

***Moringa Oleifera* Leaves Extract Attenuates Male Sexual Dysfunction**

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Abstract: Problem statement: At present, the novel agent that is effective, cheap and easy to approach for treating male sexual dysfunction is required due to the current poor therapeutic efficacy. Though *Moringa oleifera* is reputed for aphrodisiac activity in traditional folklore, no scientific evidence is available. Therefore, we aimed to determine the effect of *M.oleifera* leaves extract on male sexual behaviors in animal model of sexual dysfunction. Moreover, the possible underlying mechanisms were also investigated. **Approach:** Male Wistar rats, weighing 200-250 g, had been orally given *M.oleifera* leaves extract at doses of 10, 50 and 250 mg kg⁻¹ BW once daily at 30 min before the exposure to 12-h immobilization stress for 14 days. They were assessed male sexual behaviors including mounting, intromission and ejaculation numbers and latencies after single administration and every 7 days until the end of experiment. To further investigate the possible mechanisms of action, we also determined serum testosterone level of all rats at the end of experiment together with the determination of suppression effect of the plant extract on MAO_B and PDE-5 activities. **Results:** The results showed that after single administration, rats subjected to *M.oleifera* extract at dose of 10 mg kg⁻¹ BW significantly enhanced mounting number. When the treatment was prolonged to 7 days rats subjected to the low dose of extract showed the enhanced intromission number whereas rats subjected to high dose of extract showed the enhanced mounting number. Our data also showed no significant change in serum testosterone level. However, the extract could also suppress MAO_B and PDE-5 activities. Taken all together, the extract could enhance male sexual desire and performance via the suppression of MAO_B and PDE-5 activities. **Conclusion:** *M.oleifera* can be the potential sexual enhancer particularly for acute and short term application. However, further researches are necessary.

Key words: Numerous health products, scientific evidence, sexual enhancer particularly, Erectile Dysfunction (ED), harvested during, room temperature

INTRODUCTION

Sexual dysfunction has been recognized as one of the important social and biological relationship in human life. This condition affects the sexual life of millions of men worldwide. The prevalence of male sexual dysfunction is increased as the age advanced (Wattanathorn *et al.*, 2012; Moreira *et al.*, 2006). Therefore, the sexual dysfunction is still increasing its prevalence and importance. Sexual dysfunction caused by various factors including stress. Despite the importance of sexual dysfunction, the therapeutic efficacy is still not in satisfaction level. The most well

known drug used nowadays appears to target only at intromission phase, the main problem in Erectile Dysfunction (ED) which is the most commonly found sexual dysfunction. However, sexual dysfunction refers to a problem during any phase of the sexual response cycle. Therefore, the searching for new agent targeting at numerous phases of sexual response cycle which is more cheap has gained much concentration.

Moringa oleifera Lam. or Drumstick tree, a plant in a family of Moringaceae, is widely cultivated in Thailand. Both leaves and fruit of this tree are edible. It is believed to be miracle herbs because it can be used not only as food but also as medicine which can cure

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numerous ailments. The leaves of *M.oleifera* have been used as antiulcer, diuretic, anti-inflammatory and for wound healing (Kirtikar and Basu, 1935; Caceres *et al.*, 1992; Udupa *et al.*, 1994; Pal *et al.*, 1995). Moreover, it has been used to enhance male sexual functions including libido, improve sperm quality and anti-erectile dysfunction. Nowadays, numerous health products of *M.oleifera* are available in the market and claimed for the effect on male sexual functions as mentioned earlier, no scientific evidence was observed until now. Therefore, the current study aimed to determine the effect of *M.oleifera* leaves extract on male sexual behaviors and the possible underlying mechanism in sexual dysfunction rats induced by stress.

MATERIALS AND METHODS

Plant material: The fresh *Moringa oleifera* Lam (Moringaceae) were harvested during November to December, 2010 from the Khon Kaen province Thailand. The plant specimen was authenticated by Associate Professor Dr. Panee Sirisa-ard, Faculty of Pharmaceutical Sciences, Chiangmai University, Thailand. The voucher specimen was kept at Integrative Complementary Alternative Medicine Research and Development Group, Khon Kaen University, Khon Kaen, Thailand.

Plant material preparation: The fresh leaves were immediately cleaned, than cut into small pieces and dried at the temperature less than 50°C. The dried plant material was ground into fine coarse powder and extracted with 50% alcoholic. After that evaporation of solvent in rotary evaporator affords a crude extract of the soluble components and filtrate was lyophilized. The percent yield of extract was 17.49%. The extract contained total phenolic compounds at concentration of 86.73-93.6±0.51 mg of GAE/g extract. The extracts were stored at -25°C in a dark bottle until used. The crude extract was suspended in distilled water.

Animals: Healthy male Wistar rats (200-250 g) were obtained from National Animal Center, Salaya, Nakorn Pathom and were housed in group of 6 per cage in standard metal cages at 22±2°C on 10:14 h light-dark cycle. All animals were given access to food and water *ad libitum*. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC). The experimental protocols were approved by the Institutional Animal Care and Use Committee.

Stress procedure: The restraint stress was performed during the light cycle from 6.00 a.m. to 6.00 p.m. The restrainer was made of transparent perforated plastic tube, 20 cm long and 7 cm in diameter. The rats were put into the restrainer, head first and once in, the tubes were closed with plexiglass lids. The animals fit tightly into the restrainers and it was not possible for them to turn around. None stressed control rats were at the same time briefly handled and returned to their home cages.

Evaluation of sexual behaviors: Male Wistar rats of proven fertility were randomly divided into 4 groups of 6 animals each as following; (1) Vehicle plus stress (2)-(4) *M.oleifera* plus stress. Rats in group 1 were administered with distilled water which used as vehicle for the plant extract then expose to the 12 h-restraint stress whereas rats in group (2)-(4) were administered with the plant extract at doses of 10, 50 and 250 mg kg⁻¹ BW plus stress exposure as mentioned earlier.

All substances treatments were administered 45 m prior to the 12 h-restraint stress exposure. The treatment and the stress-exposure were performed once daily and the sexual behaviors assessments were performed blindly. The animals were allowed to rest in order to refresh the animals 3 h after the removal from restraint cage and then they were assessed the sexual behaviors between 9.00 p.m. to 12.00 a.m. at room temperature 26-28°C after single dose, 1 and 2 weeks of treatment. In order to assess the sexual behaviors, estrous female rats were paired with male treated with single or repeated doses of extract. Female rats were induced to estrous by sequential administration of estradiol benzoate (Sigma, St. Louis, MO) at dose of 2 µg kg⁻¹ BW and progesterone (Sigma, St. Louis, MO) at dose of 500 µg kg⁻¹ BW were injected before the determination of copulatory behaviors via subcutaneous route 48 h and 6 h respectively. Sexual behaviors were monitored in a separate room for 3 h in a clear plastic box via blind observer 30 min at the start of first hour whereas the whole duration of observation (3h) was recorded by digital video recording. The assessed sexual parameters were including the following parameters:

Mounting number: The number of mounts without intromission from the time of introduction of the female until ejaculation:

- Intromission number: The number of intromissions from the time of introduction of the female until ejaculation
- Mount latency: The time interval between the introductions of the female to the first mount by the male
- Intromission latency: The interval from the time of introduction of the female to the first intromission by the male

- Ejaculation number: The number of ejaculation which characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity
- Ejaculation latency: The time interval between the first intromission and ejaculation

Determination of testosterone level: Separate groups of animals were used for the measurement of plasma testosterone level of rats subjected to 12-h immobilization stress. At the end of 14-day experimental period, the rat blood samples were collected and kept on ice and then centrifuged immediately at $2000\times g$ at $4^{\circ}C$ for 15 min. The obtained plasma was kept at $-80^{\circ}C$ until analysis. Testosterone levels were measured using a commercially available radioimmunoassay (RIA) kit (TESTO-CT2, Cisbio International, France).

Determination of monoamine oxidase type B inhibition: The inhibitory action of the plant extract on monoamine oxidase type B was determined by incubating a series of concentrations of the test samples in the reaction mixture including rat brain homogenates. In brief, 2.75 mL Tris buffer (0.1 M, pH 7.4) and 100 μL of 0.1 M benzylamine was mixed in quartz cuvette which was placed in double beam spectrophotometer and followed by the addition of 150 μL solution of brain homogenate to initiate the enzymatic reaction. The change in absorbance was recorded at wavelength of 249.5 nm for 5 min against the blank containing Tris buffer and 5-hydroxytryptamine (Xu *et al.*, 2005).

Determination of Phosphodiesterase (PDE) activity: Testis was collected from healthy Wistar male rat in order to Determine Phosphodiesterase Enzyme (PDE) activity. The testis was washed with PBS and weighted before cut to small pieces. Then, it was homogenized with 5 volumes of lysate RIPA buffer. The testicular solution was centrifuged at $14,000\times g$ for 15 min at $4^{\circ}C$ and the supernatant was collected and used as PDE substrate. *M.oleifera* leaves extract at the various concentrations ranging from 2, 10, 100, 500, 2000 $\mu g mL^{-1}$ were prepared together with the positive control sildenafil at dose of 10 $\mu g mL^{-1}$. The standard curve was prepared from PDE (testicular lysate) at the various concentrations. The experiment was divide into 7 groups as following (1) control group (untreated group), (2-6) *M.oleifera* leaves extract treated groups at the various concentrations (2, 10, 100, 500, 2000 $\mu g mL^{-1}$) and (7) positive control (10 $\mu g mL^{-1}$ of sildenafil citrate). PDE-Glo™ Phosphodiesterase assays were performed in a white 96-well microplate. In brief, phosphodiesterase substrate or testicular lysate was

incubated with cGMP. Then, PDE reaction solutions were added and incubated for 20 min at room temperature. The cGMP in the mixture then drives a kinase reaction leading to a reduction of ATP levels. Following the kinase reaction, an Kinase Glo reagent was added and reactions were mixed and incubated for 10 min at room temperature. Luminescence was measured using a SpectraMax L microplate luminometer (MSD AT (US) Inc). The luminescent signal produced is directly related to the amount of ATP remaining and correlates with phosphodiesterase activity.

Statistic analysis: All data were expressed as mean \pm SEM value. The significant differences among various groups were compared by ANOVA and followed by Duncan's test. The statistical difference was regarded at $p<0.05$.

RESULTS

Effect of *M.oleifera* leaves extract on male sexual behavior: The effects of *M.oleifera* leaves extract on male sexual behavior in animal model of sexual dysfunction induced by 12-h immobilization stress were shown in Fig. 1-6. The current results showed that rats subjected to *M.oleifera* leaves extract at dose of 10 $mg kg^{-1}$ BW significantly increased mounting number after single administration whereas other parameters failed to show the significant difference (p -value <0.05 ; compared to vehicle+stress). When the treatment duration was increased further to 7 days, the rats which obtained *M.oleifera* leaves extract at dose of 10 $mg kg^{-1}$ BW showed the enhanced intromission number while the rats which exposed to high dose of *M.oleifera* leaves extract (250 $mg kg^{-1}$ BW) showed the increased mounting number (p -value <0.05 ; compared to vehicle +stress). Unfortunately, no significant changes were observed at 14 days of treatment.

Effect of *M.oleifera* leaves extract on phosphodiesterase (PDE) activity: We had determined the effect of the plant extract on the activity of MAO_B and the results were shown in Fig. 7. It was found that Sildenafil citrate or Viagra which was used as positive control in this study could significantly suppress PDE activity. The plant extract both at dose of 500 and at dose of 2000 $\mu g mL^{-1}$ also significantly suppressed PDE activity (p -value <0.05 and .01 respectively compared to control). Interestingly, *M.oleifera* leaves extract at dose of 2000 $\mu g mL^{-1}$ could suppress PDE activity at the same magnitude as Sildenafil citrate or Viagra.

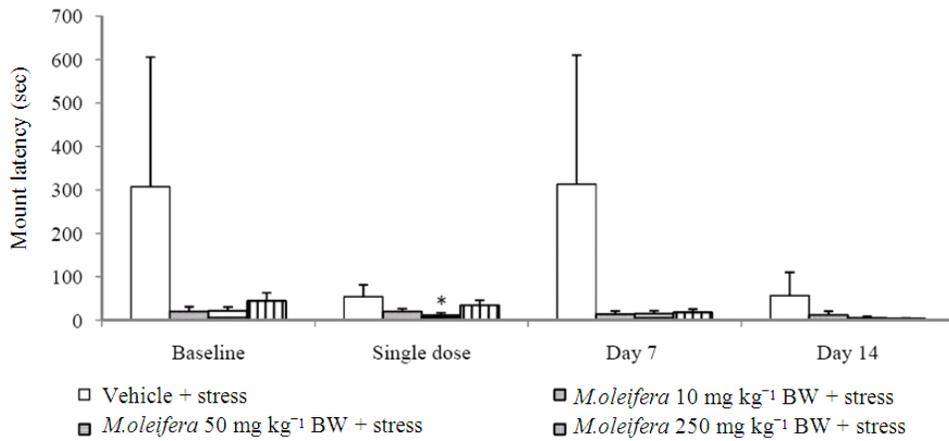


Fig. 1: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on mount latency. Data were presented as mean \pm SEM (n=6 group-1). * p<0.05 compared with vehicle plus stress treated group

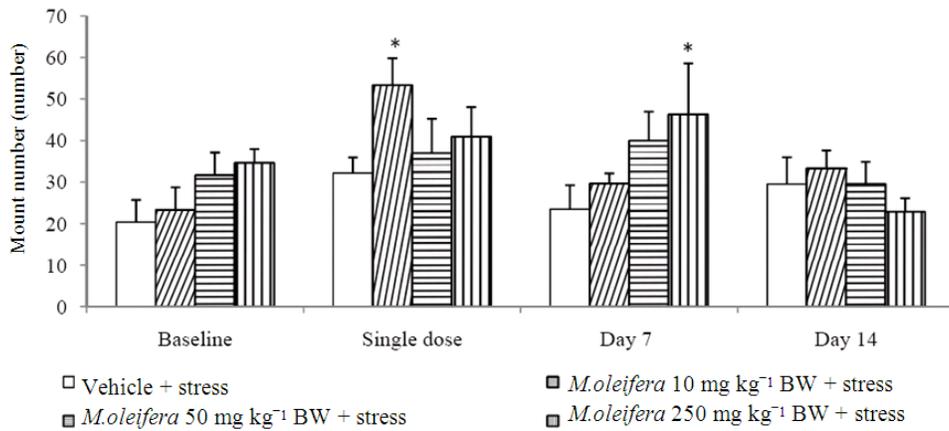


Fig. 2: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on mount number. Data were presented as mean \pm SEM (n = 6 group⁻¹). * p<0.05 compared with vehicle plus stress treated group

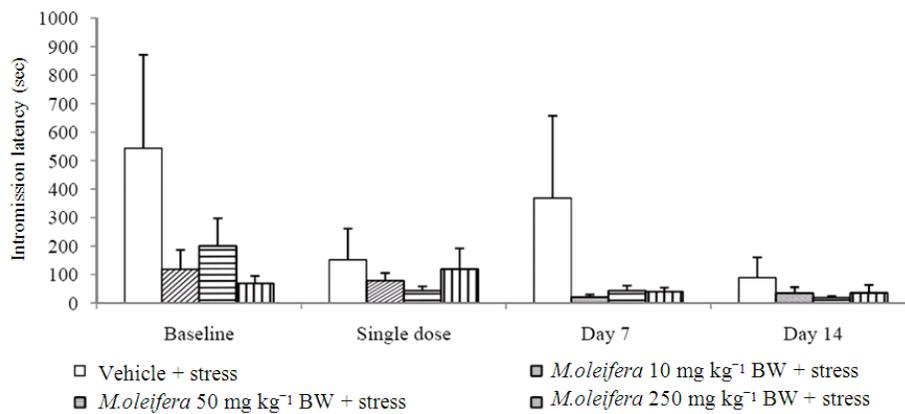


Fig. 3: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on intromission latency. Data were presented as mean \pm SEM (n = 6 group⁻¹)

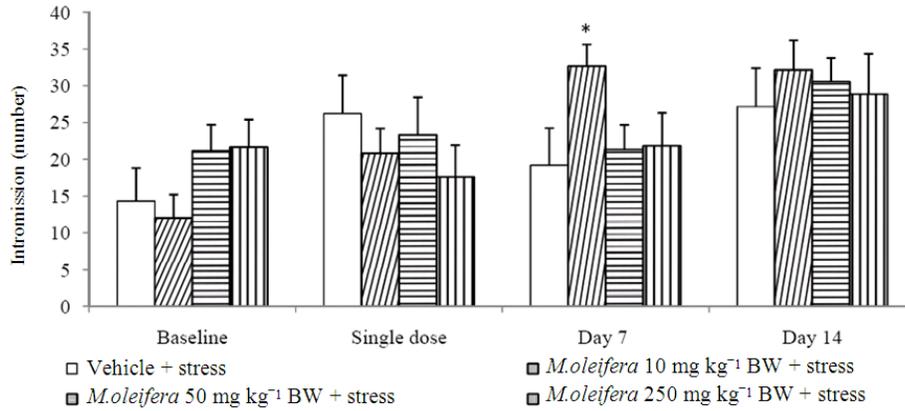


Fig. 4: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on intromission number. Data were presented as mean ± SEM (n = 6 group⁻¹). * p<0.05 compared with vehicle plus stress treated group

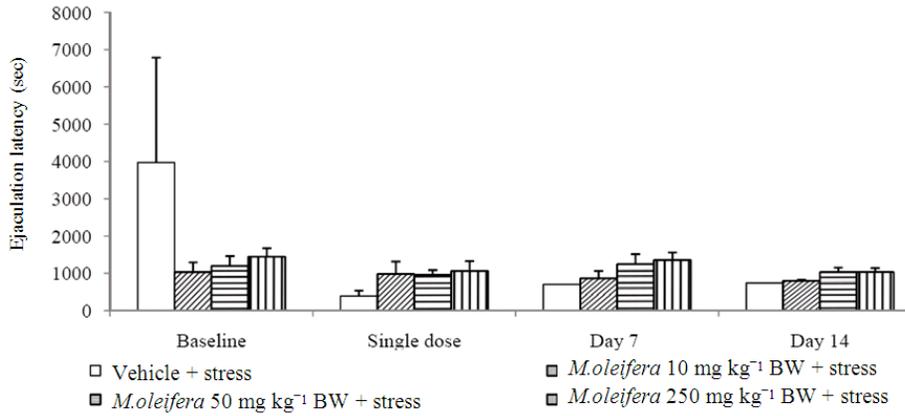


Fig. 5: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on ejaculation latency. Data were presented as mean ± SEM (n = 6 group⁻¹)

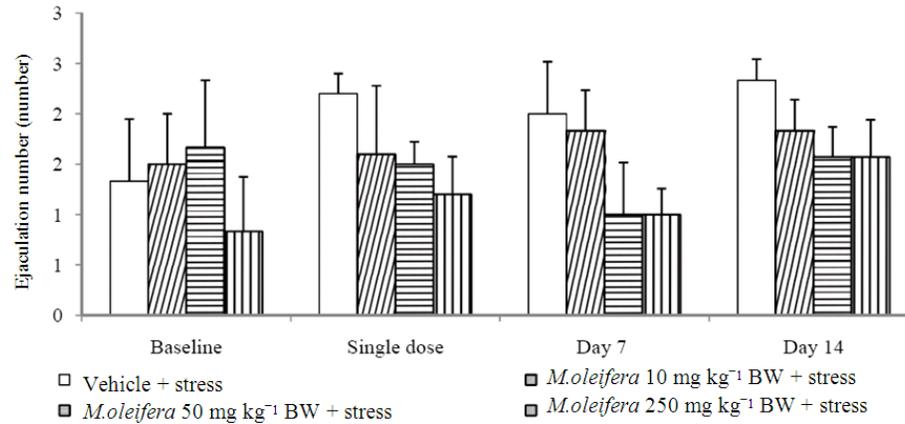


Fig. 6: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on ejaculation number. Data were presented as mean ± SEM (n = 6 group⁻¹).

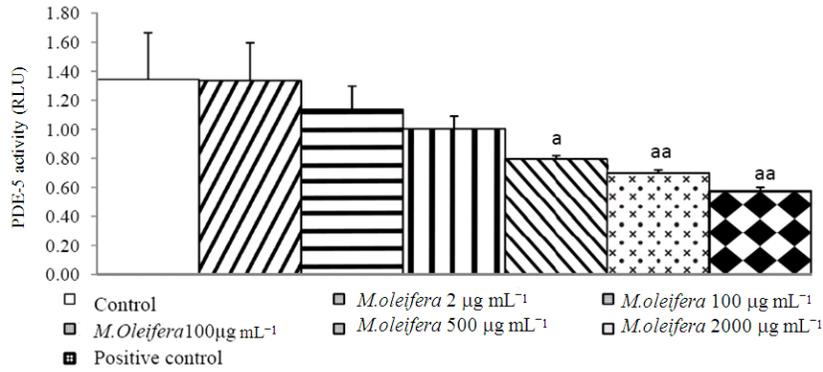


Fig. 7: Effect of *M.oleifera* leaves extract (0, 2, 10, 100, 500, 2000 µg ml⁻¹) and Sildenafil citrate (10 µg mL⁻¹) on phosphodiesterase (PDE) activity. Data were presented as mean ± SEM. ^{a, aa} p<0.05 and 0.01 respectively, compared with control group respectively

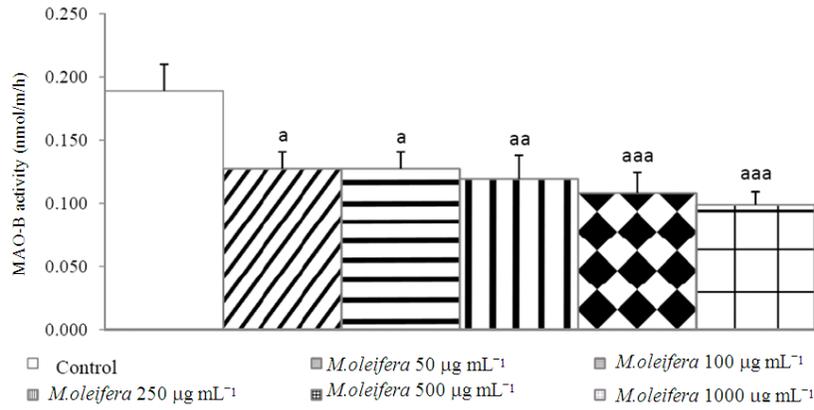


Fig. 8: Effect of *M.oleifera* leaves extract (0, 50, 100, 250, 500, 1000 µg ml⁻¹) on monoamine oxidase type B (MAO-B) activity. Data were presented as mean ± SEM. ^{a, aa, aaa} P-value < 0.05, 0.01 and 0.001 respectively, compared with control group

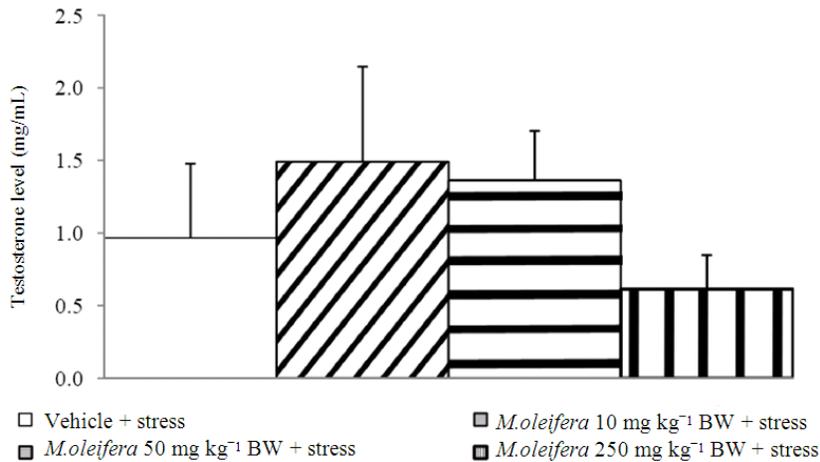


Fig. 9: Effect of *M.oleifera* leaves extract (10, 50, 250 mg kg⁻¹ BW) on testosterone. Data were presented as mean ± SEM (n = 6 group⁻¹)

Effect of *M.oleifera* leaves extract on monoamine oxidase type B (MAO_B) activity: The effect of *M.oleifera* leaves extract on MAO_B activity was evaluated and the results were shown in Fig. 8. Our data clearly revealed that the extract at concentration of 50,100, 250, 500 and 1000 µg mL⁻¹ could significantly suppress MAO_B activity (p-value<0.05, .05, .01, .01, 0.001 respectively; compared to control).

Effect of *M.oleifera* leaves extract on testosterone: Figure 9 demonstrated the effect of *M.oleifera* leaves extract on testosterone level. It was found that no significant changes in testosterone levels were observed in all rats subjected to *M.oleifera* leaves extract plus stress.

DISCUSSION

The present results provide, for the first time, information concerning the ability of *M.oleifera* leaves extract to improve male sexual behavior in rats. We have revealed that the plant extract could enhance mounting number and intromission number.

Accumulative lines of evidence have demonstrated that male sexual behavior is regulated mainly by neuroendocrine system. It has been reported that male sexual behaviors were under the influence of testosterone level (Seftel *et al.*, 2004; Wang *et al.*, 2004) and dopamine (Dominguez and Hull, 2005; Wattanathorn *et al.*, 2012). Therefore, in this study we also determined the effect of the plant extract on both parameters. Based on the information that dopamine is metabolized mainly by MAO_B, the activity of MAO_B is used to reflect the available dopamine (Glover *et al.*, 1977). Our data clearly demonstrated that rats subjected to *M.oleifera* leaves extract could enhance mounting and intromission numbers. Unfortunately, we could not detect the elevation of testosterone level. However, our *in vitro* data also demonstrated that the extract possessed monoamine oxidase type B inhibitor (MAO_BI). Since dopamine plays a crucial role on the regulation of male sexual function in many aspects including motivation and reinforcement, motor response to sexual stimuli and male genital reflex (Dominguez and Hull, 2005), it could be possible that the plant extract suppress MAO_B and gave rise to the elevation of dopamine which in turn enhanced libido and copulatory behavior both mounting and intromission numbers.

Besides dopamine, it has been found that phosphodiesterase type 5 (PDE 5) also plays a pivotal role on penile tumescence and intromission phase. PDE-5 is the enzyme contributing the important role on the hydrolysis of Guanosine 3'5'-cyclic monophosphate (cGMP), an important second messenger in cellular signal transduction processes including the smooth

muscle relaxation. PDE-5 is also abundantly present in the penile corpus cavernosum (Ballard *et al.*, 1998; Moreland *et al.*, 1998) and plays a major role in the relaxation of the corpus cavernosal smooth muscle during sexual stimulation (Andersson and Wagner, 1995). In this study, we have found that *M.oleifera* leaves extract also significantly suppressed PDE-5 activity. Therefore, the possible underlying mechanism of *M.oleifera* leaves to enhance intromission might be due to its ability to suppress PDE-5 activity together with the suppression of MAO_B activity.

Our current data failed to show the alteration of testosterone level. It has been believed that normal adult testosterone levels are not required for normal erections to occur (Bagatell *et al.*, 1994). The age of rats which used as experimental animals in this study were young adult rats and there was no pathological state and they were in eugonadal state. Since testosterone contributed minor role during this period, we have found the enhanced mounting and intromission numbers without the significant change of testosterone level.

The current results also showed that the prolonged treatment duration to 14 days failed to show beneficial effect. Although the precise mechanism was not understood, we suggested that this might be due to the adaptation of the nervous system which has been recognized as high plasticity organ.

This study provides evidence that *M.oleifera* leaves can enhance male sexual desire and performance. This enhancement can be ascribed to the suppression of MAO_B and phosphodiesterase activities. Therefore, *Moringa oleifera* may be served as the natural resource for developing functional food and food supplement to enhance sexual function particularly for acute and short term application. In order to provide optimum benefit, the screening of activities of this plant using other organic solvent should also perform in order to select the most suitable fraction as natural resource for further development of sexual enhancer food and related products. In addition, the possible active ingredient and safety evaluation are also very much necessary.

CONCLUSION

Moringa oleifera is a potential agent to manage sexual dysfunction induced by stress especially for acute and short term application. Further researches are warranted to confirm this activity before moving forward to clinical trial.

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