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Comparative evaluation of the sperm characteristics and morphology of adult Wistar rats fed either low or normal protein-energy diets and orally dosed with aqueous *Cuscuta australis* extracts

[1]Omirinde, J.O., *[1]Ozegbe, P.C. and [2]Oyeyemi, M.O.
Departments of [1]Veterinary Anatomy and [2]Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Summary: *Cuscuta australis (C. australis*) seed and stem are commonly used as dietary supplements in a maize-meal, "Ogi", by the local population for the management of male and female reproductive dysfunctions. This study, as a part of on-going efforts, therefore, evaluated and compared the effects of Low Protein-energy (LP) and Normal Protein-energy (NP) diets on the sperm morphology and characteristics of adult Wistar rats orally dosed aqueous extracts of *C. australis* seed (LPSE and NPSE) and stem (LPST and NPST), 300mg of extract/kg body weight of rat/day, for seven days. The control groups (LPWA and NPWA) received vehicle, water. Live-dead ratio and percentage of sperms with curved tail were significantly decreased ($p<0.01$) in the NPST relative to the NPWA, LPWA, LPST, NPSE and LPSE. Total abnormal sperm counts, acephalic sperms and tailless head sperms were significantly decreased ($p<0.001$, $p<0.05$ and $p<0.001$, respectively) in the LPST and NPST relative to LPSE, NPSE, LPWA and NPWA. The LPSE, LPST and NPST showed significantly decreased ($p<0.05$) percentages of sperms with either bent mid-piece or curved mid-piece relative to the LPWA. Significantly decreased ($p<0.05$) percentage of sperms with curved mid-piece was also observed in the NPSE relative to LPWA. Protein-energy diet significantly influenced (at least $p<0.05$) the effect of each extract on sperm motility and percentage of sperms with curved tail. Stem extract significantly decreased ($p<0.01$) the percentages of acephalic sperms and tailless head sperms. Diet-stem extract interaction significantly influenced ($p<0.05$) live-dead ratio. Our data suggest that orally administered aqueous extracts of *C. australis* generally enhanced the sperm morphology and characteristics of the male Wistar rat and that the stem extract maintained sperm morphology better than the seed extract. It also showed that the stem extract decreased live-dead ratio and that the efficacy of orally administered aqueous *C. australis* stem extract may be affected by variations in dietary protein-energy levels.

Keywords*: Cuscuta australis*, protein-energy malnutrition, spermiogram, sperm morphology

**Address for correspondence:* pcozegbe@yahoo.com +234 (0) 8056010341

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INTRODUCTION

Cuscuta australis R. Br, commonly called dodder, is an annual parasitic plant that forms the major flora of the tropical East and West Africa, Sudan, Madagascar, Southern Europe, Japan and Australia (Maria, 1987). Dodder is classified as a member of the Morning-Glory Family (Convolvulaceae) in older references, and as a member of the Dodder Family (Cuscutaceae) in the more recent publications (Davidson and Frey, 2005). *Cuscuta australis* has a fairly slender stem that is up to 0.5 millimetres in diameter. The flowers are about 2 millimetres long and broad (up to 3 mm. in fruit). The seeds are about 1.25 millimetres long (Verdcourt, 1963). Ye *et al*. (2002) observed that both the seeds and stems of *C. australis* contained a high amount of kaempferol, a flavonol compound.

The seed and stem of *C. australis*, locally called "Omoonigelegele", are traditionally added to a maize-meal in the South Western part of Nigeria for the management of male and female reproductive dysfunctions. Ozegbe and Omirinde (2012) reported that aqueous *C. australis* seed and stem extracts induced significant effects on the plasma concentrations of follicle stimulating hormone, luteinizing hormone, testosterone and the testicular histomorphometry of the adult male Wistar rat. However, there is dearth of literature on the effect(s) of *C. australis* extracts on the sperm characteristics and morphology.

Protein-energy malnutrition, according to Ozegbe and Omirinde (2012), induces alterations in the

morphophysiology of the seminiferous tubules of the adult Wistar rat. Decreased spermatogenesis and its' associated changes in circulating androgen and gonadotropin levels (Vawda and Mandlwana, 1990) have also been observed by Guaragna *et al*.(1986) to occur in the seminiferous tubules of malnourished rats. Malnutrition affects absorption, protein binding, distribution, biotransformation and renal elimination of xenobiotics/drugs (Krishnaswamy, 1987). However, there is no literature, to the best of our knowledge, on the effects of the interactions between protein-energy malnutrition and aqueous *C. australis* extracts on the sperm characteristics and morphology of the adult Wistar rat.

This study, therefore, evaluated the influence of protein-energy malnutrition on the characteristics and morphology of the spermatozoa of adult Wistar rats orally dosed with either aqueous *C. australis* seed or stem extracts, three possible scenarios in the developing world plagued with malnutrition and increasing incidence of male reproductive dysfunction.

MATERIALS AND METHODS

Experimental animals and their feeding

Thirty adult male Wistar rats, free from any observable ailment, obtained from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were used for this study. The rats weighed between 200-250g at the commencement of the experiment. All the rats were kept in galvanized wire mesh cages in a fly-proofed house, under hygienic conditions, in six groups of five animals each. The two rat feed formulations of Akingbemi *et al.* (1996), Normal Protein-energy (NP, 16.55% total protein, 16.04 $mJKg^{-1}$ gross energy) and Low Protein-energy (LP, 6.21% total protein, 18.11 $mJKg^{-1}$ gross energy) diets, were used throughout the experiment. The feed rations and drinking water were supplied *ad libitum* throughout the duration of the experiment.

Experimental design

The 30 adult rats were randomly assigned to two dietary protein-energy groups; Low Protein-energy (LP) and Normal Protein-energy (NP) diets. Each dietary group, LP and NP, was further divided into three groups, each containing five rats, as follows: (i) untreated control groups that received vehicle, distilled water, only (LPWA, NPWA), (ii) treated groups that received aqueous extract of *Cuscuta* seed (LPSE, NPSE) and (iii) treated groups that received aqueous extract of *Cuscuta* stem (LPST, NPST). All rats were placed on the appropriate diet two weeks before commencement of the experiment.

Preparation of aqueous extracts and dosing

Mature seeds and stems of *C. australis*, collected from Abeokuta, Ogun State, Nigeria, were identified, numbered (UIH-22351) and deposited at the Department of Botany, University of Ibadan, Ibadan, Nigeria. One hundred and fifty grams of each sample was dried, powdered, decocted, and refluxed three times with 450mL of water, and then filtered as earlier reported by Ozegbe and Omirinde (2012). The filtrates were concentrated by rotary vacuum evaporation and then lyophilized with a freeze dryer. The yield of aqueous extracts of the seeds and stems were 15.33% and 13.33% (w/w) respectively. The lyophilized powder was dissolved in distilled water (300mg of extract in 1ml of distilled water) before oral administration to the experimental animals in accordance with the procedure of Yen *et al*. (2007).

After the 14 days of stabilization, each rat in the *Cuscuta* seed-treated (LPSE, NPSE) and the *Cuscuta* stem-treated (LPST, NPST) groups was weighed using a digital balance (Scout Pro. SPU 402, OHAUS Corporation, Pine Brook, New Jersey, USA) and each rat in each group administered by oral gavage with 300mg/kg daily of the appropriate aqueous extract for seven days. The control groups (LPWA and NPWA) were also weighed and received only distilled water (1ml/kg body weight) by oral gavage.

Semen collection

The animals were weighed, deeply anaesthetized with ketamine (100mg/kg bodyweight) and xylazine (10mg/kg bodyweight) combination, and sacrificed on Day 8 of the experiment. Semen samples were collected from the *cauda epididymidis*.

Sperm motility

The percentage of sperm cells in a unidirectional progressive movement over a field on a slide was observed, using a light microscope as described by Zemjanis (1977). Briefly, a small drop of semen was placed on a warmed slide, mixed with one drop of warm sodium citrate and covered with a glass slip. Sperm cells moving in a straightforward unidirectional motion were counted while sperm cells moving in circles, in backward direction or showing pendulating movement were excluded.

Live-Dead ratio or Percentage liveability (%)

One drop of semen was mixed with one drop of eosin-nigrosin stain on a warm slide as described by Wells and Awa (1970). A thin smear was then made from the mixture of semen and stain. The smear was then air-dried and a total of four hundred sperms observed under the microscope. The live and the dead sperm cells were separately counted and the ratio of the live to dead sperm cells was calculated according to the method of Zemjanis (1977).

Sperm morphological defects

Morphological defects in a total of 400 sperm cells were determined using the method of Wells and Awa (1970). Briefly, a drop each of Wells and Awa stain and semen were placed on a warm slide, mixed, and

with another slide, a smear was made. The stained smear was then air dried and viewed under the light microscope. The defects were classified as described by Bloom (1973) and Parkinson (2001).

Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) and two-by-two random block design ANOVA. The group means were separated by Duncan's Multiple Range Test (DMRT). The level of significance was $p \leq 0.05$. Results were presented as mean ± standard error of mean (SEM).

RESULTS

Control versus aqueous seed and stem extracts

Sperm motility

There were no significant differences ($p>0.05$) between the sperm motility of NPWA, LPSE, NPSE, LPST and the NPST. There were also no significant differences between the sperm motility of the LPWA, LPSE, NPSE and the LPST. However, sperm motility was significantly decreased ($p<0.05$) in the LPWA relative to NPWA and NPST (Fig.1)

Live dead ratio or Percentage liveability (%)

Live dead ratio of sperm cells was significantly decreased ($p<0.01$) in NPST relative to NPWA, LPWA, NPSE, LPSE and LPST (Fig. 2).

Total abnormal sperm counts as a percentage (%) of total sperm count

There were no significant differences ($p>0.01$) between the total abnormal sperm cells of NPWA and LPWA. However, the NPST and LPST showed significant reductions ($p<0.001$) in the total abnormal sperm counts relative to NPWA, LPWA, NPSE and LPSE (Fig.3).

Sperms with tailless heads (normal head without tail) as a percentage (%) total sperm defects

The NPWA, LPWA, NPSE and LPSE showed significant increases ($p<0.01$) in the percentages of tailless head spermatozoa relative to the NPST and LPST (Table 1).

Sperms with headless tails (acephalic sperms or normal tail without head sperms) as a percentage (%) of total sperm defects

There were no significant differences ($p>0.05$) between the percentages of headless tail sperms of the NPWA and NPSE, as well as between those of the LPWA and LPSE. However, percentages of headless tail sperm were significantly decreased ($p<0.05$) in both the LPST and the NPST relative to the LPWA, NPWA, LPSE and NPSE as shown in Table 1.

Sperms with curved tails (% of total sperm defects)

Percentages of sperms with curved tails were significantly decreased ($p<0.001$) in the NPST relative to NPWA, LPWA, NPSE, LPSE and LPST (Table 1). Non-significant differences ($p>0.01$) were, however, observed between the percentages of sperms with curved tails in the NPWA, LPSE and LPST as well as between those of LPWA and NPSE.

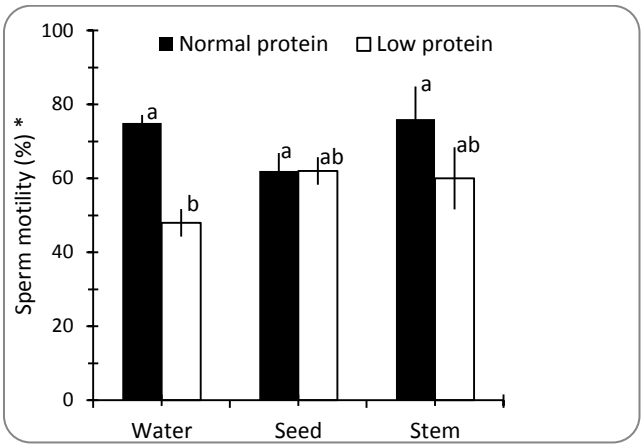

Fig.1. Mean ± SEM sperm motility (%) of *Cuscuta australis*-treated rats fed either normal or low protein-energy diets. *p < 0.05, Values with different superscripts are significantly different.

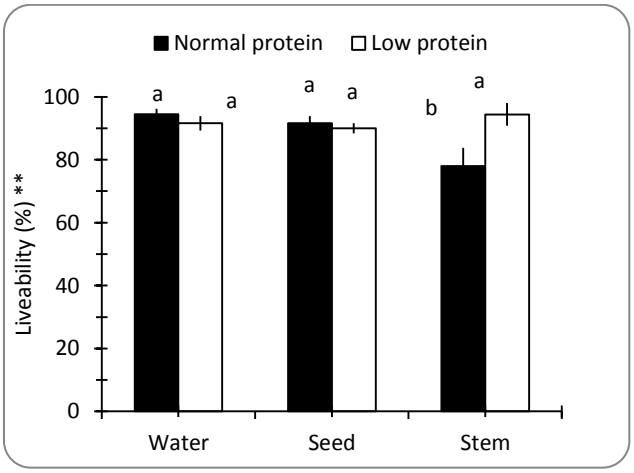

Fig.2. Mean ± SEM sperm liveability or live-dead ratio (%) of *Cuscuta australis*-treated rats fed either normal or low protein-energy diets. **p < 0.01, Values with different superscripts are significantly different

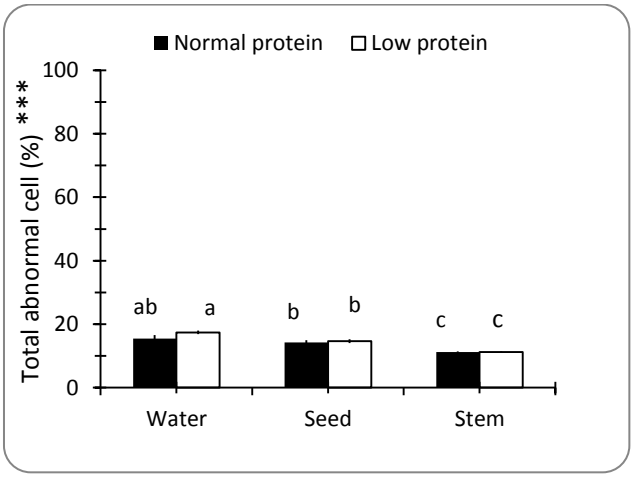

Fig.3. Mean ± SEM total abnormal sperm cells count (%) of *Cuscuta australis*-treated rats fed either normal or low protein-energy diets. ***p < 0.001, Values with different superscripts are significantly different.

Table 1: Sperm morphological defects (%) of rats that were fed either normal or low protein-energy diets and orally administered aqueous extracts of either Cuscuta australis seed or stem.

	NPWA	LPWA	NPSE	LPSE	NPST	LPST
TH**	2.24 ± 0.34^{a}	2.47 ± 0.39^{a}	1.92 ± 0.28^{a}	2.02 ± 0.27^{a}	1.07 ± 0.15^{b}	1.03 ± 0.14^{b}
HT*	1.49 ± 0.08^{abc}	1.69 ± 0.26^{ab}	1.43 ± 0.27^{abc}	1.88 ± 0.23^{a}	1.04 ± 0.93^{c}	1.12 ± 0.12^{bc}
RT	0.62 ± 0.1	0.55 ± 0.09	0.54 ± 0.12	0.64 ± 0.06	0.49 ± 0.11	0.34 ± 0.13
Bent tail	3.04 ± 0.29	2.93 ± 0.27	2.66 ± 0.19	2.48 ± 0.12	2.06 ± 0.09	3.32 ± 1.42
CT***	2.3 ± 0.14^{ab}	3.03 ± 0.12^{a}	2.37 ± 0.17^{b}	2.28 ± 0.13^{ab}	1.97 ± 0.07^{c}	2.15 ± 0.04^{ab}
LT	0.5 ± 0.14	0.50 ± 0.11	0.49 ± 0.11	0.45 ± 0.18	0.39 ± 0.12	0.34 ± 0.12
BM*	2.74 ± 0.21^{ab}	3.13 ± 0.23^{a}	2.47 ± 0.1^{ab}	2.12 ± 0.43^{b}	2.07 ± 0.06^{b}	2.0 ± 0.33^{b}
CM*	2.67 ± 0.24^{ab}	3.28 ± 0.36^{a}	2.32 ± 0.09^{b}	2.09 ± 0.45^{b}	2.17 ± 0.15^{b}	2.16 ± 0.05^{b}

Values in the same row with different superscripts are significantly different. *p< 0.05, **p< 0.01, *** p< 0.001 TH (Tailless head or normal head without tail), HT (Headless tail or normal tail without head or acephalic sperm), RT (Rudimentary tail), CT (Curved tail), LT (Looped tail), BM (Bent mid-piece), NPWA (Normal Protein-energy diet-Water), LPWA (Low Protein-energy diet-Water), NPSE (Normal Protein-energy diet-Seed), LPSE (Low Protein-energy diet-Seed), NPST (Normal Protein-energy diet-Stem), LPST (Low Protein-energy diet-Stem).

Table 2: Influence of diets and/or aqueous *Cuscuta australis* extracts on the total abnormal sperm counts and spermiogram (%) of the Wistar rat

Parameter	Diets (D)						Extracts (Ex)			D-Ex interaction	
	NP			LP							
	WA	SE	ST	WA	SE	ST	WA	SE	ST	SE	ST
Motility	75	62	76	48	62***	60***	61.5	62	68	**	ns
L:D	94.5	91.6	78	91.6	90	94.4	93.05	90.8	86.2	ns	*
TAC	15.5	14.21	11.22	17.14	14.66	11.26	16.32	14.44*	11.24***	ns	ns

ns (Not significant), *p<0.05, **p<0.01, ***p<0.001.Normal protein- energy (NP), Low protein-energy (LP), Control (WA), Seed (SE), Stem (ST), Total abnormal sperm/cell counts (TAC) and L: D (Live dead ratio).

Table 3: Influence of diets and/or aqueous *Cuscuta australis* extracts on the total abnormal sperm counts and spermiogram (%) of the Wistar rat

Parameters	Diets (D)						Extracts (Ex)			D-Ex interaction	
	NP			LP							
	WA	SE	ST	WA	SE	ST	WA	SE	ST	SE	ST
HT	2.24	1.92	1.086	2.47	2.02	1.03	2.36	1.66	1.08**	ns	Ns
TH	1.49	1.43	1.04	1.69	1.88	1.12	1.59	1.97	1.06***	ns	Ns
RT	0.62	0.54	0.49	0.55	0.64	0.34	0.59	0.59	0.42	ns	Ns
BT	3.04	2.66	2.058	2.93	2.48	3.32	2.99	2.57	2.69	ns	Ns
CT	2.3	2.37	1.97	3.03	2.28	2.15***	2.67	2.33*	2.06***	*	*
LT	0.5	0.49	0.39	0.50	0.45	0.34	0.50	0.47	0.37	ns	Ns
BM	2.74	2.47	2.07	3.13	2.12	2.00	2.94	2.3*	2.04**	ns	Ns
CM	2.67	2.32	2.17	3.28	2.09	2.16	2.98	2.2*	2.16**	ns	Ns

ns (Not significant), *p<0.05, ***p<0.001. Normal protein-energy (NP), Low protein-energy (LP), Control (WA), Seed (SE), Stem (ST), Tailless head or normal head without tail (TH), Headless tail or normal tail without head or acephalic sperm (HT), Rudimentary tail (RT), Curved tail (CT), Looped tail (LT), Bent mid-piece (BM) and Curved mid-piece (CM).

Percentages of sperm with either rudimentary tail or bent tail or looped tail (each is presented as a % of total sperm defects)

There were no significant differences (p>0.05) between the percentages of sperm with either rudimentary tail or bent tail or looped tail in the adult Wistar rats used in this study (Table 1).

Sperms with bent mid-piece (% of total sperm defects)

There were no significant differences (p>0.05) between the bent mid-piece percentages of NPWA, NPSE and LPWA (Table 1). However, the percentages of sperms with bent mid-piece decreased significantly (p<0.05) in the LPSE, LPST and NPST relative to the LPWA, NPWA and NPSE (Table 1).

Sperms with curved mid-piece (% of total sperm defects)

The NPSE, LPSE, NPST and LPST showed significantly decreased (p<0.05) percentages of sperms with curved mid-piece relative to the LPWA and NPWA (Table 1).There was a non-significant difference between the percentages of sperms with curved mid-piece in the LPWA and the NPWA (Table 1).

Control versus aqueous seed extract:

Sperm motility

Aqueous seed extract non-significantly altered (p>0.05) the percentage of sperm motility in the Wistar rat. The percentage of sperm motility was however, significantly decreased by the dietary protein-energy level (p<0.001) and influenced by the diet-seed interaction (p<0.01) as shown in Table 2. Generally, low protein-energy diet decreased sperm

motility but the seed extract ameliorated the dietary effect.

Live dead ratio / Percentage liveability (%)
Protein-energy diet, seed extract and diet-seed interaction had no significant influence ($p>0.05$) on the live dead ratio of sperm cells of the adult Wistar rat (Table 2).

Total abnormal sperm cells count
Seed extract, relative to the control, significantly decreased ($p<0.05$) the percentage of total abnormal sperm cell counts (Table 2). Protein-energy diet as well as diet-seed interaction induced non-significant effects ($p>0.05$) on the percentage of total abnormal sperm cells (Table 2).

Percentages of sperm with either headless tail (normal tail without head) or tailless head (normal head without tail) or rudimentary tail or bent tail or looped tail
The diet, the seed extract and the diet-seed interaction non-significantly altered ($p>0.05$) the percentages of sperms with either headless tail or tailless head or rudimentary tail or bent tail or looped tail in the adult Wistar rats used in this study (Table 3).

Sperms with curved tail (% of total sperm defects)
The percentages of sperms with curved tail observed in each treatment group of the adult Wistar rats were significantly altered ($p<0.05$) by protein-energy diet (increased by normal protein-energy; decreased by low protein-energy), seed extract (decreased) and diet-seed interaction (Table 3). The increased percentage of sperms with curved tail observed in the rat that received low protein-energy diet alone was ameliorated by the administration of the aqueous seed extract. Curved tail defect in the rats that received normal protein-energy diet was, however, mildly exacerbated by the administration of the aqueous seed extract.

Sperms with either bent or curved midpiece (each as a % of total sperm defects)
Seed extract significantly decreased ($p<0.05$) the percentages of sperms with bent mid-piece as well as those with curved mid-piece as shown in Table 3. Protein-energy diet alone and diet-seed interaction induced non-significant effects ($p>0.05$) on the percentages of sperms with either bent or curved mid-piece (Table 3).

Control versus aqueous stem extract:
Sperm motility
Low protein-energy diet significantly decreased ($p<0.001$) the sperm motility of the adult Wistar rats used in this study (Table 2). The effect of low protein-energy diet was antagonized by the stem extract. Aqueous stem extract and diet-stem interaction induced non-significant effects ($p>0.05$) on the percentages of sperm motility as shown in Table 2.

Live dead ratio (%)
Dietary status and stem extract treatment had no significant influence ($p>0.05$) on the live dead ratio of the sperm of adult Wistar rats used in this study (Table 2). The interaction between dietary protein-energy and aqueous stem extract significantly altered ($p<0.05$) the live dead ratio as shown in Table 2.

Total abnormal sperm counts
Total abnormal sperm count was significantly decreased ($p<0.001$) by the aqueous stem extract relative to the control (Table 2). Both the diet and diet-stem interaction induced non-significant effects ($p>0.05$) on the total abnormal sperm cell counts (Table 2).

Sperms with headless tail (% of total sperm defects)
Stem extract-treated rats showed significantly decreased ($p<0.001$) percentages of sperms with headless tail (Table 3). Dietary status and diet-stem interaction had non-significant influences ($p>0.05$) on the percentages of sperms with headless tail (Table 3).

Sperms with tailless head (% of total sperm defects)
Stem extract significantly decreased ($p<0.001$) the percentage of sperms with tailless head as shown in Table 3. Dietary status and diet-stem interaction had non-significant effects ($p>0.05$) on the percentages of sperm with tailless head (Table 3).

Sperms with either rudimentary tail or bent tail or looped tail (% of total sperm defects)
Dietary status, extract treatment and diet-extract interactions had no significant influence ($p>0.05$) on the rudimentary, bent and looped tails respectively (Table 3).

Sperms with curved tail (% of total sperm defects)
Significantly increased ($p<0.001$) percentage of sperms with curved tail was observed in the group of rats that received low protein-energy diet alone and this increase was ameliorated by the stem extract (Table 3). The interaction between dietary protein-energy and aqueous stem extract also was significant ($p<0.05$) as shown in Table 3.

Sperms with bent mid-piece (% of total sperm defects)
There was no diet and diet-stem effect ($p>0.05$) on the percentages of sperms with bent mid-piece (Table 3). Conversely, stem extract-treated adult male Wistar rats used in this study showed significantly decreased ($p<0.01$) percentage of sperms with bent mid-piece (Table 3).

DISCUSSION

Sperm morphology is an essential parameter that reflects the degree of normality and maturity of the sperm population in the ejaculate and correlates with fertility (Memon *et al.,* 1986). Defects of the head and mid-piece have been classified as primary defects of spermatogenesis (Schumacher and Moll, 2011), and arise during testicular degeneration (Bloom, 1950). Primary defects of spermatogenesis are more likely to

be associated with decreased fertility (Schumacher and Moll, 2011). The decreased percentages of total abnormal sperm counts as well as defects of the head and mid-piece of spermatozoa of the *C. australis*-treated rats observed in this study may be similar to the reported ability of *C. chinensis* seed extract to invigorate the reproductive system through its strong antioxidant properties (Peng *et* al., 1997; Qin *et al.*, 2000).

Low protein-energy diet has been reported by Ozegbe and Omoirinde (2012) to induce changes in the morphophysiology of seminiferous tubules of the adult Wistar rats. The observations of increased sperm motility in the low protein-energy diet-aqueous *C. australis* extracts-treated groups of rats indicate that protein-energy malnutrition-associated testicular dysfunction, reported by Ozegbe and Omirinde (2012) in this set of rats, rendered the adult rats sexually inactive akin to the effects of immaturity and senility that were earlier reported by Qin *et al.* (2000) to potentiate *C. chinensis*.

The significant effects of interactions between diet and aqueous extracts of *C. australis* (either seed or stem) on the percentages of spermatozoa with curved tail show that the tubular transport of spermatozoa in the *C. australis*-treated rats is directly or indirectly affected by the dietary status of the group. Heys and Gardner (1999) had earlier observed that protein-energy malnutrition elicited alterations in cellular physiology and organ function. Dietary regulation of microsomal cytochromes P450s (CYPs) has been demonstrated in monkeys, mice and rats (Rumack *et al.*, 1973; Czygan *et al.*, 1974; Adekunle *et al*., 1975). Lee *et al.* (1997) reported that such protein-energy malnutrition-induced changes in hepatic CYPs concentrations might be the cause of altered drug metabolism accompanying protein malnutrition.

Generally, fertility capacity is positively correlated to percentage liveability of the sperm cells (Oyeyemi and Okediran, 2007). The decreased live-dead ratio observed in the NPST group suggests that *Cuscuta* stem extract has an adverse effect on the fertility capacity of the sperm of recipients in the presence of normal protein-energy diet. This observed effect of the aqueous extract of the stem on sperm cell liveability in the presence of normal protein-energy diet needs further verification.

This study is part of on-going attempts to evaluate the influence of protein-energy diet on the effects of aqueous extracts of *C. australis* seed and stem on the reproductive system of the adult Wistar rat. Our work has shown that the aqueous extract of *C. australis* stem maintained sperm characteristics, except live-dead ratio, better than the seed extract. It has also shown that the efficacy of aqueous extracts of *C. australis* is influenced by the level of dietary protein-energy intake. These findings may be of value in the developing world where *C. australis* is being used in the traditional management of male reproductive dysfunction.

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