentage liveability and the haematological parameters and erythrocytic indices (10.81 ± 0.24 for group III to 12.54 ± 0.30 for group I) significantly ($p < 0.05$) decreased in group III compared to the values in the control and group II rams. Abnormal sperm morphology (bent mid-piece, curved tail, headless tail, tailless head) significantly ($p < 0.05$) increased in *D. metel* group (7.26 ± 0.12) compared to *F. syncomorus* (5.02 ± 0.04) and control groups (5.62 ± 0.01) respectively. *D. metel* aqueous extract adversely affected sperm characteristics with significant effect on semen volume, sperm morphology and counts as well as haematological parameters. Exposure of animals to *D. metel* at the dose used may impair sperm fertilizing ability, thus leading to reduced ram fertility. While *F. syncomorus* extract appears a potential drug candidate for improving fertility.

Keywords: *Ficus syncomorus*, *Datura metel*, sperm characteristics, morphology, Yankasa rams.

Introduction

There has been a geometric growth in the attractiveness of medicinal plants in many part of the world because of their acceptance as true innocuous and believe that what is natural can only be good (Okpara, 2015). The floral biodiversity of Africa provides the African traditional medical practitioners with an impressive “natural pharmacy” from which plants are selected as remedies, and ingredients to

prepare herbal medicines for an array of human and animal diseases (Oyewole, 2003; 2004). Although in Nigeria, there are no professional traditional veterinary practitioners as such some herdsmen, village elders and others who kept animals have acquired experiences for generations in diagnosing and treating animal disease conditions. Sometimes the same herbs used in treating human diseases are also used in treating corresponding animal diseases

(Nwude, 1997; Okpara, 2006). However, there are some herbs that are used mainly for veterinary purposes and some diseases peculiar to animals (Nwude, 1997, Okpara *et al.*, 2018). It has been observed that in the tropical environment, animals reared under extension husbandry systems thrive better and are usually able to withstand some of the endemic diseases to which intensively managed animals continually remain susceptible (Huffman, 2001). This apparent disease resistance has been attributed in part, to adequate exercise and the possibility of selective and unrestricted grazing whereby a wide variety of edible forages are available to animals; some of these plants have been shown to provide not only nutrition but are also of medicinal benefits (Adedapo *et al.*, 2002; Okpara, 2015). A growing body of evidence has shown the phenomenon of self-medication in animals (Huffman, 2002).

A variety of non-nutritional compounds from plants are found in the diet of animals, but little is known about the possible medicinal consequences of their ingestion. One of the challenges of interpreting self-medication in animals is to distinguish between indirect medical benefits derived from plants rich in secondary compound that are assumed to be ingested for their nutritional value versus limited and situation specific ingestion of items that are processed solely for their curative value or other physiological effects (Cousin and Huffman, 2002). *Ficus syncomorus* and *Datura metel* are employed in folkloric medicine to improve libido as well as to withstand stress (Okpara *et al.*, 2018). In Nigeria, the Hausas' referred to *D. metel* as zakami or Hankata yaro while *F. syncomorus*, is the wild fig in English and known as *Bature* in hausa language. The health, growth and productive performance of animals especially livestock are affected by the chemical composition of the plants

they consume (Nwude, 1997). To distinguish edible from poisonous plants, grazing animals were observed and plants not eaten were considered poisonous and were avoided by man. Thus, plants are generally studied either for their medicinal or toxicological effects. This study is aimed at evaluating the effects of *Ficus syncomorus* and *Datura metel* aqueous extract in the sperm characteristic of Yankasa rams.

Materials and methods

Study area

The study was carried out at the small ruminant section of the teaching and research farm of the Federal College of Animal Health and Production Technology, N.V.R.I., Vom, Plateau state.

Animals, treatment and management

Twelve 15 to 16 months old Yankasa rams of 12 to 13 kg were used for the study in a completely randomised design. Before the experiment, the animals were washed with Taktic Amitraz[®] (Intermet International, the Netherland) to rid them of ectoparasites and dewormed with albendazole solution (25mg/kg). The rams were fed with 350g concentrates supplemented with rice hay and maize silage and water was provided *ad libitum* throughout the period of the experiment. The fed concentrate comprised of maize (40kg), soybeans meal (5.5kg), groundnut cake (200g), wheat offal 25g, bone meal (2g), premix (0.5g) and salt 0.5g. The rams were randomly allotted into three (3) groups (I, II and III) of four (4) animals each. Group I was the control and Groups II and III rams were administered with 200mg/kg of *F. syncomorus* and *D. metel* aqueous extracts respectively *per OS* daily for seven (7) consecutive days. Seven (7) days post treatment blood and semen samples were collected and analysed for possible changes in haematological parameters and sperm characteristics.

Haematological analysis

Five (5ml) millilitres of blood were collected from each ram via the jugular vein into anticoagulant (EDTA) containing bottles 7 days post treatment. The blood samples were analysed using automated electronic cell counter (Abbott Haematological Analyser, Cell – Dyn, 1700, Illinois, USA) for absolute and differential white blood cell count (WBC), erythrocytes count (RBC), packed cell volume (PCV), and haemoglobin (Hb) concentration. The erythrocytic indices (MCV, MCH, MCHC) were calculated using the methods described by Schalm *et al.* (1975)

Mean corpuscular volume (MCV)
$$\text{MCV (fL)} = \frac{\text{PCV} \times 10}{\text{RBC (10}^{12}/\text{L)}}$$

MCV expresses the average volume of the individual erythrocytes

Mean corpuscular Haemoglobin (MCH)
$$\text{MCH (Pg)} = \frac{\text{Hb (g/dL)} \times 10}{\text{RBC (10}^{12}/\text{L)}}$$

Mean corpuscular Haemoglobin concentration (MCHC)
$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL)} \times 100}{\text{PCV (\%)}}$$

Mean corpuscular Haemoglobin concentration (MCHC)

MCHC (g/dL) = $\frac{\text{Hb (g/dL)} \times 100}{\text{PCV (\%)}}$

Semen collection – (Electro ejaculation method) and evaluation

The electro – ejaculator consist of a wooden rectal probe and metal electrode with a total length of 12.0m and a diameter of 1.5cm (body) and 1.0cm at the tip connected to a transformer to control delivered current at 18 Hz with output voltage of 0 – 12V. The probe was lubricated with paraffin oil before insertion into the rectum to minimize trauma. It is inserted 8 – 10 cm into the rectum and the electrode positioned vertically. The probe was inserted such that the electrode was within the pelvic cavity. The anal sphincter was also lubricated to minimise trauma. The animals were held in a standing position during the semen collection exercise and the entire procedure

lasts for approximately 2 minutes. The electricity stimulation was for interval of 4 – 5 seconds. Ejaculate from each animal was collected with a beaker and the ejaculate volume, excluding the gel read off immediately in measuring cylinder and recorded, the semen was further evaluated Zaneveld and Polakoshi, (1977). A drop of undiluted semen from each animal was placed on a clean, pre – warmed (37°C) glass slide and examined with microscope under low power to determine the mass activity of spermatozoa for each animal. The scoring was according to the intensity of the wave motion from absence of wave motion to very turbulent motion (+++), characterised by the appearance of dark prominent waves in a very rapid motion. The semen progressive motility was evaluated as described by Kastelic *et al.* (2001). The percentage live sperm as determined by counting live/dead sperm in a thin smear of a drop of semen mixed with eosin-negrosin stain (Hancock, 1952). Spermatozoa slide were also evaluated for morphological abnormalities (Maimgram and Larsson, 1984), consisting of primary defects (those affecting the head) and secondary defects (those affecting the midpiece or tail). The respective aqueous stem-bark extracts of *F. syncomorus* and *D. metel* were kept in airtight container in cool and dry place until use.

Collection, preparation and extraction of *F. syncomorus* and *D. metel* aqueous extracts

The stem-bark of the plants (*F. syncomorus* and *D. metel*) were collected from apparently healthy plants within the premises of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The stem-barks were air dried for 21 days and ground in a Hammer mill. Samples of the dried *F. syncomorus* and *D. metel* stem-barks powder were analysed for proximate and chemical composition

(AOAC, 1990). The cations (Ca, Mg, Cu) in the sample were estimated using atomic absorption spectrophotometer with wavelength: Mg = 286.4nm; Ca = 422.8nm, Cu = 325.7nm.

In extracting the plants stem-bark powder, five hundred grams (500g) each of the plant materials were mixed in a container in 2.5 L of distilled water and stirred. After 24hrs the contents were filtered coarsely using clean cotton wool and subsequently with whatmann No. 1 filter paper. The filtrates were heated to dryness in hot air oven at 40°C to obtain the solid extract (Sofowora, 1993).

Phytochemical analysis

This was carried out as described by Sofowora (1993) and Hammuet *et al.* (2014). For tannins (FeCl test); about 0.5g of the dried powdered extracts (for *F. syncomorus* and *D. metel*) were dissolved in 20mL of distilled water in a test tube, boiled and filtered; to each filtrate few drops of 1.0% Iron II chloride solution were added and observed for a blue green precipitate. *Flavonoids* test; this was conducted in two different ways. To 1.0mL of the extract 1.0 mL of 10% lead acetate was added, and observed for yellowish precipitation. Also, to 1.0 mL of the extracts 1.0 mL of dilute NaOH was added and observed for precipitation. *Alkaloids* Test: For this two different reagents (Wagners and Mayers reagents) were used to ascertain the presence of alkaloids in the sample. To 2 mL of the extract was added 10.0 mL of 2% HCl mixed thoroughly by shaking and emerged on a steam bath and filtered. Thereafter, the filtrates were divided into two equal portions. To the first portion Wagner's reagent was added in drops and observed for a brown precipitate. To the second portion of the filtrate Mayer's reagent was added also in drops and observed for white to yellow or creamy white precipitate. *Saponnins* test (Frothing test): One (1.0)

mL of the extract was boiled in 5 ml of distilled water for 5 minutes and decanted while still hot. Further, it was filtered and 1.0 mL of the filtrate was diluted with 4.0 ml of distilled water and was shook vigorously. It was observed on standing for stable froth. *Glycosides* test (FeCl test); to 5.0 mL of the extract in a test tube 2.5mL of dilute H₂SO₄ acid was added and boiled in a water bath for 15 minutes. It was cooled and neutralized with 20% KOH solution, then 5ml of a mixture of Fehling's solution A and B were added and observed for reddish brown colour at the interphase. *Cardiac glycosides* test; To 1.0 mL of the extract 80% of methanol was added and mixed with 1.0 mL of 2% solution of 3,5 dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5% NaOH. It was observed for violet colour which faded through reddish brown to brownish yellow. *Carbohydrate* test; (Molish test). The extract (0.5g) was dissolved in 3ml of distilled water, heated for 5 minutes in a hot water bath. It was filtered and allowed to cool. Five drops of Molish reagent was added to the solution and gently shaken. Furthermore, concentrated H₂SO₄ of 0.5 mL was added carefully from the side of the test tube. The presence of reddish coloured interfaced ring indicated the presence of carbohydrate in the extract (Harborne, 1998). Test for *Steroids* (Salkowaki test): One millitres (1mL) of the extract was treated with 5 drops of concentrated H₂SO₄. The presence red colouration indicates the presence of steroids.

Statistical analysis

Data obtained from the experiment were analysed using the statistical analysis of variance (ANOVA) procedure of SAS (2010) and significant level of p=0.05 was used. The treatment means were compared using the Tukey's HSD option of the same software.

Results

The results of phytochemical constituent of aqueous stem-bark extracts of *F. syncomorus* and *D. metel* in Table 1 shows that tannins, flavonoids, alkaloids and carbohydrates were present in the stem bark extracts of both *F. syncomorus* and *D. metel* while saponins was absent in *F. syncomorus* but present in *D. metel*. Glycosides and cardiac glycosides were absent in *F. syncomorus* but present in *D. metel* while steroids were observed to be absent in both *F. syncomorus* and *D. metel*. Table 2 shows the effect of *F. syncomorus* and *D. metel* stem-bark on aqueous extracts (200mg/kg) on the erythrocytic parameters and indices of Yankasa rams. The erythrocytic parameters and indices were all significantly different ($p < 0.05$) from the control, the red blood cells ($\times 10^3/L$) increasingly ranges from 10.81 ± 0.24 for *D. metel* to 12.54 ± 0.30 in the control while *F. syncomorus* value is 12.64 ± 0.56 . The haemoglobin (g/dL) values ranges from 9.54 ± 0.30 in *D. metel* to 11.30 ± 0.47 in *F. Syncomorus*, the PCV (%) values ranges significantly ($p < 0.05$) from 25.42 ± 0.45 in *D. metel* to 29.78 ± 0.81 in *F. Syncomorus*. The MCV (fL) values also varies significantly from 22.75 ± 0.10 in *D. metel* to 24.73 ± 0.42 in *F. Syncomorus* while the MCH (pg) and MCHC (g/dL) values were all significantly different across the treatments with values of *F. Syncomorus* highest compared with *D. metel* with lowest values in all the parameters measured. The results in Table 3 shows effects of *F. Syncomorus* and *D. metel* aqueous extracts (200mg/kg) on the total and differential Leucocyte counts. The WBC ($\times 10^9/L$) also increase significantly ($p < 0.05$) with *D. metel* having the lowest value of 23.04 ± 0.77 while *F. syncomorus* had the highest value of 25.30 ± 0.97 , the Neutrophil ($\times 10^9/L$) values also varies significantly ($p < 0.05$) with *D. metel* was

also observed to have the lowest value of 11.84 ± 0.19 but the control with 13.54 ± 0.84 . The Lymphocyte ($\times 10^9/L$) values also increase significantly and range from 8.05 ± 0.75 to 10.22 ± 0.86 respectively, the Eosinophil ($\times 10^9/L$) ranges from 0.64 ± 0.10 in *D. metel* to 1.02 ± 0.07 in *F. syncomorus*. The Monocyte ($\times 10^9/L$) also varies significantly ($p < 0.05$) and ranges from 0.54 ± 0.40 to 0.75 ± 0.10 while Basophil ($10^9/L$) ranges significantly from 0.20 ± 0.07 to 0.44 ± 0.08 . The result in Table 4 revealed semen characteristics of rams administered (200mg/kg) of *F. syncomorus* and *D. metel* extracts. The control (68.70 ± 4.2) was observed to have the highest volume of semen compared to *D. metel* (65.62 ± 2.00) and *F. syncomorus* (67.92 ± 2.04). Mass activity was uniform across the treatments while progressive motility was in *F. syncomorus* (86.56 ± 0.40), followed by the control (85.20 ± 1.32) while *D. metel* (84.05 ± 1.3) had the lowest motility. The sperm concentration was not significantly different across the treatments. The percentage of life sperm/dead was highest in *F. syncomorus* (78.62 ± 1.02) with *D. metel* (74.50 ± 1.25) having the lowest value, the total sperm ejaculate was highest in group 11 and lowest in group 111. The morphological abnormalities were highest in group 111 (7.26 ± 0.12) and lowest in group 11 (5.02 ± 0.04).

Discussion

The phytochemical screening of *Ficus syncomorus* and *Datura metel* stem-bark extracts revealed the presence of flavonoids, alkaloids, tannins, saponins and glycosides. Steroids was present in *D. metel* only. This agrees with previous work of Okpara *et al.* (2018) who observed the presence of these secondary plant metabolites in *F. syncomorus* and *D. metel* leaves obtained at Vyang District, Jos South

Effects of Ficus syncomorus and Datura metel stem-bark extracts on the sperm characteristics

Table 1: Phytochemical constituents of aqueous stem-bark extracts of *F. syncomorus* and *D. metel*

Constituents	<i>F. syncomorus</i>	<i>D. metel</i>
Tannins	+	+
Saponins	-	+
Flavonoids	+	+
Alkaloids	+	+
Glycosides	-	+
Cardiac glycosides	-	+
Steroids	-	-
Carbohydrates	+	+

Key: +=Present -=Absent

Table 2: Effects of *F. syncomorus* and *D. metel* stem-bark. aqueous extracts (200mg/kg) on the erythrocytic parameters and indices of Yankasa rams

Parameter/Indices	Control	<i>F. syncomorus</i>	<i>D. metel</i>
RBC (X10 ¹² /L)	12.54±0.30 ^a	12.64±0.56 ^a	10.81±0.24 ^b
Hb (g/dL)	10.84±0.12 ^a	11.30±0.47 ^a	9.54±0.30 ^b
PCV (%)	28.67±0.19 ^a	29.78±0.81 ^a	25.42±0.45 ^b
MCV (fL)	23.62±0.14 ^a	24.73±0.42 ^a	22.75±0.10 ^b
MCH (pg)	8.74±0.08 ^a	9.04±0.21 ^a	7.03±0.36 ^b
MCHC (g/dL)	37.56±32 ^a	38.06±0.14 ^a	36.13±0.18 ^b

abc means on the same row with different superscripts are significantly different (p<0.05)

rbc=red blood corpuscles, hb=heamoglobin, pcv=packed cell volume, mch= mean corpuscular haemoglobin, mcv= mean corpuscular volume, mchc= mean corpuscular haemoglobin concentration

Table 3: Effects of *F. Syncomorus* and *D. metel* aqueous extracts (200mg/kg) on the total and differential leucocyte counts (n = 3)

Parameter	Control	<i>F. syncomorus</i>	<i>D. Metel</i>
WBC (X10 ⁹ /L)	24.88±1.20 ^a	25.30±0.97 ^a	23.04±0.77 ^b
Neutrophil (X10 ⁹ /L)	13.54±0.84 ^a	13.43±0.75 ^a	11.84±0.19 ^b
Lymphocyte (X10 ⁹ /L)	9.82±0.51 ^a	10.22±0.86 ^a	8.05±0.75 ^b
Eosinophil (X10 ⁹ /L)	0.95±0.10 ^a	1.02±0.07 ^a	0.64±0.10 ^b
Monocyte (X10 ⁹ /L)	0.70±0.35 ^a	0.75±0.10 ^a	0.54±0.40 ^b
Basophil (10 ⁹ /L)	0.40±0.05 ^a	0.44±0.08 ^a	0.20±0.07 ^b

abcMeans on the same row with different superscripts are significantly different (p<0.05)

Table 4: Semen characteristics of rams administered (200mg/kg) of *F. syncomorus* and *D. metel*. extracts

Parameter	Control	<i>F. syncomorus</i>	<i>D. Metel</i>
Volume of semen	68.70±4.2 ^a	67.92±2.04 ^a	65.62±2.00 ^b
Mass activity	+++	+++	+++
Progressive motility	85.20±1.32 ^b	86.56±0.40 ^a	84.05±1.3 ^b
Sperm concentration	0.43±3.01 ^a	0.43±0.02 ^a	0.43±0.02 ^a
Life sperm/dead (%)	75.27±1.35 ^b	78.62±1.02 ^b	74.50±1.25 ^a
Total sperm/Ejaculate	27.50±1.35 ^b	28.25±1.03 ^a	26.74±0.08 ^b
Morphological abnormalities	5.62±0.01 ^a	5.02±a0.04 ^a	7.26±0.12 ^b

abcMeans on the same row with different superscripts are significantly different (p<0.05)

+++ = turbant wave motion

L.G.A, Plateau State, Nigeria. Secondary plant metabolites exact a number of biological activities such as antibacterial, anti-inflammatory, antidiarrheal, antioxidant, antiageonic, analgesic and antiallergic properties (Okpara, 2015). Tannins have been found to react with proline rich protein to form irreversible complexes (Umeh *et al.*, 2011), resulting in the inhibition of cell protein synthesis. Tannins rich herbs are astringents in nature and are used for treating gastro-intestinal disorders such as diarrhoea and dysentery (Oyewole, 2003; 2004). The presence of flavonoids and saponins in these plants lend credence to their uses in managing inflammation and stress related conditions. The decrease in RBC, PCV, HB as well as in the erythrocytic indices (MCV, MCH and MCHC) in the *D. metel* treated group compared to the control and the *F. syncomorus* groups could be attributed to the possible toxic effect of the extract (Ambali *et al.*, 2011). The RBC is susceptible to lipo peroxidative changes because of its direct association with molecular oxygen, high content of metal ions catalysing oxidative reactions and availability of high amount of polyunsaturated fatty acids (PUFA's) which are susceptible to lipid peroxidation. The decrease in the erythrocyte parameters and indices in the *D. metel* group could also be attributed to possible disruption of haemopoiesis due to intracellular oxidative stress. Haemoglobin biosynthesis takes place in the mitochondria and toxic inhibition of the activities of enzymes such as alpha-aminolevulinic acid synthase (ALA-synthase) and prophobilinogen synthase will affect the Hb synthetic pathway (Okpara, 2015). The disruption of the activities of any of these enzymes due to oxidative damage to macromolecules may interfere with haemoglobin production, thus defective and decreased erythrocyte

production. Therefore, the decrease in (MCV, MCH and MCHC) values in the *D. metel* treated groups could also be attributed to the disruption in haeme biosynthetic pathway, hence in haemopoiesis leading to the production of microcytes. The significant increase in both the erythrocytic parameters and indices in the *F. syncomorus* group showed that the extract contains phytochemical(s) with antioxidant and enzyme enhancing properties. Furthermore, the reduction in total WBC count in *D. metel* treated group could be attributed to lymphopenia and or neutropenia resulting from possible release of superoxide and other free radicals which, are toxic to cells including neutrophils and lymphocytes thereby causing a decrease in their peripheral circulation (Okpara, 2015). The elevation in the haematological parameters in the *F. syncomorus* group is speculated to be due to chain breaking antioxidant activity of one of the phytochemicals flavonoids present in the extract or combined effects of various phytochemicals (Okpara *et al.*, 2018). This played a vital role in the maintenance of cellular membrane integrity as well as stimulating the immune system (Ebaid *et al.*, 2013). *Datura metel* stem-bark aqueous extract showed a significant decrease in sperm volume and adverse effects on spermatogenesis as manifested in an increase in the number of headless tail sperm cells compared to the values obtained for the control and *F. syncomorus* group. The headless tail abnormalities according to Bloom (1948) and Oladele *et al.* (2010) is primarily caused as a result of disruption in the course of spermatogenesis which may be due to the generation of reactive species by the toxic extract (Ada and Egbunike, 2010). The decrease in the number of headless, tailless head and bent tail sperm cells observed in the *F. syncomorus* treated group showed that the extract has protective

effect on the sperm cells (Malmgran and Larsson, Kastelic *etal*; 2001). The results of the present study further lend credence to the social and medical uses of these plants in many parts of Nigeria.

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Received: 10th October, 2019

Accepted: 17th February, 2020