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In Vivo Effects of Mondia Whitei 'Mukombero' on Testicular Tissue Changes in Male Wister Rats

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Abstract**—** *In Africa, Mondia Whitei is popularly used medicinal plant to cure sexual dysfunctioning and there is still little empirical data existing in support of its therapeutic value. The main target of this research was to discuss how aqueous extract of Mondia Whitei affects testicular tissue when graded dose is administered orally brings changes in male Wister rats. Thirty-six male rats, albino weighing between 200mg -400mg were put into four groups consisting of nine rats each. There was no treatment of the rats in the first group unlike the second, third and the fourth groups, weighing 100,200 and 400mg respectively were given the aqueous extract of Mondia Whitei orally. The testes and epididymis were the examined after sacrificing the rats humanely using carbon dioxide narcosis. The testes and epididymis were studied. The gonadal tissue from the treated groups exhibited low sertoli cells in the seminiferous tubules and a thin germinal epithelium. There was degeneration of leydig cells within the interstitium and clamping of spermatozoa in the epididymis in relation to increase in extract concentration and duration. This study concludes that Mondia Whitei may be beneficial only at low dose and short duration but may alter male fertility if taken over a long time and also high doses. Therefore, Mondia Whitei can even be toxic to cells.*

I. INTRODUCTION

Infertility is an imperative component of reproductive health, and has often been omitted in many reproductive discourses (Cui, W. 2010). The incapability to have children impacts men and women throughout the globe. Infertility can lead to misery and depression, as well as discrimination and ostracism (Chachamovich*et. al* 2010). According to Jørgensen et al. (2001) For an embryo to exist during early stages, ability of human male spermatozoa to fertilize the egg is very important. Furthermore, in many animals, it is founded to be low, and in most countries, infertility rate is becoming rampant, majorly affecting one in six couples. (Sharpe et al., 2003; Kamel, 2010).

Sometimes, some men have got enough sperms with normal morphology and motility are living but still suffer from low fertility due to many reasons and inability to understand these causes poses a challenge in forming effective diagnosis through rational approach. (Wu *et al.,* 1989). According to World Health Organization [WHO, 2010], 30 million of men are affected by infertility globally. (Agarwal *et al.* 2014). Several couples are therefore looking for treatment so that they can find solution to this issue (Ikechebelu et al., 2003). Eighty percent of Africans are depending on conventional doctors and flowers that cure their illness as medical fact indicates (Johnson et al., 2007; McKay and Blumberg, 2007).There is minimal pain and suffering and vast change in medical practices by inevitable merchandize and in regard of this, new medicine are of natural products or associated with them (Demain, 1999). Because of the existence of antioxidants, researchers on conventional herbs have clearly suggested that their ability to improve male fertility is minimal. Observation of those antioxidants are being observed in order to enhance many methods of male reproductive characteristics (Nantia et al., 2009). In recognition of this, the treatment of idiopathic infertility is determined by the aggregate of plant formulations (Agrawal and Kulkarni, 2003; Devi et al., 2004; Tempest et al., 2005; Xu et al., 2003).

The genus Mondia is a perennial plant from the Apocynaceae family that grows from a large tuberous root stock and is characterized by woody, robust and vigorous aroma. Also, this plant is characterized by a large heart-shaped opposite leaves that produces reddish, purple flowers that are borne from branched inflorescences (Aremuet al., 2011). A compound of 2-hydroxy-4-methoxybenzaldehyde which is a potent tyrosinase inhibitor and an isomer of vanillin is the most common and well-known compound that is isolated from *M. whitei*is (Kubo and Kinst-Hori, 1999b). Other researchers (Oketch-Rabah, 2012) have also isolated this compound from *M. whitei*. Koorbanally*et al.* (2000) was able to isolate isovanillin. Therefore, nutritional analysis has suggested that Mondia contain many minerals and vitamins (Iwu, 2014). On the other hand, the ethanoic extract of *M. whitei* has been found through qualitative phytochemical analysis to contain the presence of triterpenes and reducing sugars (Quasie e*t al.*, 2010).

However, there is inadequate research that has been done on the impacts of *Mondia Whitei* on the testicular tissues to support it's widely use as a fertility drug. It is in this light that this study investigated the effects of *Mondia Whitei* on testicular tissue changes using male Wister rats.

II. MATERIALS AND METHODS

The study adopted a laboratory experimental design that was carried out at University of Eldoret.

The Sample Size determination for one-way ANOVA Design was employed as follows (Arifin W N et al. 2017)
n= (DF/K) + 1, Where DF= the within-subject degrees of freedom (minimum-10, K= number f groups (4), and n= number of subjects per group.
On substitution
n= (10/4) + 1 = 3 rats per group

There were 4 groups that received 100mg/kg b w t, 200mg/kg, and 400mg/kg of the aqueous extract and control group that received water only and they were sacrificed at three time points of (10^{th}, 15^{th} and 30^{th} day). Total number of animals used is 9x4=36

Normal feed was given to the rats with portable water and ad libitium during the experiment. *M. whitei was* procured from Kakamega forest with the assistance of a local botanist and transported as freshly packed roots in foil papers to maintain its moisture content and viability of the chemical composition. The specimen voucher no. CM/11/8/18/001 was deposited for identification and verification of the plant using taxonomic key at the natural herbarium of University of Eldoret .Then roots were washed, air dried (shade) for a period of 30 days, sliced into smaller pieces and ground using a laboratory mill into a fine uniform powder. Thereafter 200 g of the powdered roots was dissolved in 1.3 L of distilled water, then in 250 ml of 70% ethanol and kept for 72 h at 4° C, and occasionally stirred. Filtration of the extract was done by use of Whatman No.1 filter paper (model number 1001,150 mm) to get fine extract. It was repeated twice to ensure finer extract. Then complete evaporation was done using a rotavac control evaporator (Heidoph, Germany) at 65,100 r.p.m& 240 pascal pressure, for 30min to give 150 g of brown residue (Plate 3.2b). The aqueous extract used was prepared by dissolving 1 g of the brown residue in 10 mL of distilled water and was refrigerated for the entire research period (Gundidza et al 2009). The doses used in our study were a range of 100 mg/kg b.w (0.1ml), 200mg/kg (0.2ml) and 400 mg/kg b.w (0.4ml) of the ethanoic extract.

There were four groupings of the thirty-six male albino rats where each group contain 9 rats. For 30 days, Group I (control) was fed with normal rat feed and water *ad libitum*. On the other hand, Test groups II, III, and IV was treated with 100mg, 200mg and 400mg per kilogram per day of the extract respectively in addition to normal rat feed and water *ad libitum* for 10 days, 15 days and 30 days respectively as per the test group. The extract was administered orally and daily using syringes without needles between the hours of 8.00am and 9.00 am.

Histological preparations of the epididymis and the testes

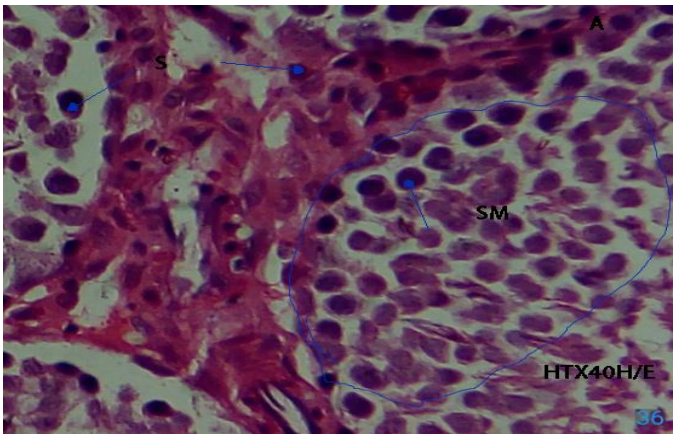
The preparation of tissues for histological studies was done at Moi University School of Medicine in the Anatomy/Histology lab as follows. It involved eight main stages: Preparation of tissues for histological studies involved Fixation, Dehydration, Clearing, and Impregnation with paraffin wax, Sectioning, Staining and Mounting. Tissues were fixed by immersing in 10% Formalin for 24 hours. This was done to preserve the tissues and prevent autolysis. After fixation tissues were treated with increasing strengths of alcohol from 70%, 80%, 90% and two changes of absolute alcohol to dehydrate them. The dehydrated tissues were then treated with two changes of xylene for two hours in each, in order to remove alcohol and prepare them for infiltration with molten paraffin wax at 60^0C. Infiltration with molten paraffin wax was done by immersing tissues in molten paraffin wax for two hours, to increase the consistency of the tissues and to facilitate sectioning of thin slices for microscopy. The tissues were fitted into suitable cassettes which were attached to a rotary microtome and one cell thick sections made (approximately 3-5µm thick). The tissue sections were attached on to glass slides and incubated in an oven for one hour at 65-70^{0}C to remove all the paraffin wax. The sections were then stained with Haematoxylin for twenty minutes and counterstained with Eosin for three to five minutes. The stained slides were mounted using DPX and studied under the microscope. Tissues were prepared for histological observation as previously described by (Cardiff *et al.*, 2014).

The sections were examined at low magnification (x400) and at high magnification (x1000) using an Olympus microscope (Japan) fitted with a Kodak camera. Several photomicrographs were taken in bright field.

Ethical clearance was sought from the University of Eastern Africa Baraton Animal Ethical Committee (UEAB/10/11/2018) and National Commission for Science, Technology and Innovation (NACOSTI/P/19/81106/27253).

III. RESULTS

The Changes in the male gonadal histology following treatments with aqueous extracts of *mondia whitei* are shown in plate's 4. Sections of testes from the control rats (A), demonstrated seminiferous tubules of normal sizes with clear outlines. Spermatogonia were also clearly evident in them (arrow). Those from treated rats (Plate B); seminiferous tubules that had thin distorted outlines and many intercellular spaces within them. Although spermatogonia were observed in both treated and untreated sections, the latter clearly showed few spermatozoa that were even clamped together and an arrest in further development as evidenced by a reduction in the number of cell layers and distortion in the cells.



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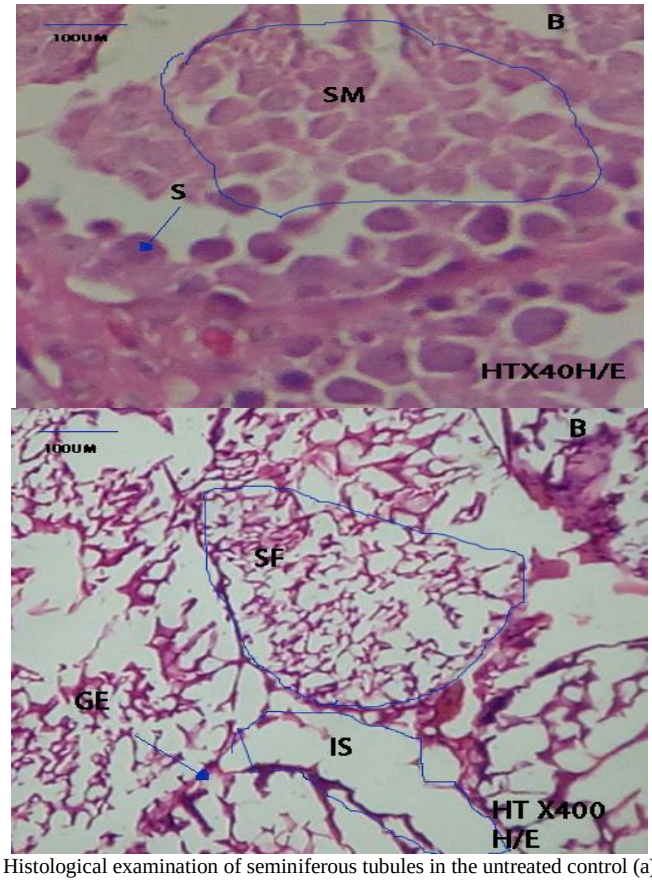


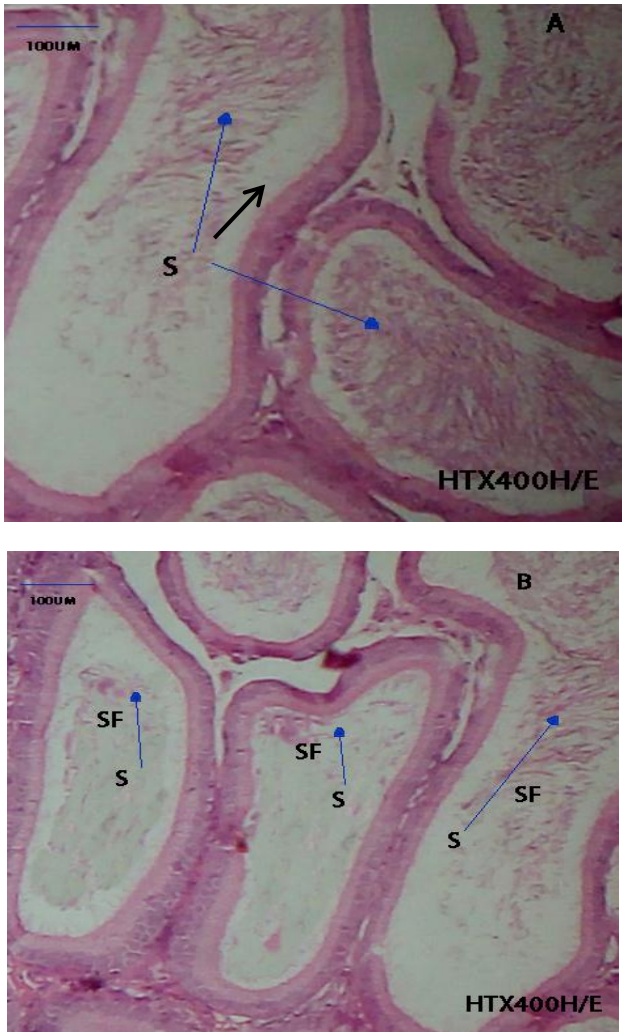
Histological examination of seminiferous tubules in the untreated control (a) and treated (b). Note regularly shaped seminiferous tubules with clear spermatogonal layer, few intercellular spaces and narrow lumens with spermatozoa, (arrow) in control (a). Enlarged seminiferous tubules with large lumens having scanty spermatozoa and many intercellular spaces, (arrow) are visible in the treated (b).GE-germinal epithelium, IS-intersitial space,SM-spermatogenesis,

Leydig cells of controls showed normal sizes typical of normal male rats while those of treated rats, were enlarged, ruptured and contained prominent lipid droplets and with many intercellular spaces. Spermatogenesis in adjoining seminiferous tubules showed arrested development. This was evident in the number of cell layers characteristic of normal spermatogenesis, in that instead of the normal four to five cell thickness, these showed one to two cell thicknesses. Some of the leydig cells appeared to be clumping.

Epididymis of testes from control rats showed clear normal outlines(black arrow) and their lumens were filled with sperm (blue arrow), (plate A) while those of testes from treated rats, showed large empty cells (blue arrows) with thin broken outlines, and detachment from adjoining cells (blue arrows) (B).

IV. Discussion and Conclusion

In this study, the seminiferous tubules had distorted outlines and few spermatogonial cells in treated male rats, which suggest diminished serum levels of Follicle Stimulating Hormone and Interstitial Cell Stimulating Hormone (ICSH). Luteinizing hormone, also known as ICSH in males, is responsible for initiation of spermatogenesis by causing growth and enlargement of spermatogonia.



Histological examination of cross sections of epididymis in the untreated control (a) and in the treated rats (b) (H and E x400). SF (seminiferous), S (spermatozoa)
Note clear outlines of epididymis cells (black arrows), and lumens filled with spermatozoa(blue arrow) in the control (a) and enlarged cells with distorted outline(blue arrows) and large empty lumens in the treated (blue arrows) (b).

It also stimulates Leydig cells to produce Testosterone which together with FSH cause maturation of sperm. Degeneration of seminiferous tubules meant that sertoli cells responsible for spermatogenesis, were being affected. The many intercellular spaces observed in seminiferous tubules pointed to the destruction of Sertoli cells. These cells produce oestrogen, Androgen Binding Protein (ABP) after priming with FSH and also provide nutrition for developing sperm. Mature sperm are transported out of the tubules by Androgen Binding Protein. Degenerated tubule outlines also meant that the germinal epithelium, that gives rise to spermatogonia, was not properly maintained, probably due to lack of FSH. Sections from control rats showed normal histological picture of tubules with prominent spermatogonia and sperm in various stages of development. Absence of large intercellular spaces in the controls suggested normal Sertoli cells. The tubules were also filled with spermatozoa, indicating normal process of spermatogenesis.

Accumulation of lipid droplets within the interstitial spaces suggested that leydig cells had also been affected. There was detachment of cells from neighbouring ones, hence a lot of vacoulation. Neaves (2014) noticed that leydig cells of non-breeding rock hyraxes were crowded with lipid droplets and a reduction in serum testosterone levels. Administration of exogenous testosterone resulted in the disappearance of the droplets and a marked increase in the levels of serum testosterone. This may have been the case in this investigation. The presence of lipid droplets suggested inhibition of testosterone production by the cells. Lipids are precursors for steroidogenesis and impairment of the process causes them to accumulate in interstitial cells. Their presence in the cells therefore meant that androgens, including testosterone were not being synthesized, leading to low levels of both testosterone and gonadotrophs. Those from control rats showed normal morphology and lacked fat deposits and vacuolation. There was significant reduction of the leydig cell nuclear area and mature Leydig cell numbers during oral administration of aqueous extract of *Mondia whitei* to male rats at the dose level of 400 mg/kg body weight per rat per day for 30 days. These findings are smilar with those of (Okon *et al.,* 2012) on degenerative changes of the heart muscles following oral administration of *Mondia whitei*.

Sections from epididymis demonstrated greatly distended cells, broken outlines and scantly filled lumens. This demonstrates low level of sperm concentration in the treated animals. The extract shows possible anti-androgenic activity as this was observed in sections of epididymis from treated rats. The extract may thus be inhibiting Follicle Stimulating Hormone, (FSH) which is responsible for the development and maturation of spermatozoa. By extension, the extract may very well be interfering with the Hypothalamic-pituitary and gonadal axis through inhibiting the tropic hormone Follicle Stimulating Hormone Releasing Hormone (FSHRH) from the Hypothalamus. This hormone causes the release of FSH that in turn maintains proper functioning of testes. *Mondia whitei* (mukombero) had negative effects on the testes, may be through suppression of Testosterone, or the tropic hormone Interstitial Cell Stimulating Hormone (ICSH), responsible for its production. The hormone is responsible for maturation of sperm and its absence, as evidenced by degeneration of interstitial cells which produce it, has a drastic effect on maturation on the process. In normal spermatogenesis mature sperm are transported out of seminiferous and temporally stored in the epididymis. The low levels of spermatozoa and clamping of the spermatozoa, indicates that spermatogenesis had been greatly interfered with by the extract resulting in possible anti-fertility changes.

According to Ogbuewu *et al.* (2011) various screened plants have been depicted to boost reproductive functions of males, however, they may also lead to deterring of testicular functions. Cruz *et al.,* (2010), Kamal *et al.,* (2003) and Ogbuewu *et al.*, (2011) in their studies examined several plants with known effects of anti-fertility effects which includes: Embeliaribes berries which showed activity of spermicidal by inhibition of sperm count and enzymes activity of energy metabolism and alteration of testicular histology (Agarwal *et al.,* 1986; Purandare *et al.,* 1979) and Azadirachtaindica (neem) affecting testis structure and function since the seminiferous tubules are damaged hence leading to germinal epithelium loosening, germ cells degeneration and germ cell types derangement (Choudhary *et al.,* 1990; D'Cruz *et al.,* 2010; Joshi *et al.,* 1996; Shaikh *et al.,* 1993).

In addition, the seeds of Crude ripe paw paw (Carica papaya) leads to the germinal epithelium and germ cells being degenerated (Udoh and Kehinde, 1999); Abrusprecatorious causes degeneration of testicular tissue which is characterized by reduction of the number of cells in the epithelium along with reduced number of sperm cells (Adedapo *et al.,* 2007); Ocimum sanctum demonstrated detrimental effects on the ultra-structure of the testis (Lohiya *et al.,* 2008; Manivannan et al., 2009); The spesiapopulnea cause enlargement of the Sertoli cells and reduction of germ cell attachment (Krishnamoorthy and Vaithinathan, 2003). In this study, degeneration of the cells was observed in higher doses indicating that the substance could have had necrotic changes in the cytoarchitecture of the testis as have been demonstrated earlier with brain tissues (Dikibo *et al.,* 2012). This study is in agreement with (Dikibo *et al.,* 2012) who found that *Mondia whitei* had cellular pyknosis, necrosis, degenerative vacoulations, infarctions and parenchymal erosion in the brain tissue following administration of *Mondia Whitei*. In another study by (Okon *et al*., 2012), revealed that *Mondia Whitei* caused severe fibrillolytic changes, myocardial necrosis, inflammatory cell infiltration and Oedema in a dosage dependent manner on heart tissues.

The mechanism of actions may not be clearly understood but known mechanism like lipid peroxidation (Myers *et al.*, 1977), damaging of mitochondria (Bier and Jaenke, 1976), and generation of reactive oxygen species like super oxide anion and nitrogen peroxide have been reported to impair cell functions (Daoud, 1992). It is also imperative to note that testosterone hormone and follicle stimulating hormone have inhibitory effects on programmed cell deaths, therefore in the current study, decrease in testosterone and FSH could have led to decrease in mitochondrial capacity for oxidative phosphorylation while simultaneously increasing reactive oxygen species production (Olanlokun *et al.*, 2018).

The study demonstrated histopathological changes in testes due to effect of *M. whitei* in treated rats including a decrease in leydig cells. Therefore, the study suggests that in addition to *M. whitei* negatively affecting fertility in male rats, it may also cause certain toxicities if used at high dose for a long period of time like hypogonadotrophic hypogonadism.

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