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Effects of *Mondia Whitei* 'Mukombero' on Sperm Parameters in Male Albino Rats

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Abstract

Infertility affects about 8 to 12% of the world's population and, in about half of cases, men are either the single cause or contribute to the couple's infertility. Many indigenous plants have been reported to be effective in male fertility regulation. Mondia whitei is a widely used medicinal plant across Africa for treatment of sexual dysfunction yet minimal empirical data exists to support its therapeutic value. The aim of this study was to evaluate the effects of aqueous extract of Mondia whitei on sperm characteristics in male albino rats following oral administration. 36 albino male rats weighing between 200mg-400mg were divided into 4 groups, each of nine rats. Group I comprised untreated controls while Groups II, III, and IV were treated with 100, 200 and 400mg/kg body weight respectively using the aqueous extract of Mondia whitei via oral gavage. At the end of experiment, rats were humanely sacrificed using Carbon dioxide, the testes and epididymis, dissected for sperm collection. Sperm count, total motility, vitality and morphology were determined using a microscope and a neubaeurs chamber. Data was analyzed using Statistical Package for Social Sciences (SPSS) -Version 20.0). Kruskal wallis test was employed in the analysis. $P < 0.05$ was considered statistically significant. The median (IQR) sperm count of group I, II, III and IV at 10^{th} day were 100.03 (100.03, 100.04) 10398 (98, 101), 96.66 (96.65, 96.68) and 100.98 (100.88, 101.47) cells/ml respectively. The difference was statistically significant (chi=8.157, p=0.043). Trend analysis indicated that within the groups, sperm count decreased significantly with increase in time (all $p<0.05$). The median (IQR) total sperm motility (percentage) of group I, II, III and IV at 10^{th} day were 91 (90, 92 84 (81, 85), 86 (84, 88) and 88 (84, 89) respectively and the difference was statistically significant (chi=7.686, p=0.049). The median (IQR) sperm vitality in percentage of group I, II, III and IV at 10^{th} day were 91 990, 91), 85 (82, 86), 87 (85, 89) and 89 (86, 90) respectively. The difference was statistically significant (chi=8.286, p=0.040). Though trend analysis indicated that it did not vary significantly within the groups (all $p>0.05$). Normal morphology percentage declined in different test groups as compared to the control groups. A statistically significant decline in normal morphology was observed within the groups with respect to time interval (p=0.027). Trend analysis indicated that within the groups, normal morphology decreased significantly with time (all $p<0.05$) while abnormal head morphology and tail increased with time p=0.05. This study concludes that Mondia whitei may alter male fertility by affecting sperm quality; it causes a decline in sperm count, morphology, motility and vitality. This shows that M. whitei might be cytotoxic and can result in hypogonadotrophic hypogonadism and oligoasthenoteratozoospermia.

Keyords: *Mondia Whitei, 'Mukombero', Sperm Parameters, Albino Rats*

INTRODUCTION

Infertility is an imperative component of reproductive health, and has often been omitted in many reproductive discourses (Cui, 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). Human male fertility is a vital issue of replica based totally at the capability of spermatozoa to fertilize and prompt the egg to assist early embryonic existence. However, it's been considered lower in most animals (Jørgensen *et al*., 2001), with increasing infertility rates in many countries affecting one in six couples (Sharpe *et al*., 2003; Kamel, 2010).

The issue of male infertility is multi-factorial with some men suffering from low fertility in spite of having adequate numbers of sperm with normal morphology and motility. Lack of knowledge on multi-factorial causes of male infertility poses a challenge on the rational approach towards development of effective therapies (Wu *et al.,* 1989). Based on the World Health Organization [WHO] 2010 standards infertility affects 30 million men globally (Agarwal *et al.,* 2014). And because of this many couples seek medical help in order to solve this problem (Ikechebelu *et al*., 2003). Medical evidence indicates that around 80 % of Africans rely on conventional healthcare practitioners and medicinal flowers for their daily healthcare needs (Johnson *et al*., 2007; McKay & Blumberg, 2007). Natural merchandise have reduced ache, suffering and revolutionized the practices of medicine. In regard of this, more than 60% of approved and pre-new drug utility (NDA) applicants are either natural products or associated with them (Demain, 1999). Studies on conventional medicinal plants have shown that their potential to improve male fertility is partially because of presence of antioxidants. Those antioxidants have been observed to enhance several methods (spermatogenesis, steroidogenesis) of male reproductive characteristic (Nantia *et al*., 2009). In recognition of this, an aggregate of plant formulations has been determined to treat idiopathic infertility (Agrawal & Kulkarni, 2003; Rama Devi *et al*., 2004; Tempest *et al*., 2005; Xu *et al*., 2003).

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 (Aremu*et al*., 2011). The most common and well-known compound isolated from *M. whitei* is 2-hydroxy-4-methoxybenzaldehyde, a potent tyrosinase inhibitor and an isomer of vanillin (Kubo and Kinst-Hori, 1999b). This compound has also been isolated from *M. whitei* by other researchers (Oketch-Rabah, 2012). In addition, Koorbanally *et al.,* (2000) isolated isovanillin. Nutritional analysis indicated that Mondia is rich in minerals and vitamins (Iwu, 2014).Qualitative phytochemical analysis of the ethanoic extract of *M. whitei* indicated the presence of reducing sugars and triterpenes (Quasie e*t al.,* 2010).

However, there is inadequate research that has been done on the effects of *Mondia Whitei* on the hypothalamo-pituitary-gonadal axis 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 fertility using male albino rats.

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 *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

$$n= (10/4) + 1 = 3 \text{ rats per group}$$

There are 4 groups that received 100 mg/kg b w t, 200 mg/kg, and 400 mg/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 and during the experiment. They were fed with normal rat feed and portable water *ad libitum*.

M. whitei was procured from Kakamega forest using a contracted vendor 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 30 min to give 150 g of brown residue. 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 (0.1 ml), 200 mg/kg (0.2 ml) and 400 mg/kg b.w (0.4 ml) of the extract.

The thirty-six male albino rats were grouped into four of 9 rats each. Group I (control) was fed with normal rat feed and water *ad libitum* for 30 days. Test groups II, III, and IV was treated with 100 mg, 200 mg and 400 mg 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 respectfully as per the test group. The extract was administered orally and daily using syringes without needles between the hours of 8.00 am and 9.00 am.

Sperm Analysis

Caudal part of the epididymis was removed and placed in a beaker containing 1 ml physiological saline solution and allowed to stand for few minutes to allow spermatozoa swim out of the solution. Sperm count was done under the microscope. The sperm count was determined using the Neubauer's counting chamber as described by Saalu *et al.* (2012). Briefly, few drops of semen was placed on a slide, two drops of eosin Y was added, slide covered with cover slip and examined under the microscope using X40 objective for sperm morphology. The sperm concentration was then calculated.

Light microscope at X400 magnification was used to evaluate sperm morphology. Five hundred sperm from the sample was scored for morphological abnormalities according to Ilbey *et al.* (2009). A sperm was considered abnormal morphologically, if it has one or more of the following features: rudimentary tail, round head, and detached head and was expressed as a percentage of morphologically normal sperm.

Vitality characteristics of the isolated sperms was analyzed as per WHO laboratory manual for the examination of human semen (1999), this is a modified Blom's technique that uses a 2-step eosin-nigrosin technique to obtain the dark background for contrast and yields reliable evaluation using ordinary microscope optics. Non-motile sperms were distinguished from other objects like dirt, leukocytes, erythrocytes, or spermatids by their size and intensity. High and low gates for these characteristics were defined as factors of the mean size or intensity of the motile sperm and were selected. 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).

Statistical Analysis

Data entry was one using Microsoft excel and later exported to SPSS V.21 for analysis. Normality test was performed using the Shapiro wilks test with Ho being that data follows the normal distribution. Since the data failed the normality test (skewed), it was summarized using median (IQR) and variance among the groups and across time was tested using the non-parametric alternative to Anova (kruskal wallis test). Trend analysis was done to establish significant changes in estimates with increase in time. Significance was set at $p<0.05$. Findings are presented in terms of bar graphs and tables.

RESULTS

Effect of *M. Whitei* Aqueous Extracts on Sperm Count Concentration after 10, 15 and 30 Days and Between Treatments of 100 (mg/kg), 200 (mg/kg) and 400 (mg/kg) and Negative Controls

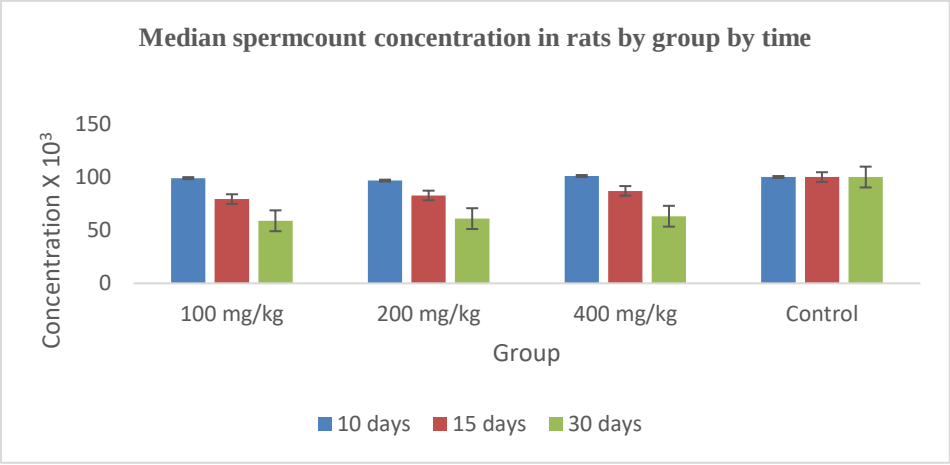

Figure 1: Effect of *M. whitei* Aqueous Extracts (100,200 and 400 mg/kg) for 10, 15 and 30 Days on Sperm Count (x1000/ml) Concentration in Albino Rats.

Table 1 indicates comparison in sperm count concentrations in rats treated with *M. whitei* extract and negative control rats after 10, 15 and 30 days and between treatments of 100 (mg/kg), 200 (mg/kg) and 400 (mg/kg) and negative controls.

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

Treatment	**Sperm count concentration x1000/ml Median (IQR)**			**Chi-value**	**p-value**
Rat Groups	**10 days**	**15 days**	**30 days**		
100 (mg/kg)	99(98, 101)	79.29(79.18, 79.51)	58.88(58.80, 58.93)	10.211	0.011
200 (mg/kg)	96.66(96.65, 96.68)	82.69(82.48, 82.83)	60.90(60.79, 60.98)	7.242	0.026
400 (mg/kg)	100.98(100.88, 101.47)	87(86, 91)	63.16(63.01, 63.20)	9.210	0.029
Control	100.03(100.03,100.04)	100.02(100.02, 100.05)	100.03(100.02, 100.03)	1.208	0.547
Chi-value	8.157	11.455	10.421		
P-value	0.043	0.010	0.015		

* IQR= Interquartile range

The sperm count concentration levels progressively decreased with time in all the three groups of rats treated with the extract (exposed). The sperm count concentration levels remained constant in the untreated (control) rats with time.

Effect of *M. Whitei* Aqueous Extracts on Percentage Motility Concentration after 10, 15 and 30 Days and between Treatments of 100 (mg/kg), 200 (mg/kg) and 400 (mg/kg) and Negative Controls

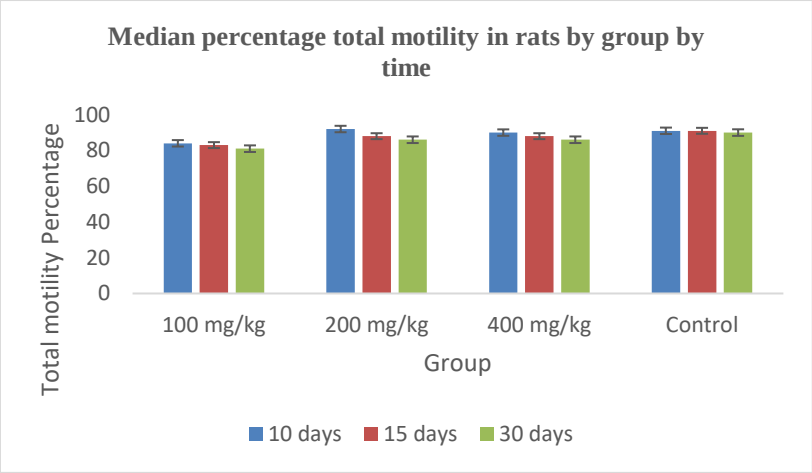

Figure 2: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Total Motility (%) in Albino Rats.

Table 2 indicates comparison in percentage motility concentrations in rats treated with *M. whitei* extract and negative control rats after 10, 15 and 30 days and between treatments of 100 (mg/kg), 200 (mg/kg) and 400 (mg/kg) and negative controls.

Table 2: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Total Motility (Percentage) in Rats

Treatment	**Total Motility (percentage) Median (IQR)**			**Chi-value**	**p-value**
Rat Groups	**10 days**	**15 days**	**30 days**		
100 (mg/kg)	84(81, 85)	83(81, 84)	81(80, 82)	2.713	0.258
200 (mg/kg)	92(91, 93)	88(87, 93)	86(84, 88)	4.497	0.106
400 (mg/kg)	90(88, 90)	88(84, 89)	86(81, 88)	3.988	0.136
Control	91(90, 92)	91(90, 91)	90(90, 91)	1.147	0.564
Chi-value	7.868	9.787	8.046		
P-value	0.049	0.02	0.045		

The decrease in sperm motility concentration levels with time in rats treated with the extracts (exposed) was **not statistically significant** in all the three groups. The sperm motility concentration levels remained constant in the untreated (control) rats with time.

Effect of *M. Whitei* Aqueous Extracts on Vitality Percentage after 10, 15 and 30 Days and between Treatments of 100 (mg/kg), 200 (mg/kg) and 400 (mg/kg) and Negative Controls

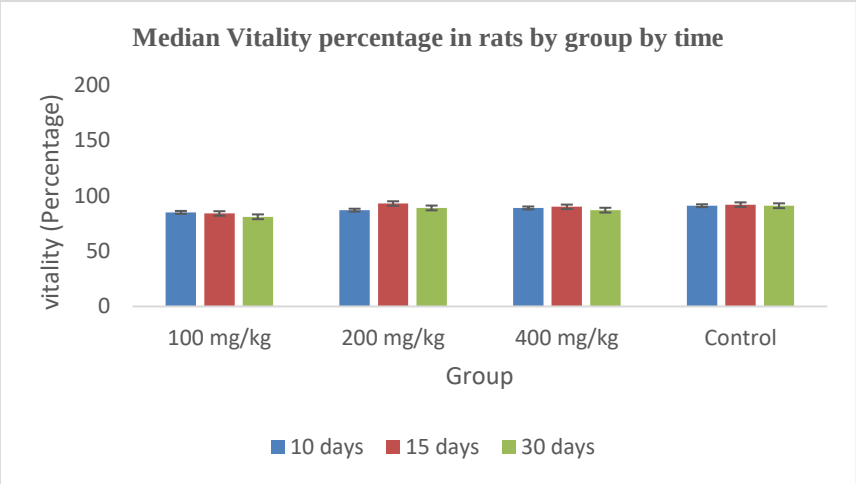

Figure 3: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Vitality (percentage) in Albino Rats.

Table 3 indicates comparison in percentage vitality in rats treated with *M. whitei* extract and negative control rats after 10, 15 and 30 days and between treatments of 100 (mg/kg), 200 (mg/kg) and 400 (mg/kg) and negative controls.

Table 3: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Percentage Vitality in Rats

Treatment	**Vitality Percentage Median (IQR)**			**Chi-value**	**p-value**
Rat Groups	**10 days**	**15 days**	**30 days**		
100 (mg/kg)	85(82, 86)	84(80, 85)	81(80, 82)	3.282	0.194
200 (mg/kg)	87(85, 89)	93(92, 94)	89(87, 94)	4.171	0.124
400 (mg/kg)	89(86, 90)	90(88, 92)	87(82, 89)	2.734	0.255
Control	91(90, 91)	92(90, 92)	91(91, 91)	1.333	0.513
Chi-value	8.286	9.000	8.372		
P-value	0.040	0.029	0.033		

There was **no statistically** significant progressive decline of the percentage of sperm vitality with time in all the three groups of rats treated with the extract (exposed). There was no statistically significant progressive changes in the % of the vitality in the controls.

Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Normal Morphology Percentage in Rats

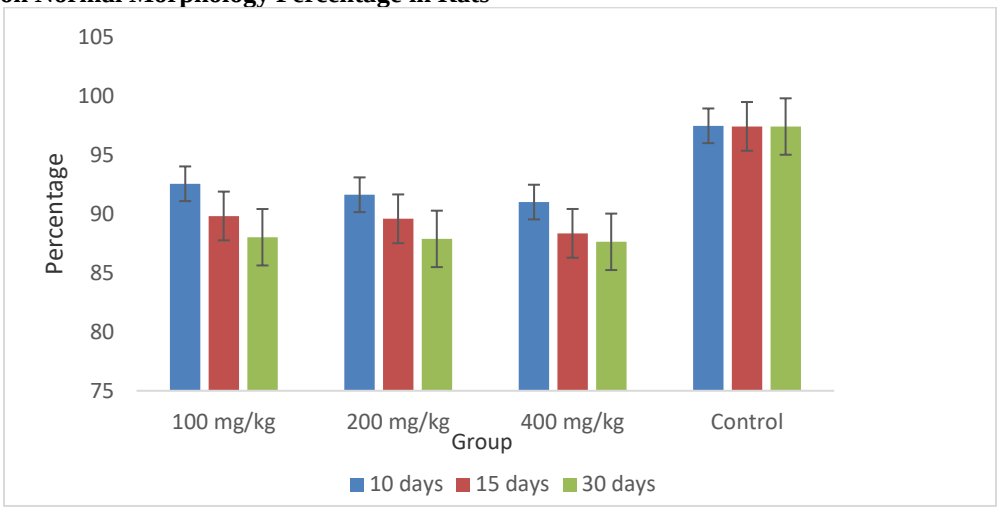

Figure 4: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Normal Morphology Percentage in Rats.

Table 4: Effect *of M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Normal Morphology Percentage in Rats

Treatment	**Normal morphology percentage Median (IQR)**			**Chi-value**	**p-value**
Rat Groups	**10 days**	**15 days**	**30 days**		
100 (mg/kg)	92.54(92.48, 92.59)	89.81(89.76, 89.83)	88.01(88.0, 88.06)	6.212	0.022
200 (mg/kg)	91.61(91.56, 91.62)	89.57(89.52, 89.58)	87.87(87.85, 87.94)	7.200	0.027
400 (mg/kg)	90.99(90.98, 90.99)	88.34(88.32, 88.37)	87.62(87.60, 87.64)	7.261	0.033
Control	97.45(97.42, 97.46)	97.40(97.32, 97.47)	97.39(97.01, 97.42)	2.711	0.258
Chi-value	10.421	9.385	7.325		
P-value	0.015	0.016	0.020		

The decrease in percentage of normal morphology with time in rats treated with the extracts (exposed) was **statistically significant** in all the three groups. The percentage of normal morphology remained constant in the untreated (control) rats with time.

Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Abnormal Head Morphology Percentage in Rats

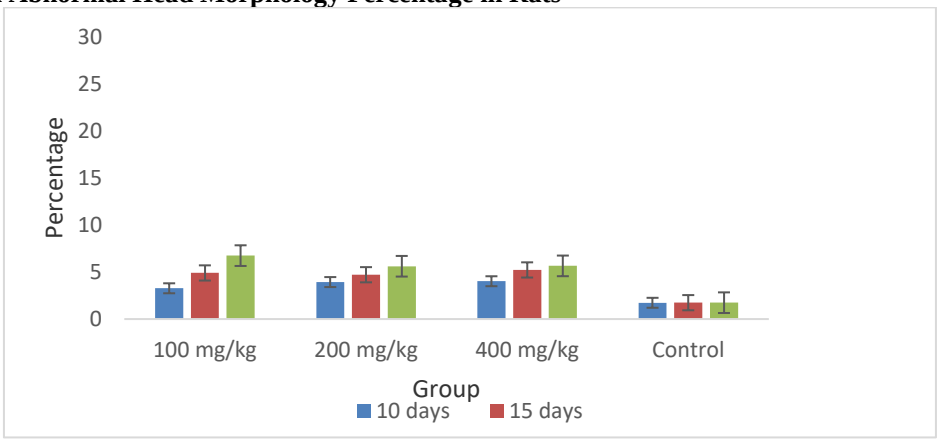

Figure 5: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Abnormal Head Morphology Percentage in Rats.

Table 5: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Abnormal Head Morphology Percentage in Rats

Treatment	**Head morphology concentration ng/ml Median (IQR)**			**Chi-value**	**p-value**
Rat Groups	**10 days**	**15 days**	**30 days**		
100 (mg/kg)	3.27(3.23, 3.9)	4.9(4.86, 4.92)	6.74(5.73, 5.80)	9.222	0.027
200 (mg/kg)	3.93(3.91, 3.95)	4.71(4.69, 4.73)	5.61(5.59, 5.63)	5.200	0.021
400 (mg/kg)	4.02(3.98, 4.04)	5.22(5.15, 5.24)	5.65(5.62, 5.68)	5.220	0.023
Control	1.73(1.70, 5.60)	1.74(1.70, 5.62)	1.74(1.73, 1.76)	0.274	0.872
Chi-value	4.446	4.866	9.974		
P-value	0.183	0.191	0.019		

Effect of *M. Whitei* Aqueous Extracts (100,200 and 400 mg/kg) for 10, 15 and 30 days on Abnormal Tail Morphology Percentage in Rats

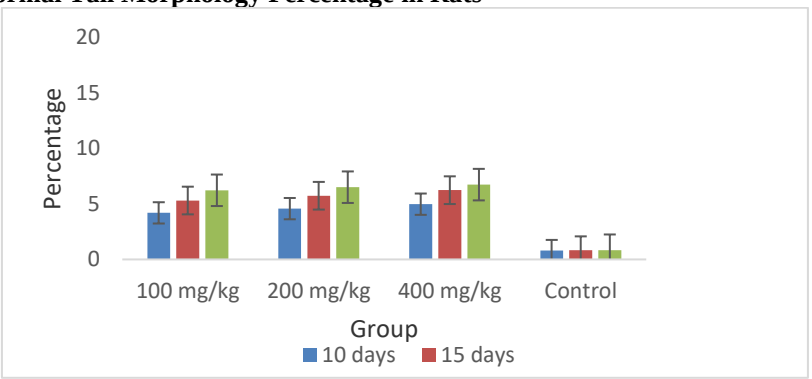

Figure 6: Effect of *M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Abnormal Tail Morphology Percentage in Rats.

Table 6: Effect *of M. whitei* Aqueous Extracts (100, 200 and 400 mg/kg) for 10, 15 and 30 Days on Abnormal Tail Morphology Percentage in Rats

Treatment	**Abnormal tail morphology in percentage Median (IQR)**			**Chi-value**	**p-value**
Rat Groups	**10 days**	**15 days**	**30 days**		
100 (mg/kg)	4.19(4.16, 4.24)	5.30(5.28, 5.34)	6.22(6.18, 6.25)	9.201	0.002
200 (mg/kg)	4.57(4.53, 4.59)	5.73(5.70, 5.76)	6.50(6.48, 6.52)	7.200	0.025
400 (mg/kg)	4.97(4.96, 5.06)	6.28(6.2s7, 6.30)	6.73(6.72, 6.74)	7.261	0.027
Control	0.80(0.77, 0.86)	0.83(0.78, 0.84)	0.83(0.78, 0.86)	0.205	0.903
Chi-value	11.385	12.300	7.315		
P-value	0.016	0.010	0.042		

The increase in percentage of abnormal tail morphology with time in rats treated with the extracts (exposed) was **statistically significant** in all the three groups. The percentage of normal tail morphology remained constant in the untreated (control) rats with time.

DISCUSSION AND CONCLUSION

Male infertility is generally attributed to insufficiencies in the semen which are mainly considered by low sperm motility and viability (Banihani *et al.*, 2012). Therefore, low sperm production (oligoozoospermia), poor sperm motility (asthenozoospermia) or abnormal sperm morphology (teratozoospermia) or a combination of all the three (oligoasthenoteratozoospermia) (Guzick *et al.*, 2001) leads to male infertility.

Findings from the present study shows that sperm concentration showed a significant difference ($p<0.05$) in sperm concentrations after 30 days in rats treated with 100, 200 and 400mg/kg of *Mondia whitei* and the control group. These findings could be attributed to the fact that certain alkaloids found in some plant extract have been implicated in reduced sperm viability. The postulated mechanism of action of such alkaloids involves releasing metabolites which bind to cell molecules and cross link DNA causing cytotoxicity (Saalu *et al*., 2010).

Previous studies done showed that decrease in sperm count and vitality is correlated with decrease in testosterone levels and oxidative damage as evident from suppressed antioxidant enzyme activities (Pandya *et al*., 2012). Further, studies have revealed spermicidal properties of plant extracts can lead to reduced human sperm motility (Harat *et al*., 2008).

Assessment of the viability of spermatozoa is one of the most important techniques of semen analysis, where one can establish the amount of dead/ live cells and employ this method as a cytotoxic marker. Eosin-Nigrosin technique was employed in the present study since spermatozoa with structurally intact cell membranes did not take up the stain as explained by Bjorndahl *et al.* (2004). Therefore, this method provided insights to the effects of *M. whitei* on the viability of spermatozoa in vivo.

When observing the effect of *M. whitei* on the viability of spermatzoa, decrease in sperm viability was recorded. In addition, viable cells decreased with increase in treatment time. These findings are in agreement with other studies where plant extracts are shown to decrease sperm cell viability at higher levels. Studies have shown that plant extracts might increase, have no effect or decrease cell viability depending on the plant's chemical composition (Cowan *et al*., 1999). On the other hand, certain alkaloids found in some plant extract have been associated with reduced sperm viability. The assumed mechanism of action of such alkaloids is said to involve the releasing of metabolites which end up binding to cell molecules and

cross link DNA initiating cytotoxicity (Saalu *et al*., 2010).

Katz *et al.,* (1982) reported that analysis of sperms morphology is a significant aspect in the assessment of sperm functions. A significant increase in incident of sperms with abnormal head and tail was detected in all the test groups. This indicates that the plant extract effects on morphological abnormalities of sperms on the basis of a dose-dependent. This could be attributed to the fact that normal sperm morphology was significantly affected by increased percentage of sperm with detached head and increased abnormal sperm tail morphology. These findings are similar to those reported from both animal studies (El-Demerdash *et al*., 2004) and research involving humans (Benoff *et al*., 2009; Wang *et al*., 2016).

Most importantly, a general improvement of observer accuracy, particularly after 1999 when stricter criteria were introduced in the WHO manual (Menkveld *et al*., 2011), may also be partly responsible for the observed decrease in normal sperm morphology. Abnormal forms have been more strictly detected over time, which would result in a decrease in the percentage of normal forms over time (Prisant *et al*., 2011). This phenomenon has already been documented and confirmed by a reanalysis of old smears but was ruled out as being the sole origin of the observed decrease (Menkveld *et al*., 2010). In conclusion, *Mondia whitei* maybe cytotoxic thus leading to male infertility. Further studies are encouraged to confirm these physiological parameters.

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