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Effects of aqueous leaves extract of *Waltheria indica* Linn on reproductive indices of male albino rats

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Wide usage of *Waltheria indica* plant has been reported but its effects on the reproductive indices of male albino rats have not been evaluated. This study investigates the effects of *W. indica* leaf extract on the sperm counts, motility, live-dead ratio and sperm morphological abnormality of male albino rats. Thirty six (36) healthy white male albino rats were randomly selected and divided into six groups. Each of the treated groups was administered with different concentrations of aqueous leaf extract for 21 days after which the semen samples were taken for analysis of reproductive parameters. The testicles were also removed for histological study. There was significant decrease ($p<0.01$) in sperm motility and sperm counts at doses 800, 1600 and 2000 mg/kg. The live-dead ratio was also significantly decreased ($p<0.01$) at 2000 mg/kg dose. There was also significant increase in total abnormal cell for all the treated groups. The extract induced periportal cellular infiltration and interstitial congestion on the testes of the treated rats.

Key words: *Waltheria indica,* motility, sperm counts, live-dead ratio, abnormal cell, histology, rats.

INTRODUCTION

Waltheria indica L. also known as sleepy morning and many other names (Burkill, 2000), belongs to the family Sterculiaceae. It is widespread in West Africa (Akobundu and Agyakwa, 1998). Locally, the plant is called 'hankufah' in Hausa, 'korikodi'' in Yoruba and 'efu-abe in Nupe (Hutchinson and Dalziel, 1958; Irvine, 1961). The uses of the plant are diverse; the plant has been used as an infusion or decoction where febrifugal, purgative, emollient, tonic, analgesic and astringent action is sought (Burkill, 2000). It is used in Northern Nigeria by the Hausas for the treatment of skin diseases, impotence, and infertility, as an aphrodisiac, and as children's medicine at birth and during teething (Mohammed et al., 2007). In the Fulani community, the aqueous extract of the root is used in relieving aches and pains during the 'Sharo' festival. Among the Yorubas, the aqueous extract of the root and stem is used in treating syphilis, internal haemorrhage, and as a restorative during the labours of harvesting (Mohammed et al., 2007).

The wide usage of *W. indica* in folk medicine has been corroborated by scientific evidences in recent time. The antioxidant effects (Saidu et al., 2012), antibacterial effects (Olajuyigbe et al., 2011), antimalarial (Clarkson et al., 2004) anti-inflammatory effects (Yerra et al., 2005) anticonvulsants effects (Hamidu et al., 2008) and trypanocidal effects (Bala et al., 2010) of *W. indica* have

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been reported. It has also been reported that medicinal plants with antimicrobial effects have tendency to adversely affect male fertility (Olayemi, 2010). Many antimalarial drugs have been implicated in male infertility. For instance chloroquine, quinine and quinacrine have been reported to inhibit Leydig cell steroidogenesis and fertility in male (Sairam, 1978). Hence, this study was designed to investigate the effect of *W. indica* leaf extract on the reproductive indices of male albino rats.

MATERIALS AND METHODS

Experimental animals

Thirty six (36) healthy white male adult albino rats (100 to 190 g) obtained from the Animal House, Faculty of Veterinary Medicine, University of Ibadan, were used for the study. The rats were fed with rat cubes (Ladokun feeds limited, Ibadan, Nigeria) and water *ad libitum*. The rats were kept at the Experimental Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan. They were acclimated to their new environment for two weeks before the commencement of the experiment. All experimental protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

Plant material

The *W. indica* plants were obtained from a farm land at Moniya in Akinyele area Council of Ibadan, Oyo state, Nigeria and identified at the Herbarium, Department of Botany, University of Ibadan with voucher number UIH-22371.

Extract preparation

The leaves of the plant were separated from the whole plant and air dried at room temperature for two weeks. A total of 200 g of the ground powder was soaked in 1 L of distilled water for 24 h at room temperature. The mixture was filtered into conical flask with Whatman filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator at 40°C to produce a gel-like extract, which weighed 43 g (21.5% yield). Appropriate concentration of the extract was then subsequently made by dilution with distilled water into graded doses and administered to the rats.

Experimental design

Thirty six (36) male albino rats were randomly divided into six groups (n = 6), labelled A to F; where, group A served as the control while the animals in the groups B, C, D, E and F served as the treated group. The treated groups were then orally administered with 200, 400, 800, 1600 and 2000 mg/kg body weight of the extract, respectively for 21 days.

After 21 days of extract administration, the rats were sacrificed and the testicles were surgically removed through a lower abdominal incision for histological study. Semen samples were collected from the epididymides for the following andrological analyses:

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 (1970). Briefly, a small drop of semen was placed on a warmed slide mixed with one drop of warm sodium citrate covered with a glass slip. Sperm cells moving in a straightforward unidirectional motion were counted while sperm cells moving in circles or in backward direction were excluded.

Sperm count

Epididymal sperm count was obtained by mincing the cauda epididymis in distilled water and filtering through a nylon mesh. The spermatozoa were counted by haemocytometer using improved Neubauer chamber (Deep 1/10mm, LABART, Germany) described by Pant and Srivastava (2003).

Live - dead ratio/percentage liveability

One drop of semen was mixed with one drop of eosin-nigrosin stain on a warm slide. A thin smear was then made from the mixture of semen and stain. The smear was then air-dried and 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 (Zemjanis, 1970).

Sperm morphological abnormalities

On a clean, warm glass slide, a drop of semen was placed as well as two drops of Wells and Awa stain as reported by Hammer (1970). The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total percentage was estimated.

Histology

All the animals from each of the treated groups B, C, D, E, F and the control were sacrificed 24 h after their respective daily doses. The rats were thereafter quickly dissected to remove the testes and then transferred into 10% buffered formalin. The organs were dehydrated in ethanol (70 to 100%), cleared in xylene and embedded in paraffin. Tissue sections (5 µm thickness) were examined under a light microscope after staining with haematoxylin and eosin (H and E) (Culling, 1963; Lillie, 1965).

Statistical analysis

The data obtained from the experiment were presented as mean ± standard error of mean (S.E.M) and analysed using the one-way analysis of variance (one-way ANOVA). The group means were separated by Duncan Multiple range Tests at 95% confidence interval using GraphPad Instat® software.

RESULTS

Effect of the aqueous extract of *Waltheria indica* on sperm parameters of rats

The result of the effect of graded doses of *W. indica* on sperm parameters of rats is presented in Table 1. There

Table 1. Effect of the aqueous extract of *Waltheria indica* on sperm parameters of rats (n=6).

Parameter	Control	B	C	D	E	F
Motility (%)	088±2.55	073±2.0[a]	074±5.10[a]	065±2.24[b]	052±3.74[b]	038±4.89[b]
Live-dead ratio (%)	097.4±0.60	094.2±1.80	095.2±1.46	089.6±3.27	089.6±2.27	086.0±2.92[a]
Sperm count (×10^6)	136.6±3.74	113.2±4.99[a]	110.0±5.05[b]	096.2±4.06[b]	082.6±7.63[b]	082.0±5.45[b]
Volume (ml)	005.18±0.02	005.14±0.02	005.18±0.02	005.14±0.02	005.18±0.02	005.16±0.02

Thirty six (36) male albino rats were randomly divided into six groups (n = 6), labelled A to F; where, group A served as the control while the animals in the groups B, C, D, E and F served as the treated group. The treated groups were then orally administered with 200, 400, 800, 1600 and 2000 mg/kg body weight of the extract, respectively for 21 days. Superscripted items indicate significant values compared to the control ([a] P< 0.05, [b] P<0.01).

Table 2. Effect of the aqueous extract of *Waltheria indica* on sperm morphological abnormality of male rats (n=6).

Parameter (%)	Control	B	C	D	E	F
Total abnormal cell	11.32±0.13	12.62±0.33[a]	13.62±0.14[b]	13.56±0.25[b]	13.54±0.40[b]	14.99±0.46[b]
Tailess head	1.11±0.14	1.16±0.14	1.14±0.13	1.08±0.15	1.O9±0.15	1.13±0.13
Headless tail	0.75±0.19	1.09±0.13	1.09±0.16	1.18±0.09	1.24±0.15	1.18±0.15
Rudimentary tail	0.40±0.09	0.61±0.10	0.65±0.06	0.64±0.06	0.50±0.08	0.44±0.09
Bent tail	2.08±0.09	2.17±0.13	2.73±0.14a	2.58±0.11	2.58±0.12	3.11±0.29[b]
Curved tail	1.93±0.08	2.34±0.06	2.68±0.08b	2.53±0.10[b]	2.53±0.14[b]	3.00±0.17[b]
Bent midpiece	2.05±0.05	2.29±0.17	2.38±0.133	2.63±0.18[a]	2.58±0.15	2.81±0.19[b]
Curved midpiece	2.18±0.14	2.74±0.07[a]	2.29±0.18	2.33±0.11	2.48±0.11	2.86±0.19[a]
Looped tail	0.49±0.08	0.49±0.08	0.65±0.06	0.55±0.09	0.49±0.11	0.39±0.10

Thirty six (36) male albino rats were randomly divided into six groups (n = 6), labelled A to F; where, group A served as the control while the animals in the groups B, C, D, E and F served as the treated group. The treated groups were then orally administered with 200, 400, 800, 1600 and 2000 mg/kg body weight of the extract, respectively for 21 days. Superscripted items indicate significant values ([a]P< 0.05, [b]P<0.01).

was significant decrease in sperm motility at doses 200 and 400 mg/kg (P < 0.05) and 800, 1600, and 2000 mg/kg (P < 0.01). The sperm count was also significantly decreased at 200 mg/kg dose (P < 0.05) and at doses 400, 800, 1600 and 2000 mg/kg doses (P < 0.01). The percentage of live-Dead sperm was significantly (P < 0.01) decreased at 2000 mg/kg b.w dose.

Effect of the aqueous extract of *Waltheria indica* on sperm morphological abnormality of rats

The result of the effect of *W. indica* on sperm morphological abnormality of rats is shown in Table 2. There was significant increase in total abnormal cell for all the treated groups (P< 0.01). The curved tailed abnormality values were also significantly increased (P< 0.01) at doses 400, 800, 1600 and 2000 mg/kg. The bent tail abnormality values were significantly increased at dose 400 (P< 0.05) and 2000 mg/kg (P< 0.01). The extract doses of 800 and 2000 mg/kg corresponding to groups D and F also caused significant increase in bent midpiece abnormality values. The abnormality values for the tailless head, headless tail, rudimentary tail and looped tail for all the treated groups were not significantly different from the control group. The curved mid piece abnormality value at 200 mg/kg dose was significantly increased (P< 0.05).

Histological effects

The result of the histological changes induced by aqueous leave extract of *W. indica* is presented in Figures 1 to 3. No visible lesion was observed in the testis (Figure 1) of the control group. Periportal cellular infiltration by mononuclear cells was observed in the testis (Figure 2) of rats at 400 mg/kg. Also, interstitial congestion was observed in the testis (Figure 3) at 1600 mg dose.

DISCUSSION

The analysis of spermiogram of the albino rats in this study shows that the administration of aqueous extracts of *W. indica* significantly reduced sperm characteristics and functions (sperm counts, live-dead ratio, motility and morphology) of male albino rats. Although, the effect of the extract of *W. indica* on male reproductive system has

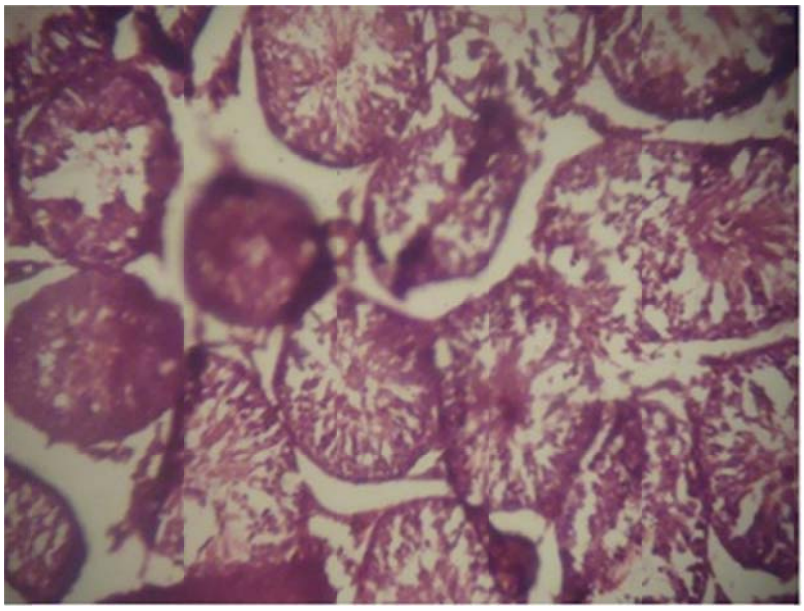

Figure 1. Testis of the control group with no visible lesion (M × 40 H&E).

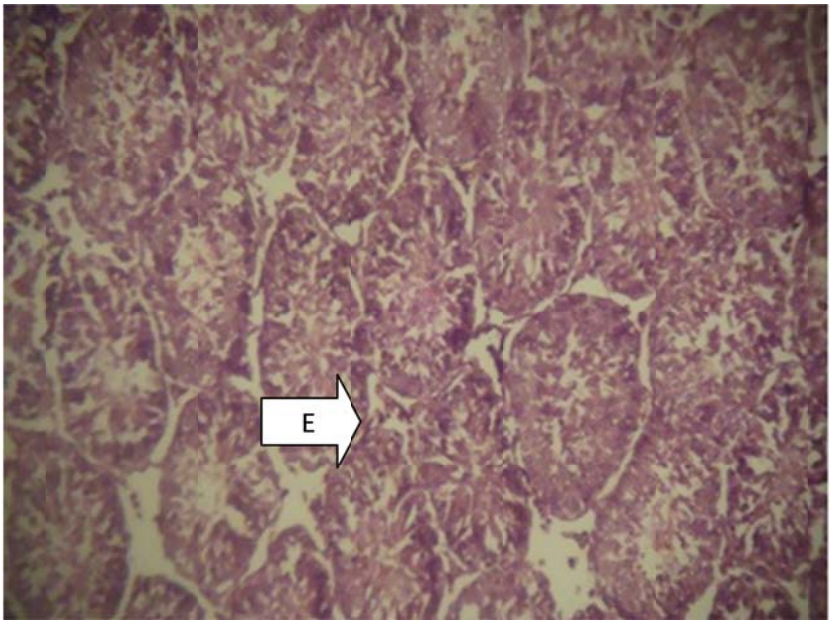

Figure 2. Testis of group C showing periportal cellular infiltration by mononuclear cells E (M × 40 H&E).

not been previously investigated, the result obtained in this study is in agreement with the findings of Raji et al. (2005) who also reported dose-dependent changes in sperm characteristics of rats treated with *Alstonia bonei.* Several other commonly used plants have been reported to adversely affect male reproductive functions in wildlife and humans. The observation with such plants has been attributed to their ability to impact adversely on spermatogenesis and steroidogenesis (D'cruz et al., 2010). Other plant extract reported to alter the morphology of sperm or to diminish its motility include *Abrus precatorious* (Adedapo et al., 2007), *Cola nitida rubra* (Adisa et al., 2010), *Croton zambesicus* (Ofusori et al., 2010), and *Kigelia africana* (Adeparusi et al., 2010). The reduction in sperm quality may be subject to the hispathological changes caused by the extract. The

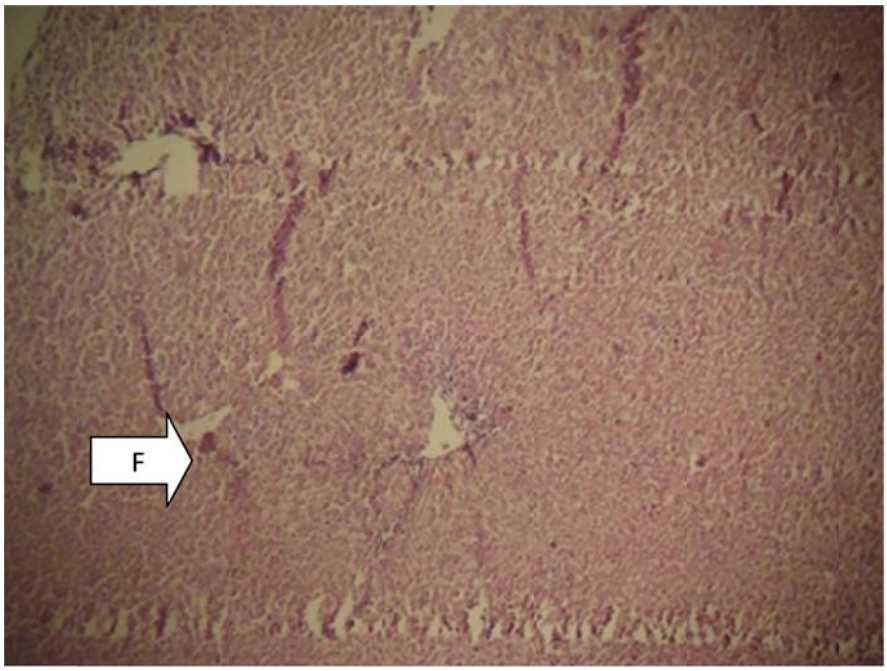

Figure 3. Testis of group E showing interstitial congestion (M × 40 H&E).

aetiology of this pathology has been traced to the presence of alkaloids (Badifu and Ogunsua, 1991). These alkaloids are bioactivated to released reactive metabolites which bind cell molecules and cross-link DNA to cause cellular damage (Cheeke, 1988). The total percentage morphological abnormalities observed increased with increasing dose. More so, the tail abnormalities account for most of these morphological abnormalities, hence significant reduction in sperm motility of all the treated groups. This suggests that treatment with *W. indica* leave extract adversely affect sperm motility. Moreover, sperm capacitation, the series of enzymatic reactions resulting in the release of acrosomal enzymes which allow for fertilization in the female reproductive tract will be adversely affected. The delay in the occurrence of capacitation had been reported to render spermatozoa nonfunctional (Nass et al., 1990).

There is also increase in mid piece abnormalities. The increase in bent mid-piece abnormality was significant at 1600 ($P<0.05$) and 2000 mg/kg ($P<0.01$) doses while the curved mid-piece abnormality was significant at 200 ($P<0.05$) and ($P<0.05$) 2000 mg/kg doses. Occurrence of high number of mid-piece spermatozoa abnormality has been traced to the period of storage in the epididymis (Oyeyemi and Babalola, 2006).

Mid-piece abnormalities had also been traced to the deficiency of zinc. Zinc and folate are involved in the synthesis of DNA and RNA. Although the exact pathophysiology of zinc deficiency leading to clinical symptoms of decreased spermatogenesis and impaired male fertility has not been known but it has been shown to cause impaired male fertility in the form of reduced sperm motility, reduced percentage motility of sperm, morphological abnormalities and reduced spermatogenesis (Wong et al., 2000).

The study concludes that excessive use of aqueous extract of *W. indica* leaf has adverse effect on reproductive parameters of male albino rats; therefore caution should be applied to the use of *W. indica* leaves despite its numerous medicinal values.

Conflict of Interest

The author(s) declared that there is no conflict of interest as regards this paper.

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