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## Effect of Short-Term Ingestion of the Methanolic Extract of *Mitragyna Speciosa* on Sperm Quality in Mice

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### Abstract

*Mitragyna speciosa* (MS) which is known as “Ketum” in Malaysia and “Kratom” in Thailand is a tropical plant indigenous to Southeast Asia. The leaves of MS have been used by natives of these countries for their opium-like effects and cocaine-like stimulant activities to overcome fatigue, enhance tolerance to hard work, prolong the duration of sexual intercourse and increase libido in males. However, no scientific studies have been carried out to assess the effect of MS consumption on the quality of sperm in animals or man. In this study, the effect of short-term ingestion of MS on the quality of sperm in mice was investigated. Forty mice were divided into 5 groups; 2 controls and 3 treatments. The negative control group received 0.9% sodium chloride (NaCl, 10 mL/kg) while the positive control received clomiphene (25 mg/70 kg). The respective treatment groups received either 50, 100 and 200 mg/kg of MS extract. The drugs and extracts were administered orally once daily for 14 d. The results showed an increase in the number of sperms in groups treated with MS. The morbidity rates of the sperm in groups treated with MS were markedly lower than that of the control groups. In addition, marked deformity in the sperm in the form of swelling at the tail was observed in the groups treated with MS. In conclusion, mice treated with MS showed an increase in the number of sperm count in spite of defect in sperm morphology and reduced sperm motility.

**Keywords:** *Mitragyna speciosa*, clomiphene, sperm quality, mice

### Introduction

In humans, male infertility accounts for an estimated 40-50% of the factors responsible for sexual dysfunction and failure of pregnancy in women (Brugh and Lipschultz, 2004). Examples of available treatments currently used to correct this

condition include Vitamin E prescription to counter the oxidative stress associated with sperm DNA damage and reduced sperm motility, a hormone-antioxidant combination and off-label use of clomiphene citrate, an anti-estrogen drug used to treat fertility problem in females.

Clomiphene citrate for example, has been shown to correct hypogonadism produced by functional suppression of pituitary gonadotropin with a modest effect on sexual function. In addition, patients treated with clomiphene citrate showed sexual improvement with significant increases in the luteinizing hormone (LH) and level of free testosterone (Guay *et al.*, 2003). However, clomiphene citrate is currently under trials for use in men and has not been approved by the U.S Food and Drug Administration for use in humans. Moreover, any enhancement of fertility in men could only be observed after 3-6 months of continuous clomiphene citrate consumption. Therefore, the need to find an alternative drug or herb which may enhance fertility rate in males in shorter period remains the focus of many reproductive researches worldwide. *Mitragyna speciosa* (MS) plant, for example, has been used in folklore medicine to treat such cases.

*Mitragyna speciosa* is a tropical plant indigenous to Southeast Asia especially in Thailand, Peninsular Malaysia and Indonesia. It has been reported that, MS taken as concoction may prolong the duration of sexual intercourse in humans although the claim has not been scientifically proven. In addition, consumption of MS leaves has been reported to increase the energy level in labourers and libido in male subjects. However, no scientific studies have been done to assess the effect of MS consumption on the sperm quality of males in general. Thus, the aim of this study was to evaluate the quality of sperm in mice treated with the crude methanolic extract of *Mitragyna speciosa* to provide pharmacological basis for treatment of male fertility disorder.

## **Materials and methods**

### ***Experimental animals***

Mice were kept under standard laboratory conditions (12h-light: 12h-dark) with an environmental temperature of 24-25°C. Feed and water were supplied *ad-libitum*. The animals were acclimatized for one week prior to the experiment. Fifty healthy male BALB/c mice (3-4 weeks old, weighing 20-25 g) were divided randomly into five groups. The negative control group received sodium chloride (0.9%, 10 mL/kg), positive control group received clomiphene (25 mg/70kg) and another three groups were treated with MS methanolic extract (MSM) at the dosage of 50, 100 and 200 mg/kg, respectively. Drugs and extracts were administered orally once daily for 14 consecutive days via intra-gastric route using an oral gavage needle attached to a syringe. Mice were euthanized on Day 14 and sperm samples were harvested from each mouse immediately after euthanasia. The quality of each sperm samples was evaluated using standard evaluation techniques.

### ***Sperm evaluation***

One drop of sperm suspension was placed on a microscope slide using a pipette. The slide was then covered with a 22 x 22 mm coverslip. Microscopic fields were observed at 400x magnification using a standard microscope and the percentage of sperm motility was determined. Sperm numbers were calculated by using a Haemocytometer and the concentration was expressed as  $\times 10^6$  sperm/mL sample. In order to observe the effect of various treatments on sperm morphology, one drop of sperm suspension was smeared onto a slide and stained with eosin-nigrosin. Samples were observed under the light microscope for presence of deformities and other abnormalities.

### ***Statistical analysis***

The one-way analysis of variance (ANOVA), followed by Tukey's test, was used to compare differences between treatments. The student's *t*-test was used to compare differences between 2 groups. Data are expressed as mean  $\pm$  SEM. Differences were considered to reach statistical significance when  $P < 0.05$ .

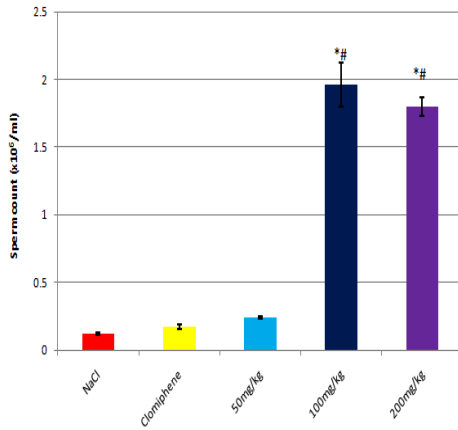
## **Results**

The effect of various treatments on the sperm count of mice is shown in Figure 1. Results showed that mice treated with 100 mg/kg of extract produced marked increase in sperm count ( $1.96 \pm 0.164 \times 10^6$  sperm/mL) as compared to negative (0.9% NaCl) and positive (clomiphene) control groups. However, the group that was treated with 200 mg/kg showed a sperm count of  $1.8 \pm 0.07 \times 10^6$  sperm/mL, which is almost similar to the group treated with 100 mg/kg extract. Despite this, there was no significant difference between these groups as indicated by the *t*-Test.

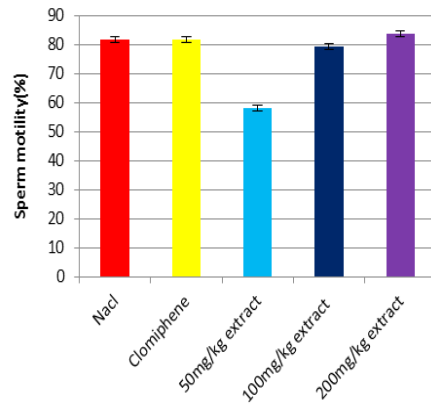
The effects of various treatments on mice sperm motility is shown in Figure 2. The group that received 100 mg/kg of extract showed a  $79 \pm 1.01\%$  sperm motility. There was no significant difference in the percentage of sperm motility as compared to negative (0.9% NaCl) and positive (clomiphene) groups except for the group treated with extract at the dosage of 50 mg/kg.

In Figure 3, the group that was treated with 100 mg/kg MSM showed an increase in the weight of testis ( $0.1 \pm 0.0$  mg) when compared to the group treated with 0.9% NaCl ( $0.015 \pm 0.002$  mg). However, there was no significant difference between groups treated with 100 and 200 mg/kg of extract.

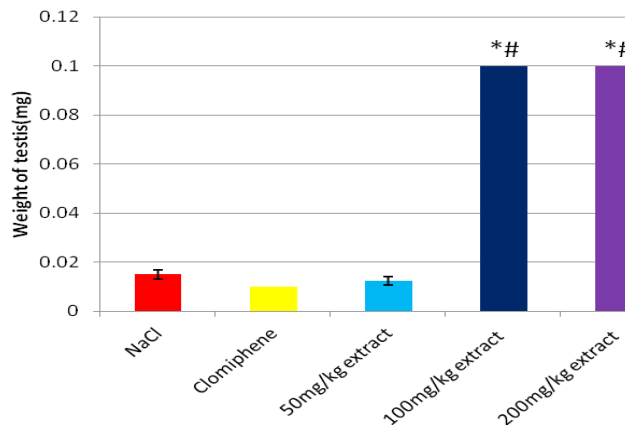
In Figure 4, no apparent abnormal findings on sperm were observed in groups treated with clomiphene and 0.9% sodium chloride. However, the groups of mice that were treated with 50, 100 and 200 mg/kg of extract showed an apparent deformity in the form of swelling at the middle tail region as shown in Figure 5.



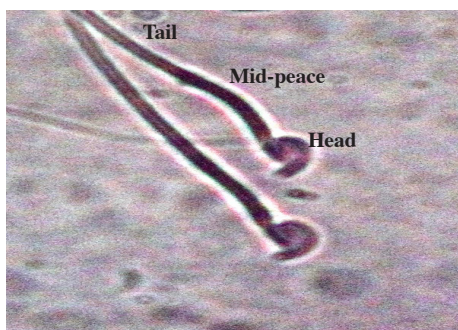
**Figure 1.** Effect of short-term ingestion of the methanolic extract *Mitragyna speciosa* on sperm count ( $\times 10^6$  sperm/mL) in mice. Values are mean  $\pm$  S.E.M ( $n=10$ ). \* Significantly different from 0.9% NaCl-treated group ( $P<0.05$ ) and # significantly different from clomiphene-treated group ( $P<0.05$ ), as determined by ANOVA followed by Tukey's test.



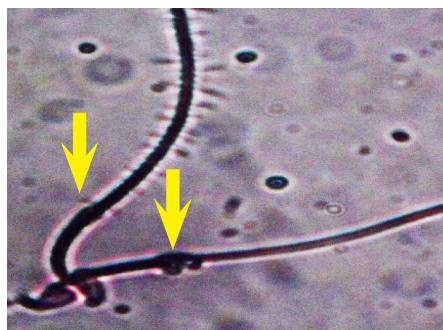
**Figure 2.** Effect of short-term ingestion of the methanolic extract of *Mitragyna speciosa* on sperm motility (%) in mice. Values are mean  $\pm$  S.E.M ( $n=10$ ). \* Significantly different from 0.9% NaCl-treated group ( $P<0.05$ ) and # significantly different from clomiphene-treated group ( $P<0.05$ ), as determined by ANOVA followed by Tukey's test.



**Figure 3.** Effect of short-term ingestion of the methanolic extract of *Mitragyna speciosa* on the testicular weight (mg) of mice 14 days post-treatment. Values are mean  $\pm$  S.E.M ( $n=10$ ). \* Significantly different from 0.9% NaCl-treated group ( $P<0.05$ ) and # significantly different from clomiphene-treated group ( $P<0.05$ ), as determined by ANOVA followed by Tukey's test.



**Figure 4.** Effect of short-term ingestion of 0.9% NaCl or clomiphene on the morphology of mouse sperm. Eosin-nigrosin, (400x). Sperms appeared normal with no marked change in morphology



**Figure 5.** Effect of short-term ingestion of the methanolic extract of *Mitragyna speciosa* on the morphology of mouse sperm. Eosin-nigrosin, (400x). Arrow indicates the deformity in the form of swelling at the middle tail region of mouse sperm.

## Discussion

In this study, the effect of short-term ingestion of MS extract showed an increase in sperm count in mice compared to control groups fed with NaCl (0.9%) and clomiphene. The results from this study also showed that there was a slight difference in weight of epididymes in control and treatment groups. Increase in the sperm count and weight of epididymes could be due to stimulation of endocrine system specifically the testis which resulted in increased spermatogenesis and consequently increased testosterone level. In addition, there may be a possible simultaneous stimulation of the hypothalamus and the pituitary gland to produce luteinizing hormone and follicle-stimulating hormone (Guay *et al.*, 2003) and thus, signals the testicles to produce testosterone and possibly more sperm through the hypothalamus-pituitary-gonadotropin axis. A possible explanation for the observed effect could be due to the synergistic effects of various secondary metabolites present in the MS leaves on these structures and not due to the effects of MS on opioid receptors. It is well-known that opiates inhibit gonadotropin secretion in experimental animals and humans (Pfeiffer and Herz, 1984). In addition, exogenous and endogenous opioids inhibit sexual arousal and erectile function in experimental animal and humans (McIntosh *et al.*, 1980; Cushman, 1972). Morphine addiction for example, has been associated with suppression of male and female LH-gonadal axis (Cicero *et al.*, 1979). Moreover, chronic exposure to morphine in cell cultures showed reduced basal and GnRH (gonadotropin releasing hormone) – stimulated LH release (Blank *et al.*, 1986). Since MS has been classified as an opioid and resembles morphine in terms of pharmacological properties and function, it is believed that the effect on sperm produced by short-term intake of MS could be

mediated by a similar mechanism. However, results obtained from this study were in contrast to this theory. However, the possible explanation for the opposite effects observed could not be determined through this present study.

Conversely, the unexpected results showed by short-term ingestion of clomiphene on the sperm motility and count analyses could be due to the short duration (14) of clomiphene intake. A more accurate result may be obtained from the study if this drug given for a longer period as clomiphene has been shown to increase fertility rate only after 3-6 mo of daily intake (Srivannaboon *et al.*, 1992; Schellen, 1982).

Human sperm cells contain  $\mu$ -opioid receptors (MOR) (Albrizio *et al.*, 2006). In males, MOR-1 gene mutation results in decreased mating behavior, sperm motility and reduced litter size (Agirregoitia *et al.*, 2006). Mitragynine, an alkaloid from MS leaves has been shown to possess an affinity towards  $\mu$ -,  $\kappa$ - and  $\delta$ - opioid receptors (Yamamoto *et al.*, 1999). Moreover, studies have demonstrated that gonadotropin have opiate receptors of relative high affinity and high capacity ( $5 \times 10^4$  sites/cell) (Fabbri *et al.*, 1985). It is probable that the compounds present in MS extract may bind to the  $\mu$ - opioid receptor and thus inducing mutation in the gene and eventually caused a deformity in the morphology of the sperm at the mid tail region. However, the exact mechanism by which MS extract induces sperm deformity could not be determined at present. Despite this, the motility of sperm especially in groups treated with 100 and 200 mg/kg of MS extract was not affected.

## Conclusion

In this study, the groups of mice treated with the methanolic extract of *Mitragyna speciosa* at the dosage of 100 and 200 mg/kg showed a marked increase in sperm count and sperm motility. The effects seen could be due to the enhancement of spermatogenesis through the stimulation of hypothalamus-pituitary-gonadotropin axis by the compounds or metabolites present in MSM. A marked increase in testicular size and weight could also be explained by the increase in sperm concentration which may be stimulated by similar mechanism stated previously. However as the doses increased, the occurrence of the deformity of the sperm at the middle of the tail region increased simultaneously. The precise mechanism underlying this effect has yet to be determined and currently under investigation, but it is likely to be associated with genetic mutations involving the interaction of MSM with the opioid receptors on the surface of each sperm.



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