Effects of *mondia whitei* on the morphometry and histological structure of the testis of the albino rat

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SUMMARY

The purpose of this study was to describe the effect of Mondia whitei on the histomorphometry of the albino rat testis. The objective of this study was to determine histomorphometric changes in the testicular parenchyma following oral administration of graded doses of Mondia whitei.

This was an experimental study in which 48 male rats of the species Rattus norvegicus were used. These rats were grouped into 4 cohorts. Control group, which was designated as group A, received distilled water via oral gavage daily. Group B received 300 mg/kg. Group C received 600 mg/kg. And Group D received 1200 mg/kg of Mondia whitei extracts daily for 28 days. At the end of the experimental period, body mass changes, histological changes, and volumes of the testis were obtained. Stereological techniques were used in the morphometric analysis of the volume densities and volumes of the parenchymal components of the testis. Statistical comparison of the differences between the groups was done using one way ANOVA followed by a Tukey post hoc test and a p – value of < 0.05 was considered statistically significant.

Administration of Mondia whitei led to hypocellularity of the seminiferous epithelium with loss of cells of the spermatogenic germ series and development of intercellular spaces; moreover, vacuolation of the Sertoli cells was also observed. There was a dose dependent decrease in the body mass of rats in the experimental groups ($p < 0.001$). This was coupled with a reduction in the volume densities of Leydig cell (LC) ($p < 0.005$), seminiferous epithelium (SE) ($p < 0.001$), seminiferous Lumen (SL) ($p < 0.013$) and interstitium (I) ($p < 0.028$). A reduction in the volumes of the SE, SL, LC, and I. Changes in the testes volumes were not statistically significant.

The observed histological changes in the seminiferous tubules and body mass draw the need for a cautious approach to the use of Mondia whitei extracts for medicinal purpose.

The hypocellularity of the seminiferous tubules may have been attributed to a reduction of the cells of the spermatogenic germ cell series with injury to the Sertoli cells, which play a pivotal role in maintaining the germ cells.

Key words: *Mondia whitei* – Albino rat testis – Volume density – Stereology – Morphometry

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INTRODUCTION

Mondia whitei is a liana plant found in the tropics of the Sub Saharan Africa distributed along the equatorial belt from West to Eastern part of the continent (Lamidi and Bourobou, 2010). *Mondia* root is traditionally used to treat many conditions such as its use as an aphrodisiac, an appetite stimulant, treatment of indigestion, sexually transmitted diseases and as a uterine stimulant among others. The most commonly cited use is as an aphrodisiac (Lampiao et al., 2008; Oketch-Rabah, 2012; Watcho et al., 2013).

Sexual dysfunction is difficulty experienced by an individual or a couple during any stage of a normal sexual activity. This includes premature ejaculation, erectile dysfunction, reduced libido, orgasm disorders and compulsive sexual behaviour (Patel et al., 2011; Prabsattro et al., 2015). Sexual dysfunction has been estimated to affect 15 – 30 million people worldwide, and it occurs in 10-32 % of men and 25-63% of women (Abudayyak et al., 2015). In Kenya, a study done by Likata et al. (2012) concluded that the prevalence of sexual dysfunction is generally high, and male sexual dysfunction can greatly affect the quality of life. It can induce depression, anxiety and debilitating feelings of inadequacy (Baldwin, 2001). Due to the high prevalence and impact of sexual dysfunctions, great efforts have been made in search of effective interventions to improve sexual performance. This has led to the use of aphrodisiacs such as *Mondia whitei.*

An aphrodisiac is defined as a substance that arouses sexual desire (Chauhan et al., 2014). Aphrodisiacs can be classified by their modes of action into three types: those that increase libido, those that increase potency and those that increase sexual pleasure. Several types of modern medicine have been used in the management of sexual dysfunction, but due to their high costs (Yakubu et al., 2005) and some of their reported side effects (Jarrar, 2011; Jarrar and Almansour, 2015; Vidale et al., 2015), individuals now search for natural supplements from medicinal plants. Various herbs have been used in folk medicine in different cultures to treat sexual dysfunctions (Kotta et al., 2013). One such plant is *Mondia whitei* (Lampiao et al., 2008). Studies done on herbal aphrodisiacs such as *Moringa Oleifera* (Zade et al., 2013), *Nigella Sativa* (Al-Sa'aidi et al., 2009) and *Psoralea corylifolia* (Dabhadkar and Zade, 2013) have shown great potential of these herbs. They have been shown to have a potential in increasing testosterone levels, which has an effect on virility and libido (Malviya et al., 2011) and also improve sperm motility and quality. Others confer histomorphological changes on the testis (Zade et al., 2013; Prabsattro et al., 2015). *Mondia whitei* has been shown to cause an increase in the levels of testosterone (Watcho et al., 2004; Lampiao et al., 2008; Oketch-Rabah et al., 2012). There are, however, conflicting reports on its effects on the seminiferous tubules and the rest of the testis parenchyma (Watcho et al., 2001; Kuo et al., 2006).

The current study thus aims to describe possible histomorphometric changes in the seminiferous tubules, Leydig cells and body mass in the rats following use of *Mondia whitei.*

MATERIALS AND METHODS

Fourty eight male rats of the species *Rattus novergicus* of comparable weights and sizes were obtained. These were grouped into 4 cohorts with 12 rats per group.

Preparation of *Mondia whitei*

Fresh roots of *Mondia whitei* were bought from the local market in Kakamega County. Botanical identification of the herb was done at the department of Botany, the School of Biological sciences, University of Nairobi. The fresh roots were diced into small cubes measuring 1cm. These cubes were air-dried for 2 weeks to avoid infestation with fungi. The dried *Mondia whitei* roots were ground into powder form and stored in air tight containers. This was weighed and macerated in Dichloromethane (DCM)/ hexane in a ratio of 1:1 for 72 hours. The macerate was filtered and the filtrate was oven dried at 55^0C, and a dark brown extract was obtained. The working solution was obtained by dissolving 100 mg of the residue in 1ml of distilled water to make a concentration of 100 mg/ml.

Determination of body mass changes

The weights of the rats were recorded at different stages of the experimental period using an electronic scale. The first set of measurements were obtained on the first day of the experimental period and this was designated as the body mass on arrival. The second set of measurements were obtained after 2 weeks of acclimatization. The third set of measurements were obtained on the last day of the experiment, and these were designated as the body mass before sacrifice.

Experimental procedure

The male rats of the species *Rattus novergicus* were used in this experiment for 28 days. A total of 48 animals following a sample size calculation were grouped into 4 cohorts: a control group and 3 experimental groups. Group A, being the control group received distilled water daily via oral gavage, group B received 300 mg/kg of *Mondia whitei* daily via oral gavage; group C received 600 mg/kg and group D received 1200 mg/kg.

Perfusion and fixation

At the end of the 28 days, the rats were put under deep anaesthesia by placing them in a lidded glass jar containing cotton wool soaked in halothane. Perfusion fixation of the rats was performed using Bouin's solution. The testicles were harvested and the volumes of the right and left testicle obtained using the Scherle method of volume displacement (Scherle, 1970). This was designated as the reference volume (V (ref)) which was the working volume when calculating the volumes of the various components of the testis thus,

V(c) = Vv (c, ref) x v (ref)

Where

V(c) = Volume of component of interest

Vv (c, ref) = Volume density of the component of interest obtained by point counting methods

The testicles were immersed in Bouin's solution in well labelled specimen bottles.

Tissue sampling and processing

From each rat, one testicle was sliced into 6 slices perpendicular to the long axis of the testicle and the slices were of approximately equal thickness. This was done by laying a transparent plastic ruler along the length of the fixed testis that was now firm, on a flat surface. Using a sharp blade, the testes was cut into 6 slices. Three slices were then sampled, using systematic random sampling with the first slice picked at random then every 2nd slice was picked. The 3 slices were picked at random and processed for paraffin embedding as shown in figure.(1). The tissues were then processed for light microscopy, using standard procedure and staining done using haematoxylin and eosin stain. These were observed under the light microscopy at X100, X400 and X1000 with acquisition of the images done using A12.0736 CX23 Olympus biological microscope.

Determination of morphometric changes

Stereological techniques were used in morphometric analysis of the parenchymal components of the testis. From each slice 6 sections were randomly selected. From each section, sampling field frames were picked in a systematic random way, starting from the top left corner of the section. Consequently, each testis had about 108 fields as shown in Fig. 1. The sampling field frames were generated using the STEPANIZER software (Tschanz et al., 2011).

Volume densities of a components of interest were calculated using the following equation:

Where: Pc = point count on profiles of the components of interest.

Pt = Total number of points on profiles of the reference space

The volume of the component of interest was calculated using the following formula:

Where: V(c) = volume of component of interest

V (ref) = volume of the reference space

Parameters that were estimated are:

Volume density of Leydig cells = Vv (LC, ref)

Volume density of the seminiferous epithelium = Vv (SE, ref)

Volume density of the seminiferous lumen = Vv (SL, ref)

Volume density of the interstitium = Vv (I, ref)

Data analysis and presentation

Data were coded and analysed by computer software, statistical package of social sciences version 23.0. Statistical comparison of the differences between groups was done using ANOVA, followed by a Tukey post hoc test. P-value of <0.05 were considered as statistically significant.

Ethical approval

Ethical approval was sought from the Biosafety, Animal use and Ethics Committee (BAUEC), of the Faculty of Veterinary Medicine, University of Nairobi.

RESULTS

Body mass changes

The mean body mass of the rats at the beginning of the study is as shown in Table 1 for group A–D. The mean body mass after acclimatization was not statistically significant between the different experimental groups. There was a statistically significant difference between the groups in their mean body mass before sacrifice: i.e., after treatment for 28 days (F (3, 20) = 10.953, p< .001, η^2 = 0.622), as shown in Table 1. The Tukey post hoc analysis revealed significant differences between group A and the other experimental groups, as shown in Table 1.

Histological features of the testis parenchyma in group A.

The testis parenchyma from the rats in the control group consisted of seminiferous tubules with a stratified epithelium, connective tissue stroma between the tubules composed of various cells and blood vessels. At X400 the seminiferous lumen (SL) was fully packed with spermatozoa, and the seminiferous epithelium (SE) was packed with various cells of the spermatogenic series at different stages of spermatogenesis (Fig. 2A).

At a higher magnification (X1000), various cells of the spermatogenic series were clearly

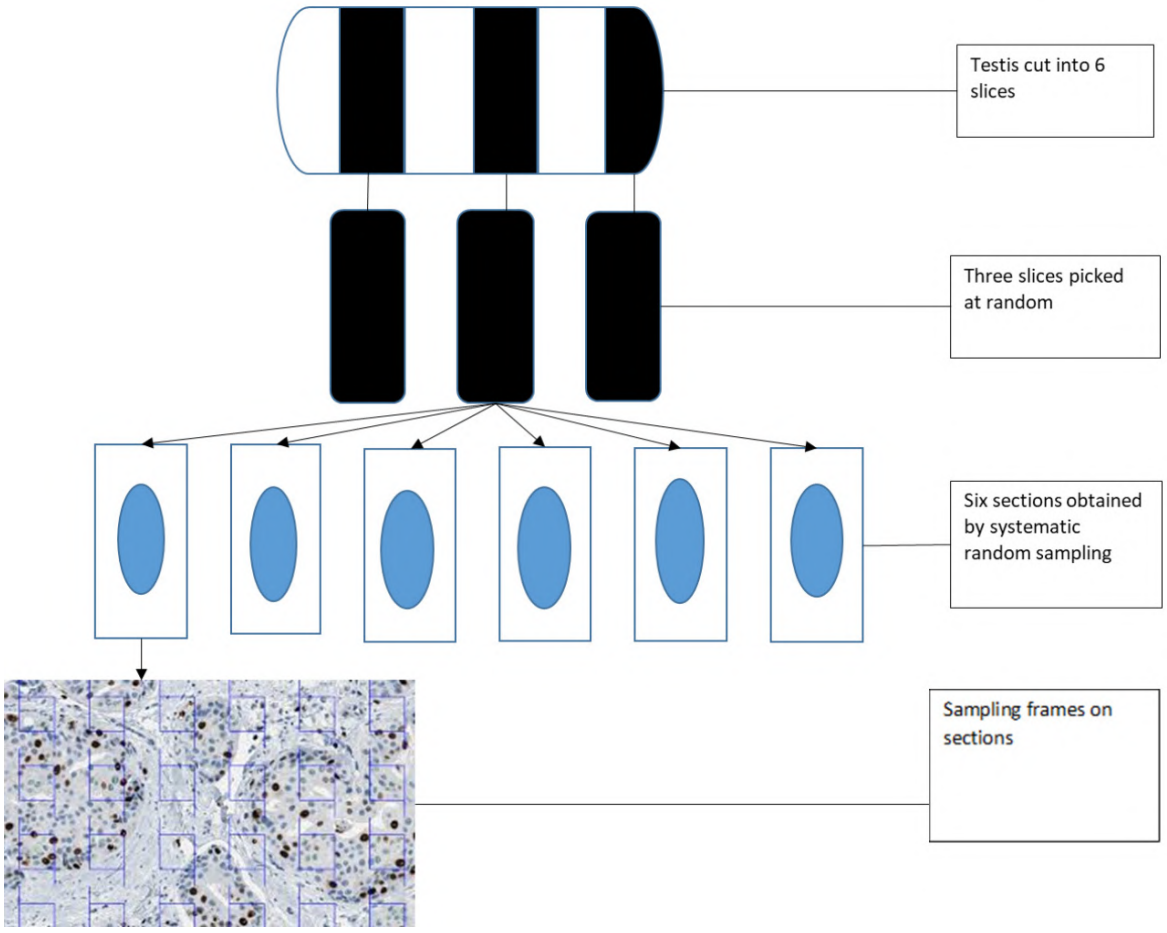

Fig. 1.- Image showing sampling of the testis

recognizable. The spermatogonia (SPG) were evident along the basement membranes, and cells were generally ovoid in shape with prominent nucleoli and dark staining cytoplasm. Sertoli cells (ST) had pale staining cytoplasm and a prominent nuclei and nucleoli. There were several spermatocytes (SPT) with disintegrated nuclei. signifying that the cells were undergoing meiosis. Early spermatids (rSPD) were round in shape with a pale staining cytoplasm, and late spermatids (eSPD) had dark elongated nuclei with inconspicuous cytoplasm buried in the cytoplasm of adjacent Sertoli cells. The connective tissue stroma consisted of Leydig cells (LC) interspersed within it. These cells were ovoid in shape clustered around blood vessels (BV) as seen in Fig. 2B.

Histological changes in the testis parenchyma of rats in the experimental groups B, C and D:

Tissue sections of the rat testes from experimental group B, C and D demonstrated development of prominent IS spaces within the seminiferous epithelium, with progressive increase in dosages. This change was most pronounced in group D, as shown in Fig. 3. Hypocellularity of the epithelium was demonstrated with increase in dosages coupled with a reduction in the cells of the spermatogenic series, as shown in Fig. 4. Microvacuolation of Sertoli cells was also observed with higher dosages of *Mondia whitei,* as shown in Fig. 5. Clamping of spermatids and fragmentation of the seminiferous epithelium was observed for group D (Fig. 6).

Table 1. Mean body mass changes in the various experimental groups.

Parameters	Mean +/- SD				P value
Groups	A (n=12)	B(n=12)	C (n=12)	D(n =12)	
Body mass on arrival	420.8 +/-13.7	393.5 +/- 16.2	414.5 +/- 35.5	406.8 +/- 44.5	0.460
Body mass after acclimatization	421.5 +/-15.2	391.5 +/- 15.5	412.3 +/- 36.8	392.5 +/- 49.3	0.319
Body mass before sacrifice	428.2 +/-11.9	353.7 +/- 17.0	346.7 +/- 42.9	352.8 +/- 31.6	0.001*
Tukey post hoc		74.5g	81.5g	75.3g	0.001**

Mean +/- SD
*P value <0.001 statistically significant difference between the experimental groups following one way ANOVA.
**P value = 0.001 following a Tukey post hoc analysis showing statistically significant differences between group A and the other experimental groups.

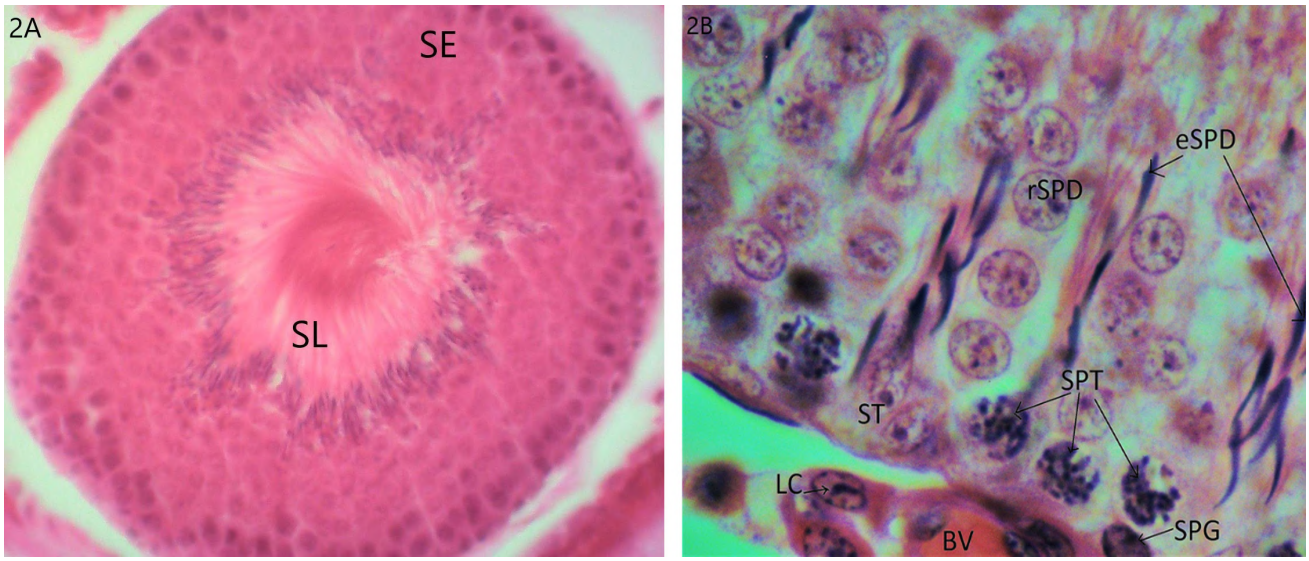

Fig. 2.- The photomicrograph shows the seminiferous epithelium (SE) with cells at different stages of the spermatogenic series and a seminiferous lumen (SL) packed with spermatozoa. Magnification = X400, stain = H&E (A). At a higher magnification a section of the seminiferous tubule shows intact seminiferous epithelium with pale staining Sertoli cells (ST), several primary spermatocytes (SPT), round spermatids (rSPD) and elongated spermatids (eSPD), spermatogonia (SPG), Leydig cells (LC), blood vessels (BV). Magnification = X1000, stain = H&E (B).

Morphometric changes of the testes

Comparative morphometric analysis of components of the testicular parenchyma

The volume densities of the various testicular components were obtained as percentages. The volume densities of the seminiferous epithelium between the experimental groups were significantly different (F (3, 20) = 86.487, p = 0.001, η^2 = 0.928) (Table 2). Tukey post hoc analysis revealed significant differences between group A and the other experimental groups, as shown in Table 2.

The volume densities of the seminiferous lumen between the experimental groups were significantly different (F (3, 20) = 4.615, p = 0.013, η^2 = 0.409) (Table 2). Tukey post hoc analysis revealed significant differences between group A and the other experimental groups as shown in Table 2.

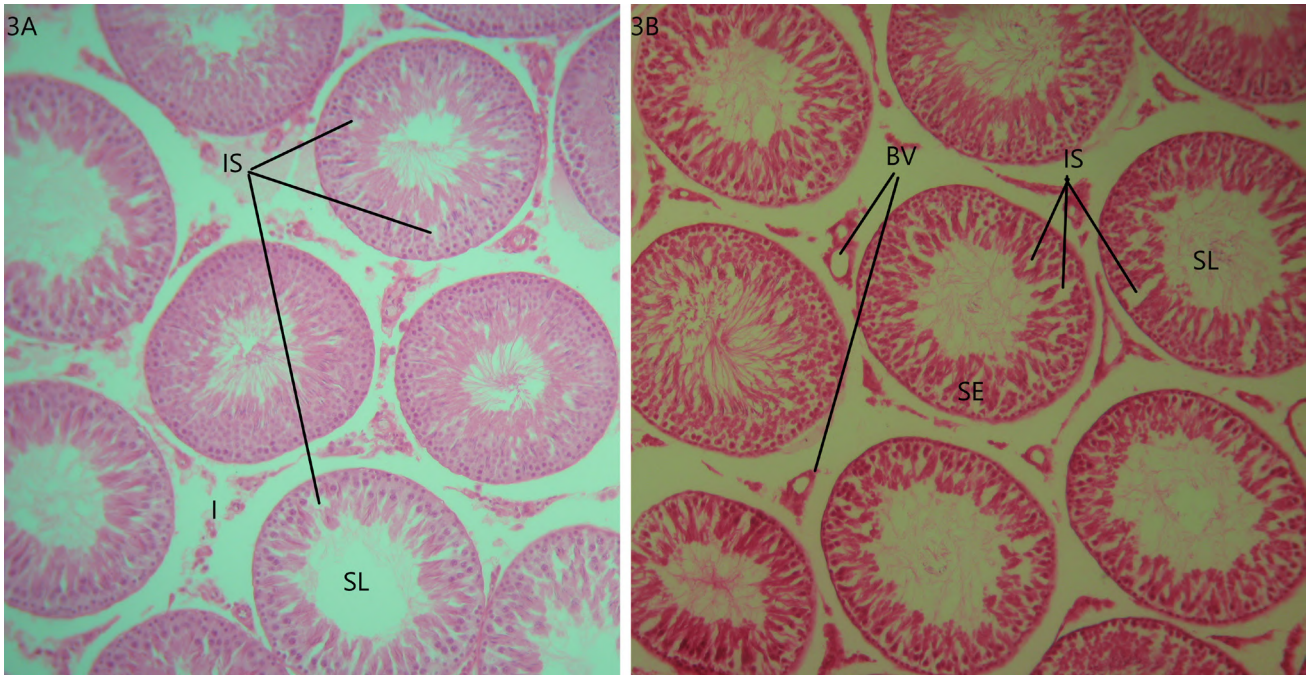

Fig. 3.- The photomicrograph shows subsequent development of interstitial spaces = IS within the seminiferous epithelium of rats in group B. Magnification = X100; Stain = H&E. SL = seminiferous lumen, I = interstitial tissue (A). As a comparison a section of the seminiferous epithelium of a rat from group D shows grossly dilated blood vessels = BV, with prominent interstitial spaces = IS. Magnification = X100, stain = H&E. SL = seminiferous lumen, SE = seminiferous epithelium (B).

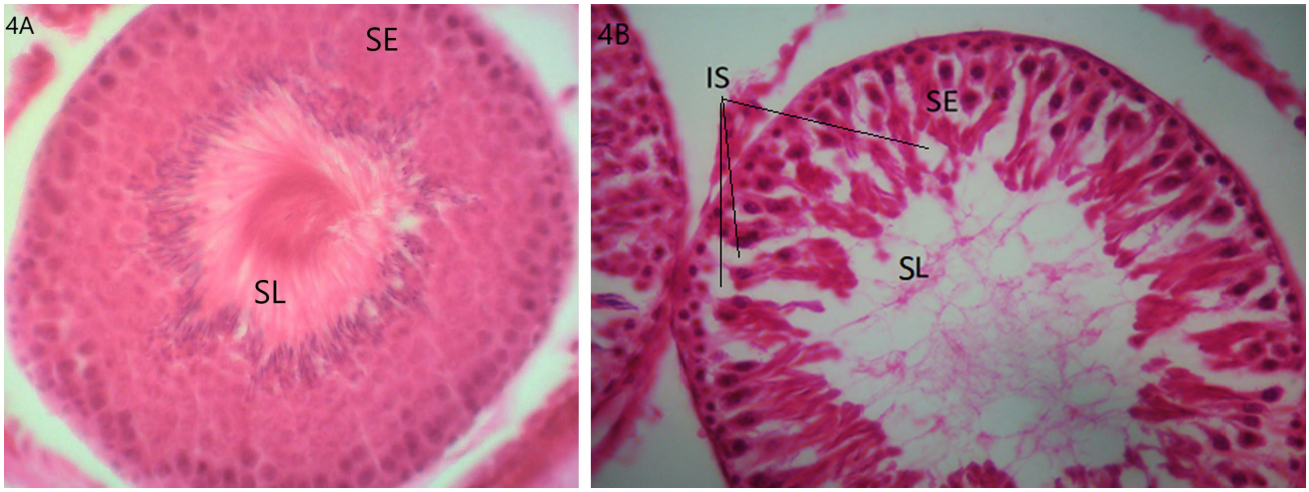

Fig. 4.- The photomicrograph shows a seminiferous tubule of a rat testis from group A with presence of a cellular epithelium (SE) and a packed seminiferous lumen (SL). Magnification = X400, stain = H&E (A). In comparison to a section of the seminiferous tubule of a section of a rat from group D showing presence of a hypocellular seminiferous epithelium (SE) and a less packed seminiferous lumen (SL) with development of intercellular spaces (IS) within the epithelium. Magnification = X400, stain = H&E (B).

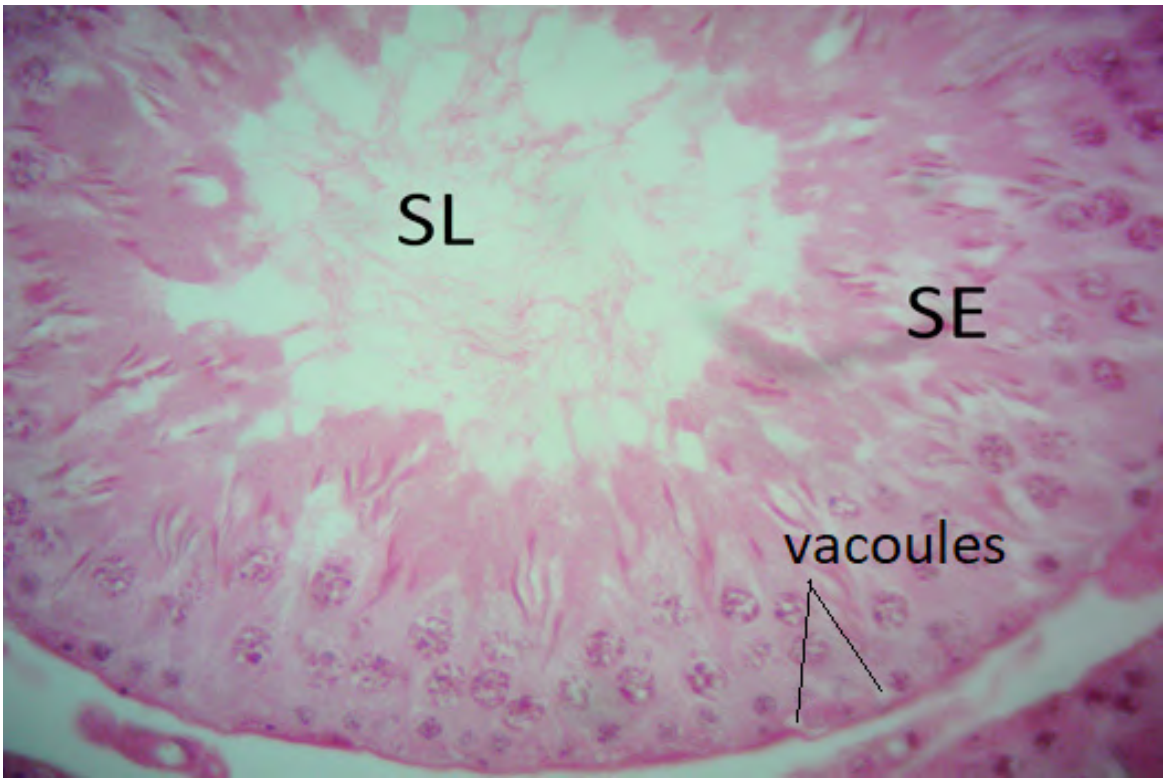

Fig. 5.- The Photomicrograph of a rat testis from group B showing presence of vacuolations in the sertoli cells. Magnification = X400; Stain = H&E. SE = seminiferous epithelium, SL = seminiferous lumen.

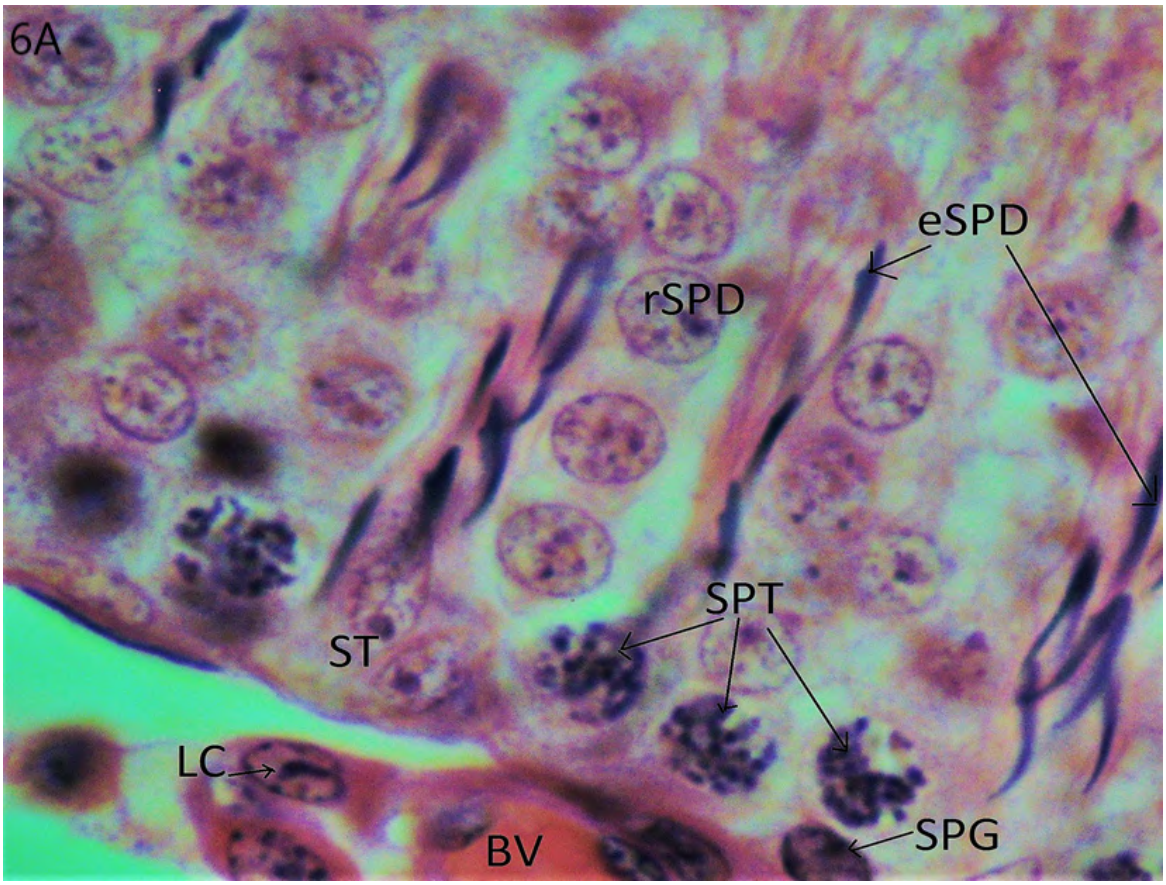

Fig. 6.- The photomicrograph of a section of the seminiferous epithelium of a rat testis from group A shows components of the intact seminiferous epithelium namely pale staining Sertoli cells (ST), several primary spermatocytes (SPT), round spermatids (rSPD) and elongated spermatids (eSPD), spermatogonia (SPG), Leydig cells (LC), blood vessels (BV). Magnification = X1000, stain H&E (A). In comparison to a section of the seminiferous tubule of a rat testis from group D which showed a hypocellular seminiferous epithelium with loss of cells of the spermatogenic series with presence of sparse spermatocytes (SPT), clamping of elongated spermatids (eSPD) and development of intercellular spaces (IS). Magnification = X1000, stain H&E (B).

The differences in volume densities of Leydig cells were statistically significant between the experimental groups (F (3, 20) = 5.807, p = 0.005, η^2 = 0.466) (Table 2). Tukey post hoc analysis revealed significant differences between group A and the other experimental groups, as shown in Table 2.

The volume densities of the interstitium between the experimental groups were significantly different (F (3, 20) = 3.712, p = 0.028, η^2 = 0.358) (Table 2). Tukey post hoc analysis revealed significant differences between group A and C, as shown in Table 2.

Volumes of the components of the testes parenchyma

The volumes of the various testicular components for the different experimental groups were obtained in centimeters cubed (cm^3). The volumes of the seminiferous epithelium was statistically significant between the experimental groups (F (3, 20) = 27.33, $p < 0.001$) (Table 3). Tukey post hoc analysis revealed significant differences between group A and the other experimental groups, as shown in Table 3.

The volumes of the seminiferous lumen between the experimental groups were significantly different (F (3, 20) = 3.959, p = 0.023) (Table 3). Tukey post hoc analysis revealed that the statistical significance was within groups.

The volumes of interstitium between the experimental groups were significantly different (F (3, 20) = 3.268, p = 0.043) (Table 3). Tukey post hoc analysis revealed the statistical significance was within groups.

The volumes of the Leydig cells between the experimental groups were significantly different (F (3, 20) = 5.833, p = 0.005) (Table 3). Tukey post hoc analysis revealed significant differences between group A and the other experimental groups, as shown in Table 3.

Testicular volumes

The right and left testis volumes were obtained using the Scherle method of volume displacement, and the volumes were as shown in 4. The volumes were not significantly different among the experimental groups, p = 0.478 for the right testis and p = 0.158 for the left testis.

DISCUSSION

This study investigated the effect of *Mondia whitei* on the rat testis following oral administration of the herb. The qualitative evaluation of the

Table 2. Volume densities of the components of the testis parenchyma.

Groups	**Mean +/- SD**			
Parameters	**Vv (SE)**	**Vv (SL)**	**Vv (LC)**	**Vv (I)**
A (n = 12)	58.2 +/- 4.1	13.2 +/- 0.8	0.89 +/- 0.1	34.2 +/- 1.5
B (n = 12)	39.0 +/- 2.0	12.5 +/- 1.6	0.66 +/- 0.3	31.5 +/- 2.6
C (n = 12)	35.5 +/- 3.4	10.1 +/- 2.3	0.47 +/- 0.3	29.7 +/- 3.9
D (n = 12)	33.8 +/- 1.5	10.1 +/- 2.1	0.48 +/- 0.1	28.5 +/- 3.9
*P values	0.001	0.013	0.005	0.028
Tukey post hoc				
Group A - B	↓19.2	↓3.02	↓0.43	
Group A - C	↓22.7	↓3.07	↓0.42	↓5.7
Group A - D	↓24.3			
** P values	< 0.001	P = 0.041	P = 0.008	P = 0.025
		P = 0.037	P = 0.010	

Mean +/- SD
*P values, statistically significant differences following a one way ANOVA.
**P value, Tukey post hoc test for the various experimental groups with statistically significant differences.
↓Statistically significant decrease between the various experimental groups following a Tukey post hoc test
Vv = volume density, LC = Leydig cells, SE = seminiferous epithelium, I = interstitium, SL = seminiferous lumen, SD = standard deviation.

rat testis showed noticeable alterations on the tissue sections of the testis at different dosages. The assessment of the quantitative parameters showed a dose-dependent reduction in the volume densities and volumes of the parameters. The changes noted included a reduction in the gross body mass of the rats, a noticeable reduction in the cellularity of the seminiferous tubules, a reduction in the volume densities and volumes of the SE. SL, I and Leydig cells.

Following administration of different dosages of *Mondia whitei,* the body mass of the rats in the experimental groups reduced throughout the duration of the experiment, while for the control group the body mass increased steadily. Body mass may provide some indication of the general health status of animals. Body mass loss in animal studies have been attributed to rejection of food or water by the animals (Thomas et al., 1983; Summers et al., 1990; Uko et al., 2001; Nottidge et al., 2008), reduced food palatability, treatment induced anorexia and systemic toxicity (Abdulazeez et al., 2009). However it is important to note that the rats in this study received the herb via oral gavage.

Concurrent with these observations, Ihongbe et al. (2012) noted a reduction in the body weight of Wistar rats following administration of *Mondia whitei,* although the authors stated that the mechanism for the weight loss was largely unknown. Contrary to this, Watcho et al. (2001) observed an increase in body weights of rats following administration of *Mondia whitei,* although they equally did not highlight the possible mechanisms for the weight gain observed. In a different study, Watcho et al. (2005) observed no increase in body weights of rats despite administration of higher dosages of *Mondia whitei* up to 1000 mg/kg, and with this the possible mechanisms still remained unknown.

Table 3. Mean volumes of the components of the testis parenchyma.

Groups	**Mean +/ - SD**			
Parameters	**Vc (SE)**	**Vc (SL)**	**Vc (I)**	**Vc (LC)**
A (n = 12)	1.101 +/- 0.15	0.25 +/- 0.03	0.65 +/- 0.06	0.02 +/- 0.002
B (n = 12)	0.78 +/ - 0.05	0.25 +/- 0.02	0.63 +/- 0.05	0.013 +/- 0.005
C (n = 12)	0.68 +/ - 0.11	0.19 +/ - 0.05	0.57 +/- 0.09	0.009 +/- 0.005
D (n = 12)	0.64 +/- 0.03	0.19 +/- 0.04	0.53 +/- 0.07	0.009 +/- 0.002
*P values	<0.001	0.023	0.043	0.005
Tukey post hoc				
A – B	↓0.32			↓0.008
A – C	↓0.42			↓0.008
A - D	↓0.46			
**P values	P < 0.001			P <0.009
				P = 0.011

Mean +/- SD
*P values, statistically significant differences following a one way ANOVA.
**P value, Tukey post hoc test for the various experimental groups with statistically significant differences.
↓Statistically significant decrease between the various experimental groups following a Tukey post hoc test
LC = Leydig cells, SE = seminiferous epithelium, I = interstitium, SL= seminiferous lumen, SD = standard deviation.

Table 4. Table showing means and standard deviations of the testis volumes of the right and left testis in the various study groups.

Parameters	**Mean +/- SD**				**P value**
Groups	A (n =12)	B (n =12)	C (n =12)	D (n =12)	
Right testis volume (cm^3)	1.89 +/- 0.20	2.03 +/-0.14	1.83 +/-0.38	1.87 +/- 0.08	0.478
Left testis volume (cm^3)	1.89 +/- 0.14	1.98 +/- 0.14	1.99 +/-0.08	1.89 +/- 0.10	0.158

Aphrodisiacs have been shown to cause an increase in the body weights of animals (Watcho et al., 2004; Mohammad et al., 2009; Prabsattro et al., 2015). Authors have proposed that the weight gain observed could be a result of the androgenic effect of the aphrodisiacs (Gauthaman et al., 2003). Androgens increase lean muscle mass (Forbes et al., 1992) and appetite, which has been proposed to contribute to the weight gain observed in rats (Wilson, 1996). The loss in body weight observed may have been due to a reduced appetite, reduction in androgenic hormones as evidenced by the reduction in the volumes, and volume densities of the Leydig cells or systemic toxicity, use of different species of *Mondia whitei* roots which may have been influenced by the different ecological patterns (Van Wyk, 2015), and lastly due to use of a different methodology.

Consequently, the constituents of *Mondia whitei* and the dose administered cannot be ignored, as there was an observed body mass changes in relation to the dose administered and the duration of administration. Whether or not the toxicity observed might be a cause for concern in humans was not examined here.

Administration of *Mondia whitei* led to noticeable alterations on the seminiferous epithelium with a reduction in the cellularity of the epithelium noted, microvacuolations of the Sertoli cells.

Concordant with the current observations, findings by Salisu, who studied a herb known as alomo bitter, whose major constituent was *Mondia whitei,* showed disorganization of testicular tissue germinal cells (Salisu et al., 2012). Another study showed that extracts from *Mondia whitei* had inhibitory effects on spermatogenesis and reduced fertility (Watcho et al., 2001). Other authors also showed cellular changes commensurate (corresponding) with cardiotoxicity (Okon et al., 2012) and neurotoxicity (Dikibo et al., 2012) in rats following administration of *Mondia whitei.* Okon et al. (2012) postulated that the changes observed in the heart may have been due to free-radical-induced myocardial injury, while Dikibo et al. attributed the cellular necrosis observed to changes in the intercellular physiological conditions. In a study done by Choumessi et al. (2012), *Mondia whitei* depicted cytotoxic effects on breast cells, and he proposed that this toxicity involved caspase 3-like signalling, which suggested apoptosis. He stated that *Mondia whitei* resulted in nuclei showing condensed chromatin; thus he concluded by noting that caution should be observed when using high doses of the herb (Choumessi et al., 2012).

Microvacuolation of the basal Sertoli cells was seen, and this is the most common morphological response of Sertoli cells to injury (Creasy, 2001). This is especially so, as Sertoli cells are extremely resistant to cell death. Injury to these cells has consequences, because of its pivotal role in supporting spermatogenesis. Sertoli cells are important in maintaining the internal milieu of the testis. These cells secrete important transport substances such as metal ions, important proteases and protease inhibitors, which play a role in tissue remodelling that takes place during spermiation and secretion of regulatory glycoproteins (Griswold, 1998). Consequently, germ cell degeneration, disorganization or exfoliation are generally seen as subsequent to the injury to Sertoli cells.

The characteristic of germ cell toxicity is rapid apoptosis and phagocytosis of the affected cells by the Sertoli cell, leaving a tubule depleted of a generation of germ cells (Creasy, 2001; Creasy and Chapin, 2015). This could also be the reason for the observed loss of the spermatogenic cells affecting the round spermatids and the elongated spermatids.

The major function of Leydig cells is steroidogenesis, and any substance that interferes with this pathway will produce functional disturbances in hormone balance. Intratesticular concentration of testosterones are usually maintained at higher levels than circulating plasma levels. Sertoli cell function and germ cell development during stage VII and VIII of the spermatogenic cycle are dependent on adequate levels of testosterone. If testosterone levels are reduced, cells in this stage will show increased levels of apoptosis and degeneration. This could be another reason for the observed loss of cells of the spermatogenic series.

Volumes of the testes in the control group increased gradually over the 4-week duration. This can be attributed to normal increase in volume with growth. The volume of the testes in experimental groups that received 300 mg/kg, 600 mg/kg and 1200 mg/kg reduced over the 4-week duration. Contrary to this finding, Ihongbe et al. (2012) observed an increase in testicular weight of the rats, and attributed this increase to increased secretory activity of the testis. Watcho et al. (2001) equally observed an increase in testicular weight, and also attributed this increase to increased levels of testosterone.

Seminiferous tubules make up to 90% of the wet weight of the normal testis; testicular weight loss observed in the different groups may be attributed to the spermatogenic disruption and degeneration of the germinal epithelium (Mishra and Singh, 2008). Sperm production is highly correlated with testicular weight. Alterations in the testis weight may suggest that sperm production and fertility were affected (Monteiro et al., 2012). To the best of our knowledge, there are no published reports that assessed volumetric densities of the testis parenchyma following use of *Mondia whitei.* Nonetheless, stereological measurements of the testis parenchyma have been shown to be reproducible and reliable (Wing and Christensen, 1982; Liu et al., 2009).

Stereology has been shown to be an accurate and reproducible method for morphometric analysis of tissues (Brown, 2017; Rahimi et al., 2021).

There was a reduction in the volume densities and volumes of the components of the testicular parenchyma following administration of *Mondia whitei,* which corresponded with the dosages that were administered.

In interpreting the findings of this study, some limitations need to be considered: we were unable to determine whether the reduction in the number of cells of the seminiferous tubules were due to apoptosis or atrophy or both. Seven um thick sections were used in studying the histology of the testis. Being a highly cellular tissue, this predisposes to tissue overlap and may contribute to underrepresentation of the changes observed.

CONCLUSION

The findings in the current study demonstrated changes in the seminiferous epithelium, which highlight possible effects of *Mondia whitei* on the testes.

There was a decrease in the volume densities and volumes of the components of the testicular parenchyma and the body mass of the rats. These histological changes, in addition to the loss of body mass, may justify the need for a cautious approach when using *Mondia whitei* extracts for medicinal use.

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