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Effect of *Mondia whitei* (mukombero) on Testosterone Levels, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) in Male Albino Rats

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Abstract **Original Research Article**

This paper draws from the findings of a study conducted to establish the effect of *Mondia whitei* on the production of testosterone levels, Luteinizing Hormone (LH), and follicle stimulating hormone (FSH) in male albino rats. The target population comprised 32 albino male rats weighing between 200mg-300mg bought from University of Nairobi's Chiromo Campus and housed at University of Eldoret's animal house. '*Mondia whitei'* was obtained from Kakamega town market and ground into powder using a laboratory mill. The rats were grouped into 4 categories, each of which consisted of 8 rats with Group I as the control and Groups II, III, and IV as test groups. The *Mondia whitei* extract was administered orally and blood sample collected through cardiac puncture after anaesthetizing the rats. Thereafter, the animals were sacrificed and their testes, epididymis, seminal vesicles and ventral prostrate dissected. Serum hormones was determined using ELECSYS (Cobas, USA). By use of SPSS Version 21.0, the analyzed data produced results showing insignificant differences ($p>0.05$) in serum concentrations after 10, 15 and 30 days in rats treated with *M. whitei* extract and negative control rats. After 10 days of treatment, highest serum testosterone of 6.03ng/ml was recorded in *M. whitei* treated rats with 100 mg/kg of the extract. Serum testosterone levels after 30 days of treatment were highest in negative control (4.67 ng/ml); nevertheless, this was insignificantly from those of rats treated with 200mg/kg (1.68ng/ml), 400mg/kg (2.64 ng/ml) and 100mg/kg (0.97ng/ml) of the extract.

Keywords: *Mondia whitei*, Albinism, Male Rats, Luteinizing Hormone, Follicle Stimulating Hormone, Animal House, Motility, Testosterone.

INTRODUCTION

The genus *Mondia* of the Apocynaceae family is a woody, robust and vigorous aromatic perennial plant that grows from a large tuberous root stock. It has large heart-shaped opposite leaves and produces reddish, purple flowers borne in branched inflorescences [1]. It is distributed in the sub Saharan Africa and flowers in Southern part of Africa from October to March, and from May to August in the Northern part of Africa [1]. For easy harvesting, the roots spread laterally beneath the soil surface. They have a ginger-like taste and a vanilla aroma. In West Africa, it is found in Nigeria, Guinea, and Ghana; Cameroon in Central Africa; and in East Africa, it is found in Kenya, Uganda and Tanzania [1].

Many indigenous plants have been reported to be effective in male fertility regulation [2, 3]. Phytochemical studies in *Mondia whitei* have revealed the presence of glucosides, alkaloids and 2-hydroxy-4-methoxybenzaldehyde [4]. Toxicity studies in mice showed a LD50 of 11.9 g/kg for the aqueous extract, thus indicating a very low toxic level [5]. Studies have indicated that at least 15% of couples around the globe, quantified by 48.5 million couples, are infertile. Of these, it has been established that males contribute to infertility cases ranging from 20-30% and an overall of 50% [6]. The World Health Organization [7] defines infertility as a reproductive system disease explained by the failure to conceive or achieve a clinical pregnancy 12 months or more after regular unprotected sexual intercourse. According to the World Health Organization [7], male factor contributes 40%, female factor 40%, both male and female 15% and unknown factors 5% of cases of infertility.

While there has been skepticism on the biological effects of plant extracts, advances in scientific research have started to demonstrate the efficacy of these extracts using animal models to elucidate mechanism of action on fertility. However, these studies have been inconclusive. *M. whitei* contain

antioxidants that have been recognized as having the potential to reduce disease risk that can scavenge free radical. Although studies have been done on the effect of various plant extracts on spermatogenesis, few studies exist on *M. whitei*. This paper therefore attempts to fill the existing gap by expanding knowledge on the effect of *M. white* extracts on testosterone levels.

Literature Underpinning

Testosterone and Other Reproductive Hormones

Cholesterol is the major substrate responsible for the anabolic effect of testosterone in males [8]. As observed by Vijaykumar *et al*. [9], a significant decrease in the intra-testicular concentration of cholesterol was observed in rats treated for 30 days with the hexane suspension of *Mondia whitei* suggesting its conversion into androgens (mainly testosterone) depending on the availability of Luteinizing Hormo (LH)). At high dose of about 1000 mg/kg b.w, a significant increase in the relative weights of the caput epididymis ($p<0.001$), ventral prostate ($p<0.001$) and seminal vesicles ($p<0.001$) associated with the increase in their total protein contents was observed and could then support the increase in androgen levels.

Assessment of sexual behavior in male rats encompasses components of arousal, erection and ejaculation. In the presence of a receptive female, male rats undergo a predictive series of mounts and intromissions culminating in ejaculation. Male rat mating behavior involves interaction of olfactory stimulation by female pheromones that augment actions of androgens centrally with possible stimulation of prosexual central neurotransmitters such as dopamine [10, 11]. Normal function of the testes and reproductive functions are regulated by the interaction between highly complex endocrine hypothalamus-pituitary-gonadal axes. The onset of puberty in the human male is strongly characterized by the production of testosterone, which is influenced upon stimulation of Luteinizing Hormone (LH) and Leydig cells [12, 13]. Endocrine regulation of spermatogenesis is then maintained through the relationship between gonado-trophins, steroid and testicular somatic cells (Sertoli and Leydig cells).

Production of Testosterone

The onset of puberty in the human male is strongly characterized by the production of testosterone, which is influenced upon stimulation of Luteinising Hormone (LH) and Leydig cells [12, 13]. The interstitial connective tissue contains Leydig cells that function in-producing and releasing testosterone [12] while foetal Leydig cells differentiate and begin to produce testosterone [14]. This differentiation takes place in the interstitial space between the seminiferous cords [15]. Testosterone produced within the Leydig cells in the developing testis act to stimulate the Sertoli cells proliferation [14].

Besides, the number of sperm cells produced in adulthood is determined by the number of Sertoli cells within the testis [14]. According to Plant and Marshall [16], the importance of Sertoli cell proliferation and factors that regulate this process during the different periods of development may differ: for instance, during foetal and neonatal period; testosterone seems to play an important role. The amount of testosterone produced in male is thought to be approximately directly proportional to LH [15]. Both FSH and testosterone contribute to the final maturation of spermatozoa, and protect the germ cell line against apoptosis [18]. In early postnatal testis development, an increase of inhibin B levels is seen leading to down-regulation of the hypothalamic-pituitary-gonado axis which results in the changes in testosterone, LH and FSH levels [12].

Functioning of Testosterone

Testosterone is the principle male steroid hormone from the androgen group [19]. In the male, testosterone is mainly secreted in the testes by interstitial Leydig cells. Small amounts are also secreted by the adrenal glands [20]. The major function of testosterone in the testes is the maintenance of spermatogenesis via stimulation of Sertoli cells; its actions are mediated through intracellular androgen receptor which acts as a transcription factor [19]. Testosterone produced by Leydig cells passes into the Sertoli cells and binds to receptors. The combination of testosterone with the receptors is required for the Sertoli cells to function normally. In addition, testosterone is converted to two other steroids in the Sertoli cells: estrogen and dihydrotestosterone [18].

The Sertoli cells also secrete a protein called androgen-binding protein into the seminiferous tubules [20]. Testosterone and dihydrotestosterone bind to androgen-binding protein and are carried along with other secretions of the seminiferous tubules to the epididymis. Estradiol and dihydrotestosterone are active hormones that promote sperm cell formation [18]. According to Rizzo [20], other functions of testosterone include: promotion of growth, differentiation, and function of accessory organs of reproduction; maintenance of normal reproductive function in the adult, stimulation of transport and delivery of sperm and increase of sexual drive (libido) in both men and women.

Derangements of the hypothalamic–pituitary–gonadal (HPG) axis are associated with abnormalities of spermatogenesis and sexual function. Men with azoospermia secondary to testicular failure often present with small, soft testes measuring less than 10 ml in volume with small, flat epididymis. In primary testicular failure, decreased testosterone production diminishes negative feedback inhibition and gonadotropin production is stimulated - hypergonadotropic hypogonadism [21]. These men

often have excessive conversion of testosterone to estradiol by the aromatase enzyme and respond to aromatase inhibitor therapy with normalization of testosterone levels and improved spermatogenesis. Men with azoospermia secondary to obstruction have normal testosterone production and the hormone profile reflects the normal state. Although a significantly elevated FSH is consistent with spermatogenic failure, not all men with abnormal spermatogenesis have elevated FSH levels. In a study by Schoor *et al*. [22], 96% of men with obstructive azoospermia had FSH levels less than or equal to 7.6 mIU/ml or testicular long axis greater than 4.6 cm, whereas 89% of men with nonobstructive azoospermia had FSH levels greater than 7.6 mIU/ml or testicular long axis less than or equal to 4.6 cm [22].

Low testosterone, low FSH, and low LH are typical findings in Hypogonadotropic Hypogonadism (HH) wherein the pituitary does not produce adequate levels of gonadotropins to maintain adequate testosterone production. HH may be congenital or acquired. Kallmann's syndrome, a congenital disorder of the hypothalamus with failure of GnRH secretion, is HH associated with midline abnormalities such as anosmia and less commonly cleft palate and unilateral renal agenesis. Congenital HH may be associated with cryptorchidism and micropenis [21].

Mondia whitei and its Impact on Testosterone Levels, LH, Estrogen and FSH

According to Watcho *et al.* [5], administration of *Mondia whitei* over a period of 55 days caused an increase in serum and intratesticular testosterone levels after treatment, suggesting an androgenic effect of the *Mondia whitei* aqueous extract. Kamtchouing *et al.* [5] also observed an increase in testosterone concentration by Zingiber officinale, Pentadiplandra brazzeana Hibiscus macranthus and Basellaalba treatment in rats. However, in another study by Watcho *et al.* [23], a treatment of rats for over 55 days with the same dose of *Mondia whitei* yielded no change in testosterone concentration. It could then be assumed that as the duration of the treatment is prolonged, the sensitivity of the steroidogenic mechanism to the bioactive molecules present in the plant extract may be decreased [5]. The observed increase in testicular protein content and weight may be the result of testosterone action. An androgenic effect of the extract is also suggested by the increased sperm density in cauda epididymis of treated rats [24]. An increase in the testicular weight without accompanying changes in the weights of the secondary sex organs may signify a selective effect of *Mondia whitei* [25]. The main finding of this study suggests that the aqueous extract of the dried roots of *Mondia whitei* possesses sex-stimulant property.

METHODOLOGY

The study adopted a longitudinal experimental design and it was conducted at the animal laboratory of University of Eldoret, anatomy/histology laboratory of Moi University and Immunology laboratory of Moi Teaching and Referral hospital. Thirty-two male albino rats weighing between 200-300g and of age six to seven weeks were bought from University of Nairobi's Chiromo Campus. This is the age at which rats are able to produce viable sperms. The animals were maintained at room temperature (22-23 °C), with a reverse natural light-dark cycle in the animal house of University of Eldoret and used for the research that lasted for 60 days (Plate 1). University of Eldoret has a well-equipped animal laboratory. This enabled accurate and real time data collection. Thirty days were also adequate for absorption of *Mondia whitei* phytochemicals. The rats were housed in a conducive environment, allowed three weeks to acclimatize and their health status closely monitored before and during the experiment. They were fed with normal rat feed and portable water *ad libitum*.

Plate-1: Experimental Animals

M. whitei was purchased from Kakamega town and transported in freshly packed roots (Plate 2a) to maintain its moisture content and enable keep proper viability of the chemical composition. Identification and verification of the plant using taxonomic key in the natural herbarium of UoE was done to establish that it is *Mondia whitei*. Then 'Mukombero' roots were sliced into pieces, dried under ambient temperature (shade) for a period of 30 days and grinded using Laboratory Mill. Then 200g 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 was done by use of Whatman No.1 filter paper (model number 1001, 150mm) to get fine particles. It was done

twice to ensure fine particles. 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 2b). The aqueous extract used was prepared by dissolving 1 g of the brown residue in 10 mL of distilled water. The doses used in this study arranged between 100 mg/kg b.w and 400 mg/kg b.w.

Plate-2: Roots of *Mondia whitei* (a) and Rotavac Control Evaporator (b)

The thirty-two male albino rats were grouped into four of 8 rats each. Group I (control) was fed with normal rat feed and water *ad libitum* for 30 days. Test groups II, III, and IV were 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.

Blood samples were collected after 10, 15 and 30 days by cardiac puncture after anaesthetizing the rats with carbon dioxide. Then 2ml of blood was collected from the rats in each group including the control group. The samples were carefully introduced into plain vacuum containers free from anticoagulant and properly labeled. The blood samples were allowed to clot, retract and then centrifuged for 5minutes at a speed of 3000 revolutions per minute. The serum was then aliquoted and refrigerated at -20°C. The hormonal assay was done at Moi Teaching and Referral Hospital in the immunology laboratory.

The animals in all the four Groups were then sacrificed and the testes, epididymis, seminal vesicle and ventral prostate was dissected, cleared of the fat and connective tissue and weighed. Sperm samples were collected from the distal region of the right cauda of the epididymis by diffusion method as described by Klinfelter *et al.* [26]. The epididymis was clamped at the corpus-cauda junction by a hemostat angled to clamp the vas deferens as well and severed to isolate the cauda and the cut edge blotted dry. The hemostat was then released and the cauda placed in a petridish containing 2ml of medium. The petri dish was then gently swirled and further diluted.

The serum and testicular testosterone, Luteinizing Hormone (LH) and follicle stimulating hormone (FSH) was measured using Elecsys (Cobas, Indianapolis-USA) according to the procedure provided in the manual. The within assay variation was 3% and the sensitivity was 13.5 pg/tube. The results obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 for Windows. Two-way Analysis of Variance (ANOVA) was used for comparison between different treatments and duration. Means that were statistically significant were separated using Tukey's test. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

The study sought to establish the effect of *Moundia Whitei* on the production of testosterone luteinizing hormone (LH) and follicle stimulating hormone (FSH) among male rats with albinism. Results in Table 1 indicate insignificant differences ($P>0.05$) in serum testosterone concentrations after 10, 15 and 30 days in rats treated with *M. whitei* extract and negative control rats. In addition, extract by time interaction recorded an insignificant effect on the concentration of serum testosterone levels of rats.

Table-1: Effect of *M. whitei* Aqueous Extracts (100,200 and 400 mg/kg) for 10, 15 and 30 Days on Serum Testosterone Concentration in Rats

Treatment	Testosterone concentration ng/ml Mean±SD		
Rat Groups	10 days	15 days	30 days
100 (mg/kg)	6.03±0.01b	1.06±0.53a	0.97±0.01a
200 (mg/kg)	1.53±0.71a	3.22±1.48a	1.68±1.17a

400 (mg/kg)	1.64±2.64a	1.82±2.43a	2.64±1.29a
Negative Control	-	-	4.67±3.65a
Source of variation		F-Value	P-Value
Extract (E)		1.281	0.313NS
Duration(D)		0.501	0.615NS
E×D		0.861	0.479NS

Means followed by same letters within a column are insignificantly different at p<0.05, NS denotes not significant.

After 10 days of treatment, serum testosterone in *M. whitei* treated rats with 100, 200 and 300 mg/kg were 6.03, 1.53 and 1.64 ng/ml, respectively. Highest serum testosterone level of 3.22ng/ml was recorded in rats treated with 200mg/kg of the extract after 15 days of treatment. However, this was not significantly different from serum levels of rats treated with 100mg/kg (1.06ng/ml) and 400mg/kg (1.82mg/kg). Serum testosterone levels after 30 days of treatment were highest in negative control (4.67ng/ml) nevertheless, this was insignificantly from those of rats treated with 200mg/kg (1.68ng/ml), 400mg/kg (2.64ng/ml) and 100mg/kg (0.97ng/ml) of the extract. It is worth noting that the concentrations of both Luteinizing hormone (LH), and follicle stimulating hormone (FSH) were below the standard (<0.01) as indicated in Table 2.

Table-2: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Serum LH and FSH (ng/ml) Concentration in Rats

	LH (ng/ml)			FSH(ng/ml)		
Treatment	10 days	15 days	30 days	10 days	15 days	30 days
100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
200	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
400	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Control	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Concentration of testosterone, LH and FSH

Spermatogenesis is a complex process, covering production of the spermatogonia, long-lasting process of the tissue meiosis and several alterations in the spermatids in the course of their pre-formation [27]. During germ cell development, gonadotropins and testosterone are the major regulators. Kerr and de Krester [28] have explained that the successful and complete male germ cell development is associated the balanced endocrine interplay of hypothalamus, pituitary and the testis. FSH binds with receptors in the sertoli cells and stimulates spermatogenesis. LH plays a significant role of stimulating the production of testosterone in Leydig cells, which in turn may act on the cells of seminiferous tubules hence triggering spermatogenesis [29]. It has been postulated that the maintenance of testicular atrophy is associated with an elevation of intra testicular testosterone hence; agents reducing the intra testicular testosterone levels triggers the regaining of spermatogenesis [29].

Male fertility in mammals is controlled by the two adeno hypophyseal hormones, Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) through synthesis of testosterone in the interstitial cells of Leydig. The regulated release of the hypothalamic gonadotropin-releasing hormone (GnRH) ensures normal functioning of the hypothalamo-hypophysio-gonadal axis, through secretion of gonadotropins and testosterone in systemic circulation, necessary for spermatogenesis, maturation of spermatozoa, and reproductive behavior [30]. Testosterone is a steroid hormone from the androgen group. In mammals, testosterone is secreted primarily in the testicles of males although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. Testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics in mammals. Testosterone plays a pivotal role in sexual maturation, behavior and maintenance of accessory sex organs [31]. Threshold levels of required testosterone might be different for the different accessory organ functions [32].

Findings from the present study show that the *M. whitei* aqueous extract reported decreased serum levels of testosterone. However, the decrease was not significantly different from control. Similarly, the extract had recorded a significant decrease in the levels of LH and FSH. The decreased level of FSH reveals that *M. whitei* aqueous extract lacks the potential to influence the release of gonadotrophic hormones from the pituitary since FSH by itself is of critical importance in the initiation and expansion of spermatogenesis in mammals, as is generally agreed [15]. The decrease levels on FSH may probably be due to failure to suppress negative feed-back inhibition of anterior Pituitary [33, 34]. This study also demonstrates that *M. whitei* aqueous extract has no effect on the mean serum LH in male rats. This finding is contrary to what other studies have reported [35, 36]. The decreased serum LH and FSH levels observed may probably be due to failure to suppress the negative feed-back inhibition of anterior pituitary [37].

Therefore, the observed decrease in serum testosterone level of rats treated with the *M. white* aqueous extract is associated with a decrease in LH thus suggesting that the etiology is associated with a disorder in the pituitary and also testicular dysfunction, which is indicated when low serum testosterone levels are accompanied by low levels of serum LH. This could explain that the plant extract affected the hypothalamus which produces the Gonadotropin-releasing hormone (GnRH) which acts on the anterior pituitary gland to release LH and FSH. As FSH stimulates the sperm production in the testicles and LH stimulates the production of testosterone by the leydic cells, then the whole reproduction process is impared by the treatment. It is well-known that testosterone production by Leydig cell is primary under the control of LH and stimulation of LH is usually followed by stimulation of testosterone [38]. Leydig cells secrete testosterone by the stimulatory effect of LH [37] in males' reduction of testosterone level may impair spermatogenesis and cause male infertility [34]. Thus the extract shows the inhibitory activity on the proliferation of spermatogonia.

Findings from the present study are similar to the earlier findings [39, 27, 33, 34]. Nevertheless, the findings differ with other studies. For example, Watcho *et al.* [23] originally recommended that the aqueous root extract of *M. whitei* has contraceptive properties. This finding was founded on the experimental *in vivo* inhibition of spermatogenesis and reduced fertility. The researchers further assessed the *in vivo* androgenic activity of the same extract. Their findings recorded an increase in serum and intra-testicular testosterone levels after chronic exposure to *M. whitei* aqueous root extract [5].

Watcho *et al.* [40] used three different extraction solvents; hexane, methylene chloride and methanol for the extraction of *M. whitei* roots. Their study was conducted both *in vivo* and *in vitro* androgenic activity. Findings indicated that the methanolic extract showed revocable androgenic properties. On the other hand, the hexane fraction had important inhibitory effects against KCl-and adrenaline-induced contractions in isolated deferent ducts *in vitro*. Another study by Watcho *et al.* [41] found that the extracts increased the number of intromissions and erectile occurrences of inexperienced male rats. From these studies, it was presumed that the extract demonstrated both contraceptive properties as well as properties associated with enhancing male fertility. The difference in their results and the present studies could be attributed to the extraction solvent and the duration of treatment. Their study exposed rats to *M. whitei* hexane, methylene chloride and methanol root extract for a longer period of time than the present study did.

CONCLUSION AND WAY FORWARD

The study recorded an insignificant effect of the *M. whitei* extract on serum testosterone concentrations after 30 days of treatment. In addition, the extract recorded insignificant increase in the concentrations of both Luteinizing hormone (LH) and follicle stimulating hormone (FSH). Therefore, this paper concludes that the extract of *M. whitei* has no significant effect on the levels of serum testosterone, LH and FSH.

Based on the findings presented in this paper, the study focused on male rats hypothesizing that infertility is most caused by male than females hence the authors recommendI a study of *Mondia whitei* on female reproductive system and its effect both on the female gonads and hormones. Further studies on the effects of *M. whitei* on other body hormones, such as adrenal gland and male reproductive system should be done.

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