CHAPTER IV
EFFECT OF APHRODISIAC HERBS ON MALE SEXUAL DYSFUNCTION IN IMMOBILIZATION STRESS RATS

4.1 Introduction

Sexual feeling and sexual activity are inevitable parts of life which play an important role in the survival of the human race (Kothari, 2001). Since sex is a most intimate, indispensable and integral part of every individual's life and can be a source of pleasure and fulfillment, sexual dysfunction can induce depression, anxiety and debilitating feelings of inadequacy (Baldwin, 2001; Kennedy *et al.*, 1999). Although male sexual dysfunction is not a life threatening disorder, it can greatly affect the quality of life. It has been reported that about 20-30% of men suffer from sexual dysfunction (Lewis *et al.*, 2004). Because of its high impact and high prevalence, a great effort has been made to search for effective interventions to protect against sexual dysfunction.

Sexual function is a complex process involving the brain, hormones, emotions, nerves, muscles and blood vessels. It is very sensitive to stress. Therefore, stressful lifestyles or events usually induce sexual dysfunction. It has been reported that chronic physical and psychological stresses modulate neurotransmission in the median preoptic area and decrease penile blood flow, resulting in erectile dysfunction (Santosh *et al.*, 2011). Experimental stress induced by restraint can suppress testicular steroidogenesis giving rise to a reduction in plasma testosterone (Orr and Mann, 1990). Long term exposure to stress destroys interstitial cells of Leydig and decreases serum testosterone and spermatogenesis (Rai *et al.*, 2004). Stress also increases the activity of the hypothalamo-pituitary-adrenal axis (HPA-axis) leading to the enhanced plasma cortisol and increased sympathetic system function (Carrasco and Van de Kar, 2003). This leads to excessive oxidative stress and stress-related disorders (Ahmad *et al.*, 2012) including sexual dysfunction (Orr and Mann, 1990).

To date, most available drugs in the market target at the intromission phase, the most common phase of sexual dysfunction. The most popular drugs are sildenafil

citrate, vardenafil and tadalafil citrate, which target the suppression of phosphodiesterase type 5 (PDE5), which in turn increases penile blood flow and penile tumescence. Unfortunately, these drugs have serious side effects such as sudden hypotension, hypersensitivity reaction, myalgia, abnormal vision, and infertility (Kasper, 2005). Since the current therapeutic drugs do not target all phases of the sexual responsive cycle and produce serious side effects, a novel strategy is required which is cheap, easy to apply and less toxic.

Various herbs known to be aphrodisiacs have long been used for enhancing sexual desire and sexual performance in traditional folklore. They can exert their actions at various targets such as the neuro-endocrine system, which plays a crucial role in sexual motivation and function (Hull *et al.*, 1997; Meston and Frohlich, 2000), and phosphodiesterase type 5 (PDE5), an important enzyme in the signal pathway which regulates penile erection via the regulation of cavernous smooth muscle tone, which in turn controls penile blood flow (Andersson, 2001). Several lines of evidence show that testosterone and dopamine can control male sexual function both at the nervous system and at the penis (Hull *et al.*, 1997). In addition, a recent study has shown that oxidative stress also plays a role in the impairment of carvernosal function and the pathophysiology of erectile dysfunction (Agarwal and Prabakaran, 2005), and that antioxidants can improve erectile function (Zhang *et al.*, 2011).

Thailand also contains abundant of medicinal plants reputed for aphrodisiac property such as *Moringa oleifera* (Lalas and Tsaknis, 2002; Priyadarshani and Varma, 2014), *Anacardium occidentale* (Gupta *et al.*, 2012) and *Nelumbo nucifera* (Mukherjee *et al.*, 2009). Despite the long term reputation, no scientific data were available. In addition, previous studies also demonstrate that these plants also possess antioxidant activity (Durairaj and Dorai, 2014; Kongkachuichai *et al.*, 2015; Siddhuraju and Becker, 2003) which can improve arteriogenic erectile dysfunction (Zhang *et al.*, 2011). Based on the reputation and the antioxidant activity of medicinal plants mentioned earlier, the beneficial effects of these medicinal plants on male sexual dysfunction have been raised. To elucidate these issues, the effects and possible underlying mechanisms of *M.oleifera* , *A.occidentale* and *N.nucifera* on male sexual behaviors in animal model induced by restraint stress were investigated.

4.2 Materials and Methods

4.2.1 Plant Materials and Preparation

4.2.1.1 Preparation of *Moringa oleifera* leaves extract

Fresh leaves of *M. oleifera* were harvested during November-December, 2010 from Khon Kaen province, Thailand. After the authentication, the herbarium specimen was deposited at Integrative Complementary Alternative Medicine Research Center, Khon Kaen University (voucher specimen 2010002). The fresh leaves were cleaned, cut in to small pieces and dried in oven at 40°C. The dried plant material was ground into powder and extracted with 50% hydro-ethanolic by maceration technique. The extract was filtered through Whatman filter paper number 1 and concentrated with rotator evaporator at 45°C. Then, the yielded extract was kept at 4°C till used. The percentage yield of the extract was 17.49%. The concentration of total phenolic compounds was 62.333±0.008 mg GAE·g^{-1} extract. In addition, the contents of ferulic acid and quercetin were 0.003±0.0001 mg FAE·mg^{-1} extract and 0.444±0.0001 mg QE·mg^{-1} extract respectively.

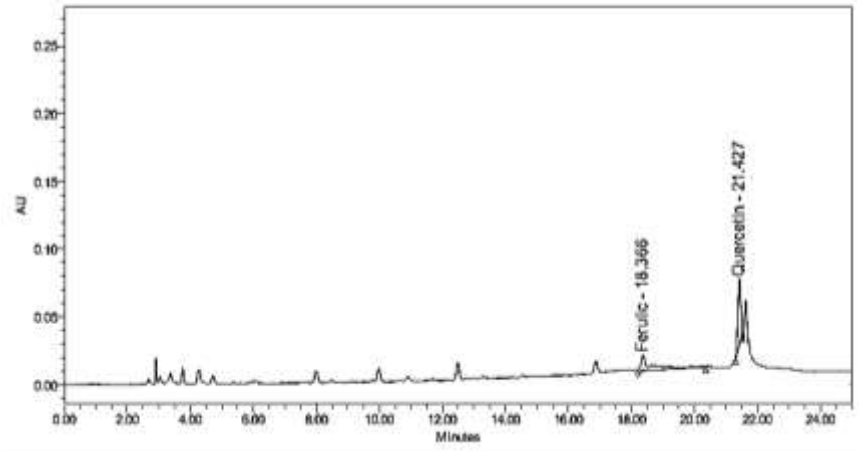


Figure 4-1 The fingerprint chromatogram of 50%hydroalcoholic extract of *Moringa oleifera* leaves used in this study

4.2.1.2 Preparation of *Anancardium occidentale* leaves extract

A. occidentale leaves were collected from Phuket province and authenticated by Associate Professor Panee Sirisa-ard, Faculty of Pharmacy, Chiang Mai University. After the authentication, the herbarium specimen was deposited

there. The fresh leaves were cleaned, cut in to small pieces and dried in oven at 40°C. The dried plant material was ground into powder and extracted with 95% hydro-ethanolic by maceration technique. The extract was filtered through Whatman filter paper number 1 and concentrated with rotator evaporator at 45°C. Then, the yielded extract was kept at 4°C till used. The percentage yield of the extract was 17.32%. The concentration of total phenolic compounds was 102.963±0.006 mg GAE·g^{-1} extract. In addition, the contents of gallic acid and quercetin were 7.771±0.003 mg GAE·mg^{-1} extract and 0.617±0.0001 mg QE·mg^{-1} extract respectively.

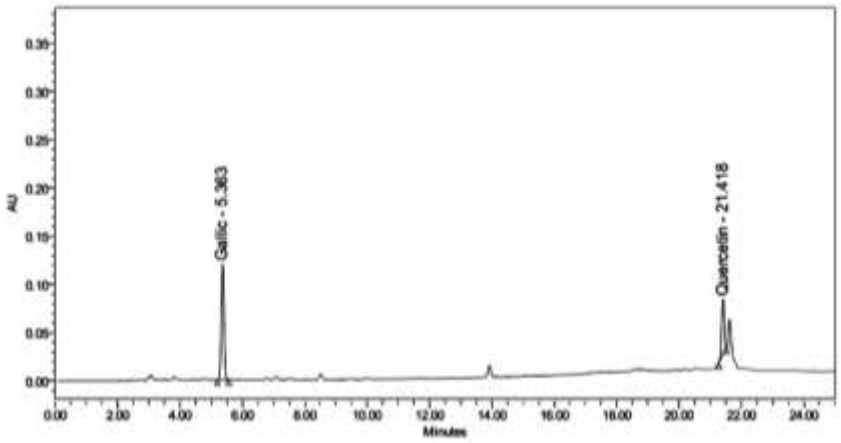


Figure 4-2 The fingerprint chromatogram of 50%hydroalcoholic extract of *Anacardium occidentale* leaves used in this study

4.2.1.3 Preparation of *Nelumbo nucifera* flower extract

Fresh flower of *N.nucifera* were harvested during November-December, 2012 from Khon Kaen province, Thailand. *N.nucifera* were authenticated by Dr. Nopachai Chansilp, Rajamangala University of *Technology* Tawan-ok. After the authentication, the herbarium specimen was deposited at Integrative Complementary Alternative Medicine Research Center, Khon Kaen University. The fresh flowers were cleaned, cut in to small pieces and dried in oven at 40°C. The dried plant material was ground into powder and extracted with 50% hydro-ethanolic by maceration technique. The extract was filtered through Whatman filter paper number 1 and concentrated with rotator evaporator at 45°C. Then, the yielded extract was kept at 4°C untill used. The percentage yield of the extract was 10.23%. The concentration

of total phenolic compounds was 152.963±0.009 mg GAE·g^{-1} extract. In addition, the contents of quercetin were 0.456±0.0001 mg QE·mg^{-1} extract.

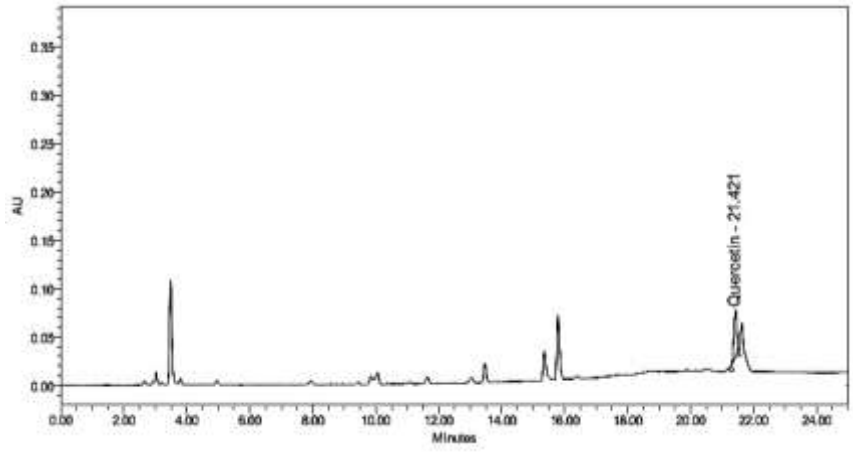


Figure 4-3 The fingerprint chromatogram of 50%hydroalcoholic extract of *Nelumbo nucifera* flowers used in this study

4.2.2 Animal Treatment

Adult male Wistar rats, weight 250–350 g were obtained from National Laboratory Animal Center, Salya, Nakorn Pathom province. They were housed, six per cage, under standard conditions and maintained on a 12:12 dark-light cycle. Temperature was controlled at 24±1°C. Food and water were available *ad libitum* throughout the experiments.

4.2.3 Experimental Protocol

This study was divided into 3 parts. The first part was designed to evaluate the sexual enhancing effect of *Moringa oleifera* Lam leaves in restraint rats whereas the second and the third parts were set up to determine the sexual enhancing effect of *Anacardium occidentale* Linn leaves and *Nelumbo nucifera* Gaertn flower in restraint rats.

Adult male Wistar rats, weighing 250-350 g (8-12 weeks of ages) (n = 6/group) were trained for sexual behavior since 7 days. The trained animals were divided into various groups as following; naïve control, vehicle treated group, Sildenafil citrate treated group, Tianeptine treated group and 3 various doses of plant

extract (*M.oleifera;* at dose of 10,50 and 250 mg/kg BW, *A.occidentale;* 25,100 and 200 mg/kg BW and *N.nucifera*; 10, 100 and 200 mg/kg BW) treated groups. Rats in all groups except naïve control were orally administrated the assigned substances before exposed to 12 hours immobilization stress.

Animals were placed in a fit immobilization cage container (5 cm diameter) this procedure effectively restricted movement. The start of immobilization stress began at 6 A.M. to 6 P.M. for a period of 12 hours each day. Female rats were induced to estrous phase by injection of estradiol benzoate at dose of 2 μg/kg BW via subcutaneous route and 500 μg/kg BW of progesterone at 48 h and 6 h respectively, before the copulatory study (Agmo, 1997; Ramachandran *et al.*, 2004) as shown in figure 4-4.

4.2.4 Sexual Behaviors

Sexual behavioral testing was performed under dim red lights 3 hours after refreshing time of male rat. Sexual behaviors were assessed by placing the male in a Plexiglas arena (45 cm diameter) 5 min before the presence receptive female was presented. After the presentation of the female rat, sexual behaviors were recorded for 30 minutes. The following parameters were recorded; mount, intromission and ejaculation latency and frequency (Retana-Márquez *et al.*, 2003) at baseline, single dose, 7 days and 14 days of experimental period.

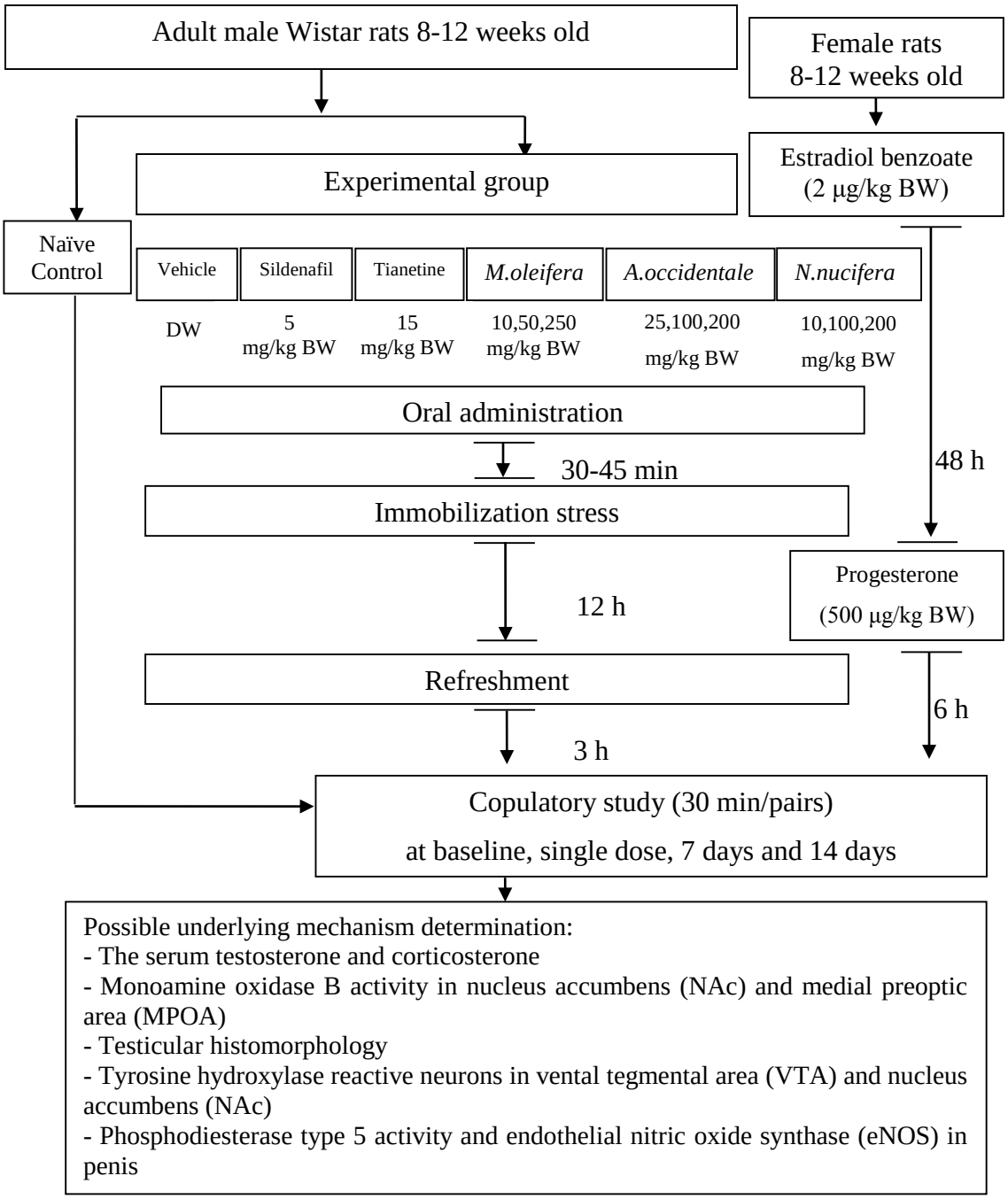


Figure 4-4 The schematic diagram represents the experimental protocol

4.2.5 Serum Testosterone and Corticosterone Assays

Venous blood of each animal was prepared as serum by the centrifugation at 2000*g*, 4 °C for 15 minutes. The serum was stored at −80 °C until used. Testosterone levels were measured using a radioimmunoassay (RIA) Kit

(TESTO-CT2, Cisbio International, France) and corticosterone levels were measured using a Corticosterone Double Antibody Radioimmunoassay Kit (MP Biomedicals) for the quantitative determination of corticosterone in rat and mice serum. The results are expressed as ng/ml. Both assays were performed at the Radiology Department, Srinakarindhra Hospital, Faculty of Medicine, Khon Kaen University, Thailand.

4.2.6 Determination of phosphodiesterase type 5 (PDE5)

The level of PDE-5 was determined using a PDE-Glo™ Phosphodiesterase Assay Kit (Promega Corp., Thailand). The penis was washed with PBS, cut into small pieces and prepared as homogenate using lysate RIPA buffer. The sample was subjected to a 14,000*g* centrifugation at 4 °C for 15 minutes. The supernatant was separated and served for the determination of PDE-5 activity. The PDE-Glo™ phosphodiesterase assay was carried out in a 96-well plate. The assay was performed according to the guidelines of the kit. In brief, the penis was incubated with cyclic guanosinc monophosphate (cGMP) substrate in reaction buffer until the phosphodiesterse reaction was complete. PDE-Glo™ termination buffer was incubated with PDE detection solution containing adenosine triphosphate (ATP) and protein kinase A (PKA). The amount of ATP consumed by this reaction was directly correlated with the cGMP level and was evaluated using the luciferase-based Kinase-Glo reagent. After a 10-min incubation period at room temperature, the optical density of the sample was determined using a SpectraMax® L microplate luminometer (MDS AT (US) Inc.) and expressed as relative light units (RLUs) and as a percentage of the control.

4.2.7 Determination of Monoamine Oxidase Enzyme Activity

Monoamine oxidase type A and B (MAO-A and MAO-B) were determined by the continuous peroxidase-linked photometric assay modified from Holt *et al.* (Holt *et al.*, 1997) in the 96-well plate. Medial preoptic area (MPOA) and nucleus accumbens (NAc) of each brain were collected and prepared as a homogenate with RIPA buffer (50 mM TrisHCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.5% sodium deoxylcholate, 0.1% SDS and 1% TritonX-100). Brain homogenate was centrifuged at 14, 000*g*, 4 ^{0}C for 20 minutes. After the centrifugation, the supernatant was harvested to determine MAO-A and B activities. Briefly, the well contained 120 µl amino substrated (2.5 mM tyramine (Sigma-Aldrich) in potassium phosphate

buffer), 40 µl chromogenic solution (1 mM vanillic acid (Sigma), 0.5 mM 4-aminoantipyrine, 4 U/ml peroxidase in potassium phosphate buffer, 40 µl of brain homogenate and then incubated for 30 minutes at 37 °C. Distilled water was used as the blank. In order to determine the specific MAO-B activity the brain homogenate was pre-incubated with clorgyline (selective MAO-A-I) for 30 min at 37 °C. While MAO-A activity was pre-incubated with pargyline (irreversible MAO-B-I). The reactions were read with microplate reader at 490 nm.

4.2.8 Histological and Immunohistochemical Studies

4.2.8.1 Tissue preparation

Rats were sacrificed and perfused transcardially with NSS. Brain and testis were fixed in the 4% paraformaldehyde in 0.1 mol/L phosphate buffer pH 7.4 overnight at 4°C following by immersing sequentially for 48-72 h each in a cryoprotectant containing 30% sucrose. Tissues were cut by cryostat sectioning (Micro HM 525) at 10 µm and mounted on slides which were coated with a 0.5% gelatin solution. The serial sections were either stored at 4 °C or processed immediately.

4.2.8.2 Histomorphological study of testis

The testes were dissected out, freed from surrounding tissues and weighed quickly on a sensitive balance. They were fixed and cut as a 10 µm-sections and stained using hematoxylin and eosin (H&E). Histomorphological analysis was performed using Olympus BX51 light microscope.

4.2.8.3 Tyrosine hydroxylase immunohistochemical study

The sections were evaluated for tyrosine hydroxylase expression (Decressac *et al.*, 2011) using the Polymer immunochemical staining method. In brief, brain sections were retrieved antigen via heat-induced epitope retrieval (HIER) method in Tris-EDTA retrieval buffer (containing 0.05% tween 20, pH 9.0) by using microwave for 20 min and then blocked endogenous peroxidase with 3% H_2O_2 in PBS containing 0.1% sodium azide for 1 h. Non-specific binding was blocked by 1% normal goat serum in PBS containing 0.25% BSA, 0.1% gelatin and 0.3% Triton X-100 for 1 h. Sections were incubated with anti-tyrosine hydroxylase antibody at a ratio of 1:400 in 1% normal goat serum containing Triton-X-100 for 24-48 h at 4 °C. Sections were washed 3 times at 5 min each in cold PBS and followed

by the incubation for 2-4 h with The Dako REAL™ EnVision™/HRP, Rabbit/Mouse (ENV) kit consisting of a dextran backbone which is a large number of peroxidase (HRP) molecules coupling with secondary antibody molecules. The reaction is visualized by the Dako REAL™ DAB+Chromogen in Dako REAL™ Substrate Buffer for 10 min at room temperature. Sections were dehydrated with serial dilution of alcohol from 70 to 100% followed by the clearance with xylene. Then, the slides were mounted and covered by coverslip using MERCK KGaA mounting medium (Kee *et al.*, 2002). Images were captured by microscope in the area of nucleus accumbens (NAc; bregma +1.6 mm, interaural 10.6 mm) and ventral tegmemtal area (VTA; bregma -4.80 mm, interaural 4.20 mm) according to the stereotactic rat brain atlas of Paxinos and Watson by using the Olympus BX51 light microscope. TH-Immunopositive (IR) neurons density was counted in VTA whereas that in NAc was measured by pixel density using the ImageJ software (Version 1.48V, National Institutes of Health). The density values were corrected with non-specific background from the cortex. The data are expressed as a pixel density.

4.2.9 Determination of Endothelial Nitric Oxide Synthase by Western Blotting

The level of endothelial nitric oxide synthase (eNOS) in penis was determined by Western blotting (Smith and Titheradge, 1998). In brief, penis of rat was collected and homogenized in ice cold RIPA buffer with protease inhibitors. The solution was then centrifuged at 14,000*g*, 4 °C for 20 min. The supernatant was collected and measured the level of protein by using NANO drop Spectrophotometers. Equal amounts of protein (100 µg) were fractionated by 8% SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA). Each step was preceded by rinsing (three times for 5 min) in 0.05% Tris-buffer saline with Tween-20. Membranes were blocked with 5% skim milk in Tris-buffer saline with 0.05% Tween-20) and incubated overnight in primary antisera against eNOS (1:500). The membranes were then incubated with horseradish peroxidase-linked secondary antibody (1:4,000) for 1 h at room temperature. Then the signal was enhanced with a Thermo Scientific™ Pierce™ ECL Substrate chemiluminescence kit (Pierce™ ECL Western Blotting). Images were acquired by ImageQuant LAS 4000, GE Healthcare. Band densities were quantified

with NIH-ImageJ (Version 1.48V; National Institutes of Health,USA). The PVDF was reprobed with the beta actin antibody (1:2,000) as a loading control.

4.2.10 Statistical Analysis

The data was expressed as mean ± SEM. The significance of differences among the groups were assessed using one way analysis of variance (ANOVA) test followed by LSD multiple comparison test using SPSS, version 13. P-values < 0.05 were considered as significant.

4.3 Results

4.3.1 Effect and Possible Underlying Mechanism of *M.oleifera* Leaves Extract on Sexual Dysfunction in Immobilization Rats

4.3.1.1 Effect of *M.oleifera* leaves extract on sexual behaviors

The effects of *M. oleifera* leaves extract on sexual behaviors were presented in Figure 4-5 –Figure 4-10. The data showed that rats which received vehicle and subjected to a 12 h-immobilization stress significantly increase mounted latency after single administration and at 14 days of intervention (P-value<.001 all; compared to control group) but increased intromission latency after the single administration and at 7 days of intervention (P-value<.001 and .05 respectively; compared to control group). In addition the decreased intromission number was also observed in rats which received vehicle and subjected to 12 h-immobilization stress throughout the 14 day-study period (P-value<.01, .05 and .05 respectively; compared to control group). No significant changes of ejaculation latency and number were observed. Sildenafil citrate, Tianeptine and *M.oleifera* at all doses used in this study significantly attenuated the elevation of mounting latency after the single dose administration (P-value<.001, .001, .01, .001, .05 respectively; compared to vehicle+stress treated group) and at 14-day study period (P-value<.001 all; compared to vehicle+stress treated group). After the single administration, Sildenafil citrate, Tianeptine and *M.oleifera* at all doses could attenuated the enhanced intromission latency (P-value<.001 all; compared to vehicle+stress treated group). However, only Sildenafil citrate and *M.oleifera* at dose of 10 mg.kg-1 BW could alleviated the elevation of intromission latency at 7-day study period (P-value<.05 all; compared to

vehicle+stress treated group). Sildenafil citrate also attenuated the decreased intromission number in stress rats after the single dose of administration and at 7 and 14 days of treatment (P-value<.05, .05, .01 respectively; compared to vehicle+stress treated group) whereas Tianeptine produced the significant mitigation effect on this parameter after the single dose of administration and at 14 days of treatment (P-value<.05 all; compared to vehicle+stress treated group). *M.oleifera* leaves extract significantly mitigated the decreased intromission number of stress-exposed rats at 7 and 14 days of treatment (P-value<.01 and .05 respectively; compared to vehicle+stress treated group). At 14 days of treatment, the extract at doses of 50 and 250 mg.kg^{-1} BW also mitigated the reduction of intromission number of stress-exposed rats (P-value<.05 all; compared to vehicle+stress treated group). Sildenafil citrate, Tianeptine and *M.oleifera* at all doses failed to modify the changes of both latency and number of ejaculation in stress-exposed rats.

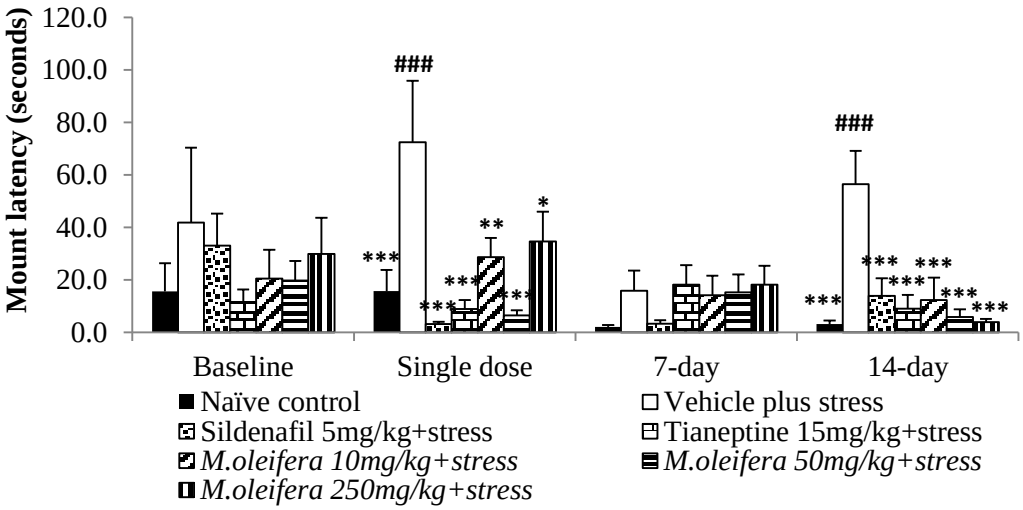


Figure 4-5 The effect of hydro-alcoholic extracts of *M. oleifera* leaves extract on mounting latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **, ***}$*P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{###}$ *P*-value <0.001; compared with control group.

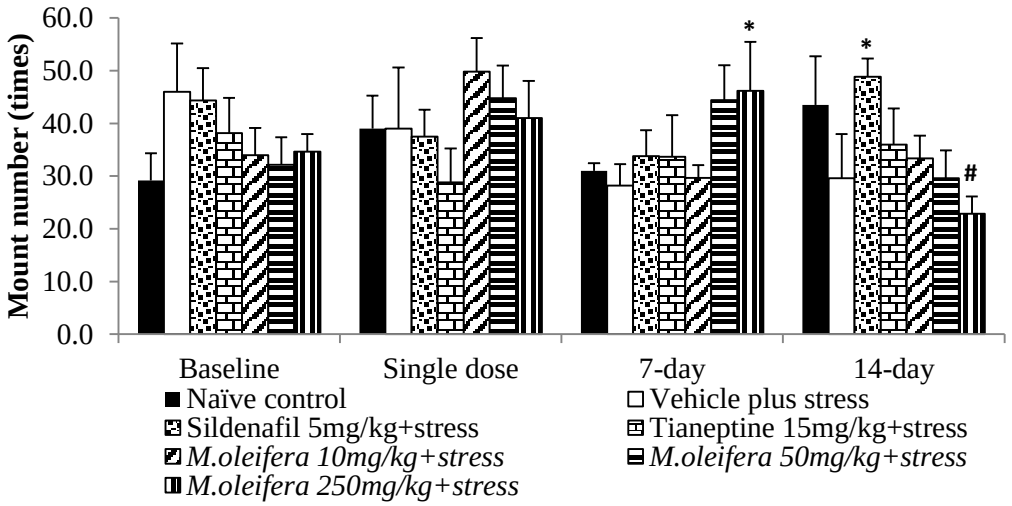


Figure 4-6 The effect of hydro-alcoholic extracts of *M. oleifera* leaves extract on mounting number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*}P$-value <0.05; compared with vehicle plus stress. $^{\#}P$-value <0.05; compared with control group

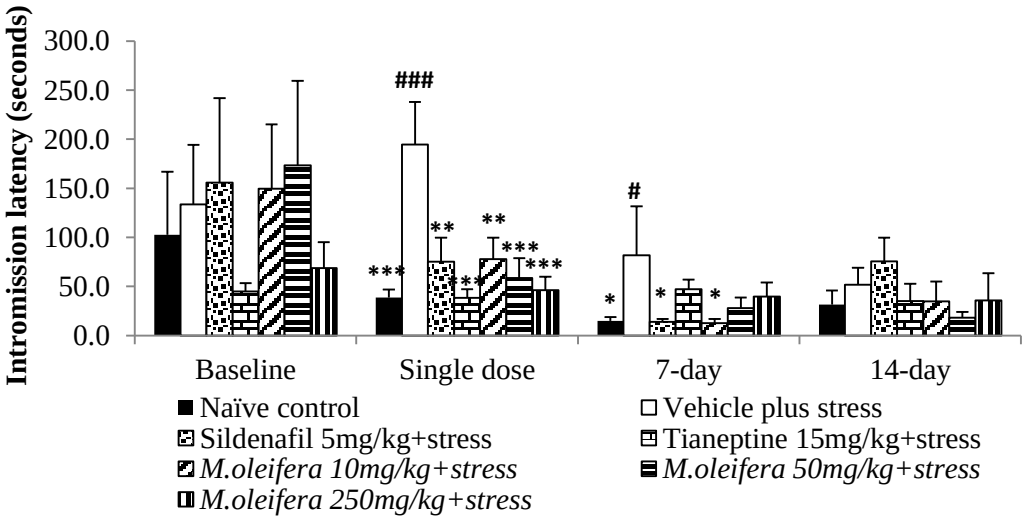


Figure 4-7 The effect of hydro-alcoholic extracts of *M. oleifera* leaves extract on intromission latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **, ***}P$-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#, \#\#\#}P$-value <0.05 and 0.001; compared with control group

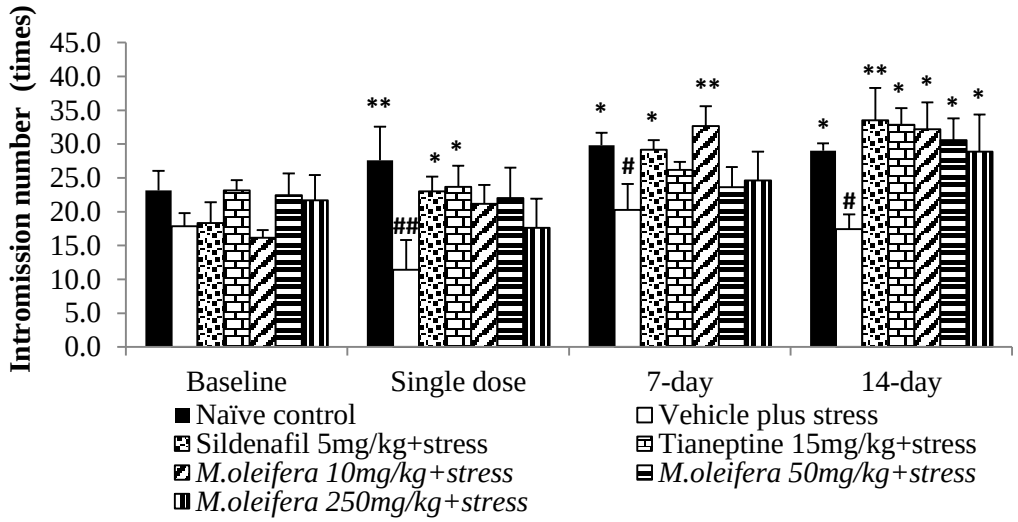


Figure 4-8 The effect of hydro-alcoholic extracts of *M. oleifera* leaves extract on intromission number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **}$*P*-value <0.05 and 0.01; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0.05 and 0.01; compared with control group.

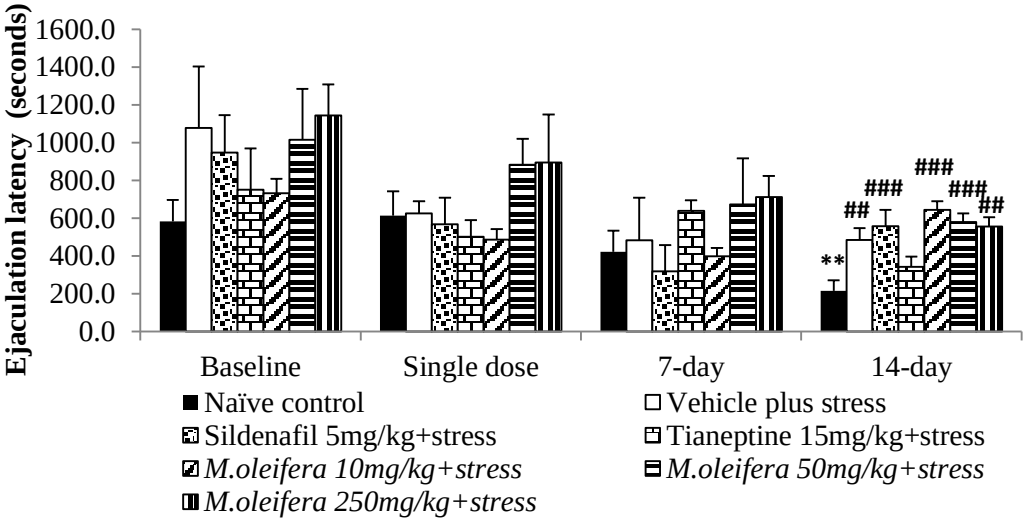


Figure 4-9 The effect of hydro-alcoholic extracts of *M. oleifera* leaves extract on ejaculation number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). ***P*-value < 0.01; compared with vehicle plus stress. $^{\#\#,\#\#\#}$ *P*-value <0.01 and 0.001; compared with control group

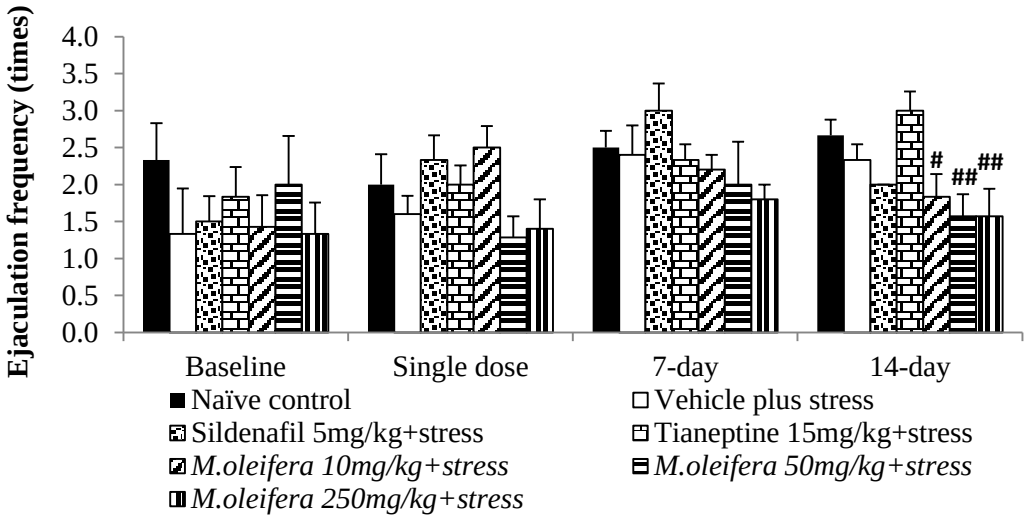


Figure 4-10 The effect of hydro-alcoholic extracts of *M. oleifera* leaves extract on ejaculation frequency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{\#,\#\#}$ *P*-value <0.05 and 0.01; compared with control group.

4.3.1.2 Effect of *M.oleifera* leaves extract on serum testosterone and corticosterone levels

Rats which received vehicle and exposed to stress significantly decreased the serum testosterone level bot at 7 and 14 days of treatment (P-value <.001 all; compared to control group). Both Sildenafil citrate and Tianeptine failed to mitigate the reduction of testosterone in stress-exposed rats throughout the study period. *M.oleifera* leaves extract at dose of 50 mg.kg-1 be could significantly mitigate the decrease of testosterone in stress-exposed rats at 7 days of treatment (P-value<.05; compared to vehicle+stress treated group) but it failed to produce the significant change of this parameter at 14 days of treatment as shown in Figure 4-11.

The effect of the extract on the serum corticosterone level was also evaluated and the results were shown in Figure 4-12. It was found that stress exposure significantly enhanced serum corticosterone levels both at 7 and 14 days of treatment (P-value<.001 all; compared to control group). Both sildenafil citrate and Tianeptine failed to attenuate the elevation of testosterone in stress-exposed rats throughout the study period. Interestingly, *M.oleifera* leaves extract at doses of 5 and

250 mg.kg^{-1} BW significantly mitigated the elevation of corticosterone level in stress-exposed rats both at 7 (P-value<.01 and .05 respectively; compared to vehicle+stress treated group) and 14 days of treatment (P-value<.05and .01 respectively; compared to vehicle+stress treated group) whereas the extract at dose of 50 mg.kg-1 BW could produce the significant mitigation effect on the enhanced corticosterone level in stress-exposed rats only at 7 days of treatment (P-value<.001; compared to vehicle + stress treated group).

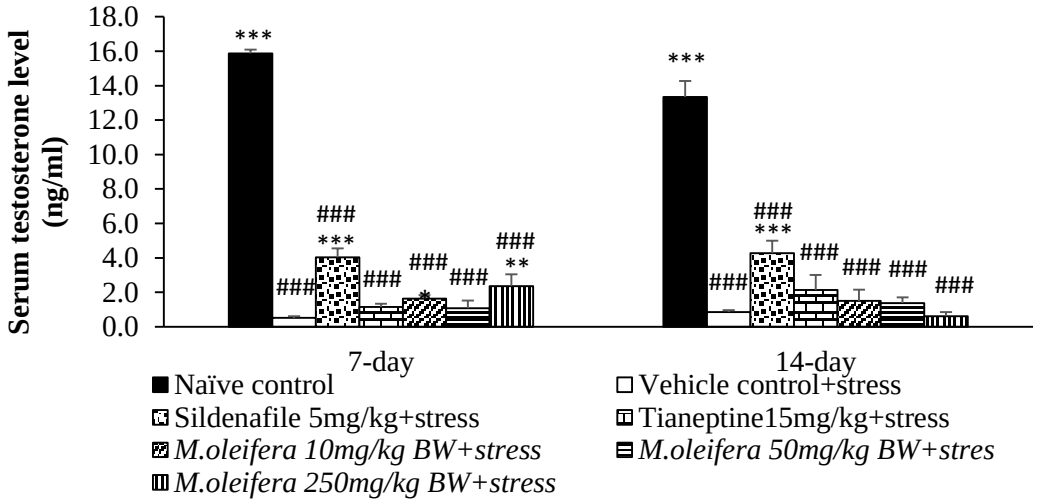


Figure 4-11 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on serum testosterone level of stress-exposed rats at 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{###}$ *P*-value <0.001; compared with control group.

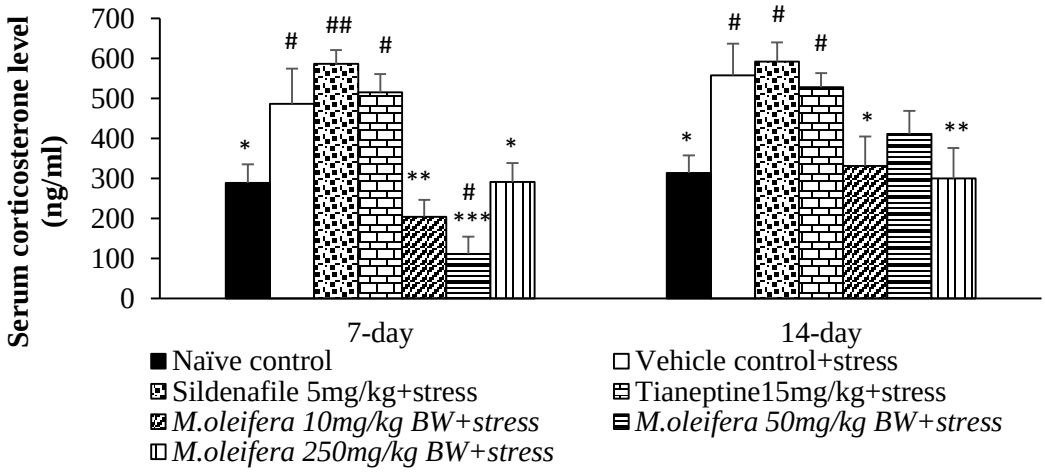


Figure 4-12 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on serum corticosterone level of stress-exposed rats at 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0.05 and 0.01; compared with control group.

4.3.1.3 Effect of *M.oleifera* leaves extract on phosphodiesterase-5 (PDE-5) activity in penis

Since PDE-5 plays a critical role on the penile blood flow which in turn induces penile erection, the effect of *M.oleifera* leaves extract on PDE-5 in penis was also evaluated and the results were shown in Figure 4-13. Rats which received vehicle and exposed to stress showed the increased PDE-5 activity in penis (P-value<.01; compared to control group). However, this change was attenuated by Sildenafil citrate, Tianeptine and *M.oleifera* leaves extract at dose of 10 $mg.kg^{-1}$ BW (P-value<.01, .05 and .05 respectively; compared to vehicle+stress treated group).

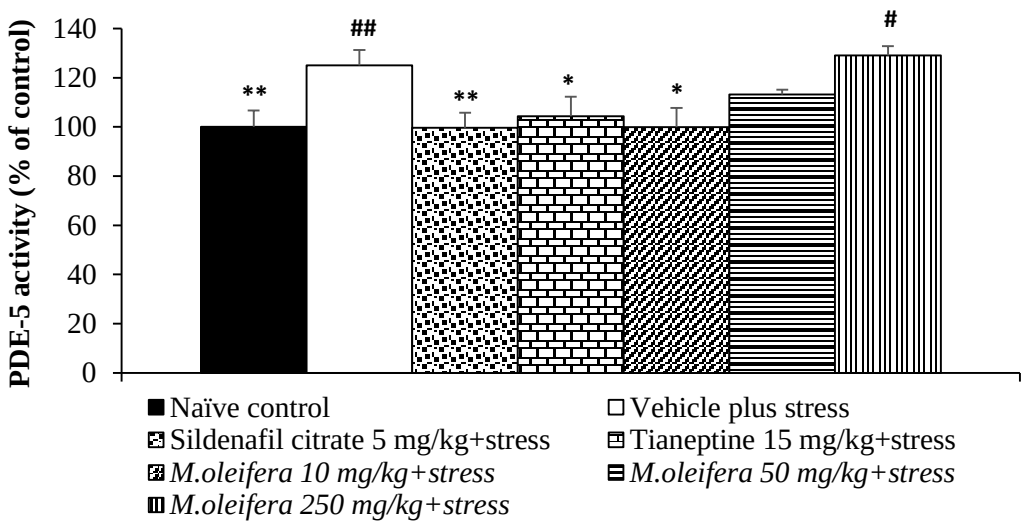


Figure 4-13 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on phosphodiesterase-5 activity in penis of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,** *P*-value <0.05 and 0.01; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0,05 and 0.01; compared with control group.

4.3.1.4 Effect of *M.oleifera* leaves extract on monoamine oxidase B activity

Based on the findings that dopamine plays an important role on the regulation of male sexual function and MAO-B is an indirect indicator which indicates the available of dopamine level, the effects of *M.oleifera* leaves extract on MAO-B in medial preoptic area (MPOA) and nucleus accumbens (NAc), the areas contributing the important role on male sexual behaviors were determined. The results were shown in Figure 4-14. Stress- exposed rats significantly enhanced MAO-B in NAc (P-value<.001; compared to control group) but not in MPOA. Sildenefil citrate significantly decreased MAO-B both in MPOA and NAc (P-value<.001 all; compared to vehicle+stress treated group) whereas Tianeptine produced the significant reduction of MAO-B only in NAc (P-value<.001; compared to vehicle+stress treated group). Both low and medium doses of the extract significantly decreased MAO-B in MPOA

(P-value<.001 and .05; compared to vehicle+stress treated group). No significant changes of this parameter were observed both in MPOA and NAc.

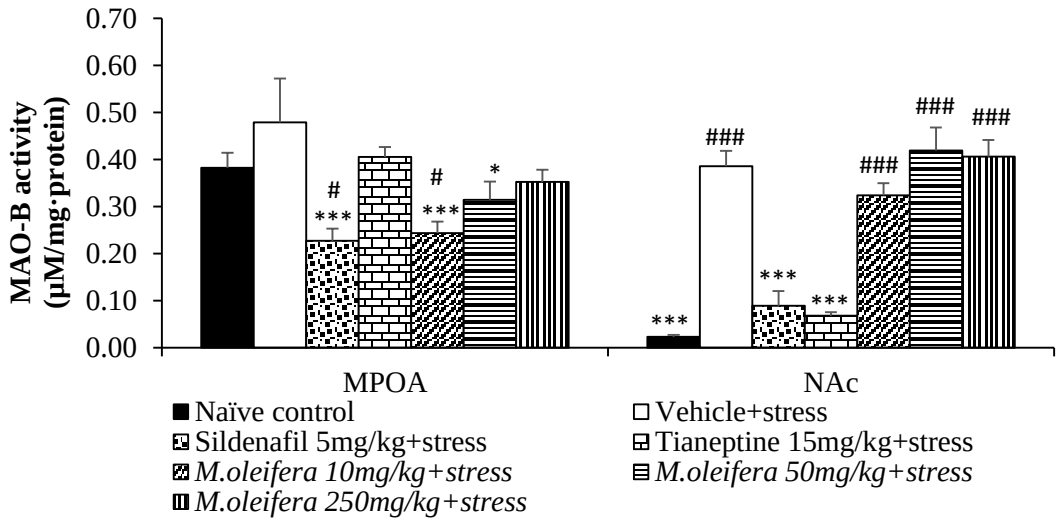
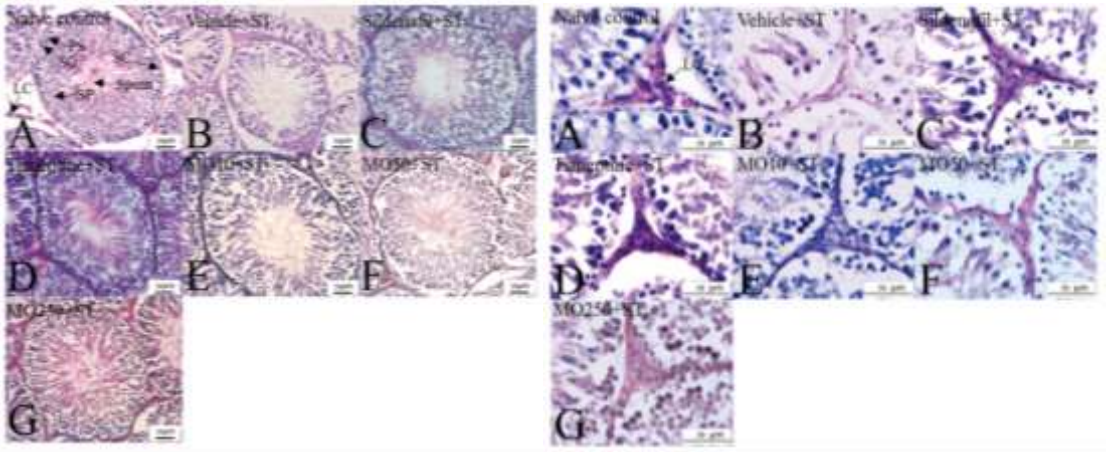


Figure 4-14 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on monoamine oxidase-B in medial preoptic area and nucleus accumbens of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,*** *P*-value <0.05 and 0.001; compared with vehicle plus stress. $^{\#,\#\#\#}$ *P*-value <0,05 and 0.001; compared with control group.

4.3.1.5 Effect of *M.oleifera* leaves extract on testicular histomorphology

The histological morphology of the testis was evaluated and the results were shown in Figure 4-15. In the vehicle+stress treated group, the seminiferous epithelium was disorganized and fewer interstitial cells of Leydig were observed. Rats subjected to stress and sildenafil, or stress plus Tianeptine, or stress plus *M. oleifera* extract at all doses used in this study showed a more organized seminiferous epithelium than those treated with the vehicle plus stress. In addition, rats in all these groups appeared to have more interstitial cells of Leydig and more spermatozoa in the lumen of the seminiferous tubules than those in the vehicle+stress treated group.



1) Seminiferous tubules **2) Interstitial cells of Leydig**

Figure 4-15 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on histomorphology of rat testis stained with Haematoxylin and eosin (H&E). The cross section photographs showed the 1) seminiferous tubules (40x magnification) and 2) interstitial cells of Leydig (100x magnification), Sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS), spermatids (SP), sperm and Leydig cells (LC) in the following treatment groups: A) naïve control B) vehicle+stress C) Sildenafil citrate 5mg/kg +stress D) Tianeptine 15mg/kg +stress E) - G) *M. oleifera* at doses of 10, 50 and 250 mg/kg+stress, respectively.

4.3.1.6 Effect of *M.oleifera* leaves extract on tyrosine hydroxylase immunoreactive neurons

Since tyrosine hydroxylase plays the crucial role on the conversion of L-DOPA, a precursor of dopamine synthesis, the density of tyrosine hydroxylase immunopositived neurons was used as indicator to reflect the density of dopaminergic neurons density. Figure 4-16 and Figure 4-17 showed the effects of *M.oleifera* leaves extract on the density of tyrosine hydroxylase immunopositived neurons in vental tegmental (VTA) and nucleus accumbens, the areas playing the pivotal roles on sexual behavior (Hull *et al.*, 1990; Matsumoto *et al.*, 2012). Rats which received vehicle and stress exposure significantly decreased density of tyrosine hydroxylase immunopositived neurons in NAC both in core (C) and shell (S) areas

(P-value<.05 and .01 respectively; compared to control group). Both Sildenefil citrate and Tianeptine could mitigate the decreased density of tyrosine hydroxylase immunopositived neurons in NAcC (P-value<.01 all; compared to vehicle+stress group) and NAcS (P-value<.001 and .05 respectively; compared to vehicle+stress group). It was found that *M.oleifera* leaves extract at doses of 10 and 250 $mg.kg^{-1}$ BW significantly mitigated the decreased tyrosine hydroxylase immunopositived neurons in NAcC (P-value<.05 and .01 respectively; compared to vehicle+stress group) of stress-exposed rats. However, the extract at all doses used in this study (10, 50 and 250 mg.kg-1 BW) could mitigate mitigated the decreased tyrosine hydroxylase immunopositived neurons in NAcS (P-value<.01, .05 and .001 respectively; compared to vehicle+stress group) of stress-exposed rats.

The reduction of tyrosine hydroxylase immunopositived neurons in VTA of rats which received vehicle and subjected to immobilization stress (P-value<.05; compared to control group). Interestingly, this change was attenuated by Tianeptine and the extract at dose of 250 mg.kg-1 BW (P-value<.05 and .001 respectively; compared to vehicle+stress treated group) as shown in Figure 4-15.

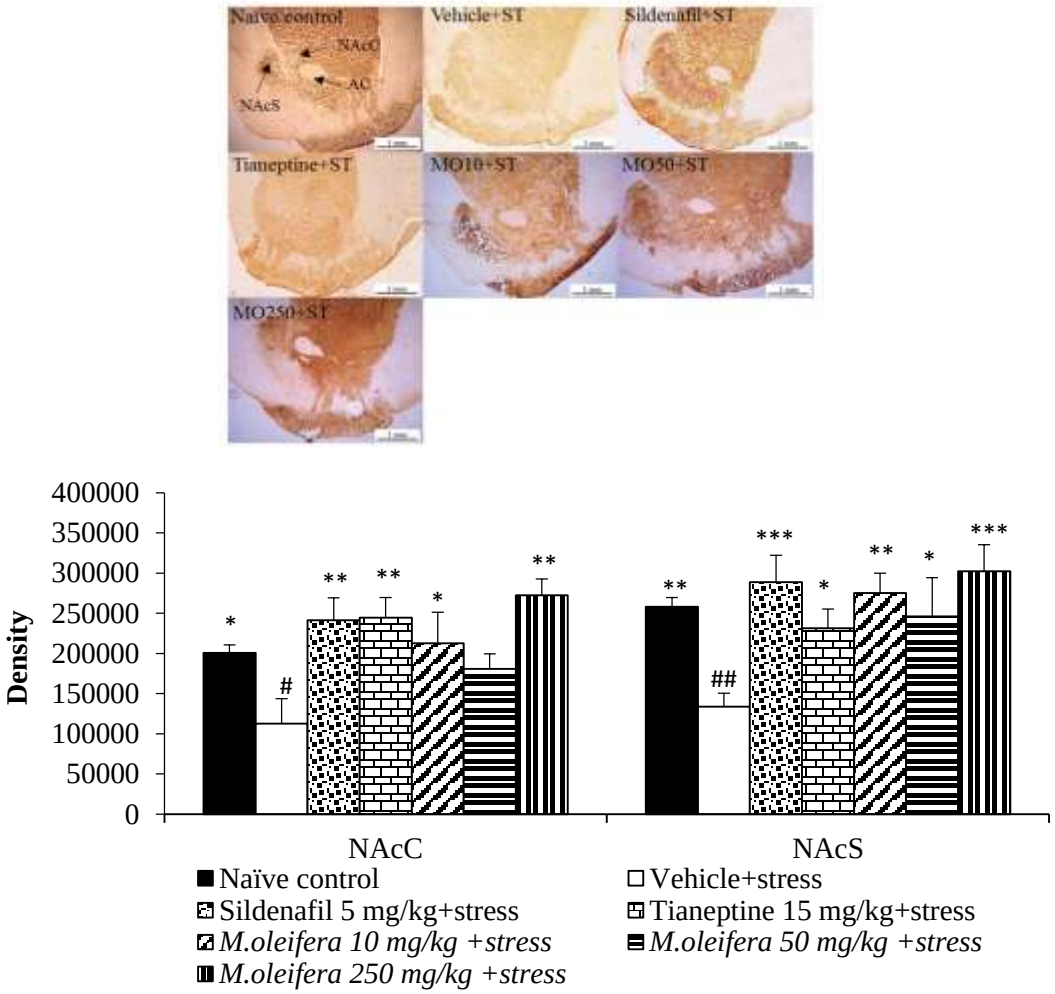


Figure 4-16 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on tyrosine hydroxylase immunoreactive neurons in core and shell of nucleus accumbens of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0,05 and 0.01; compared with control group

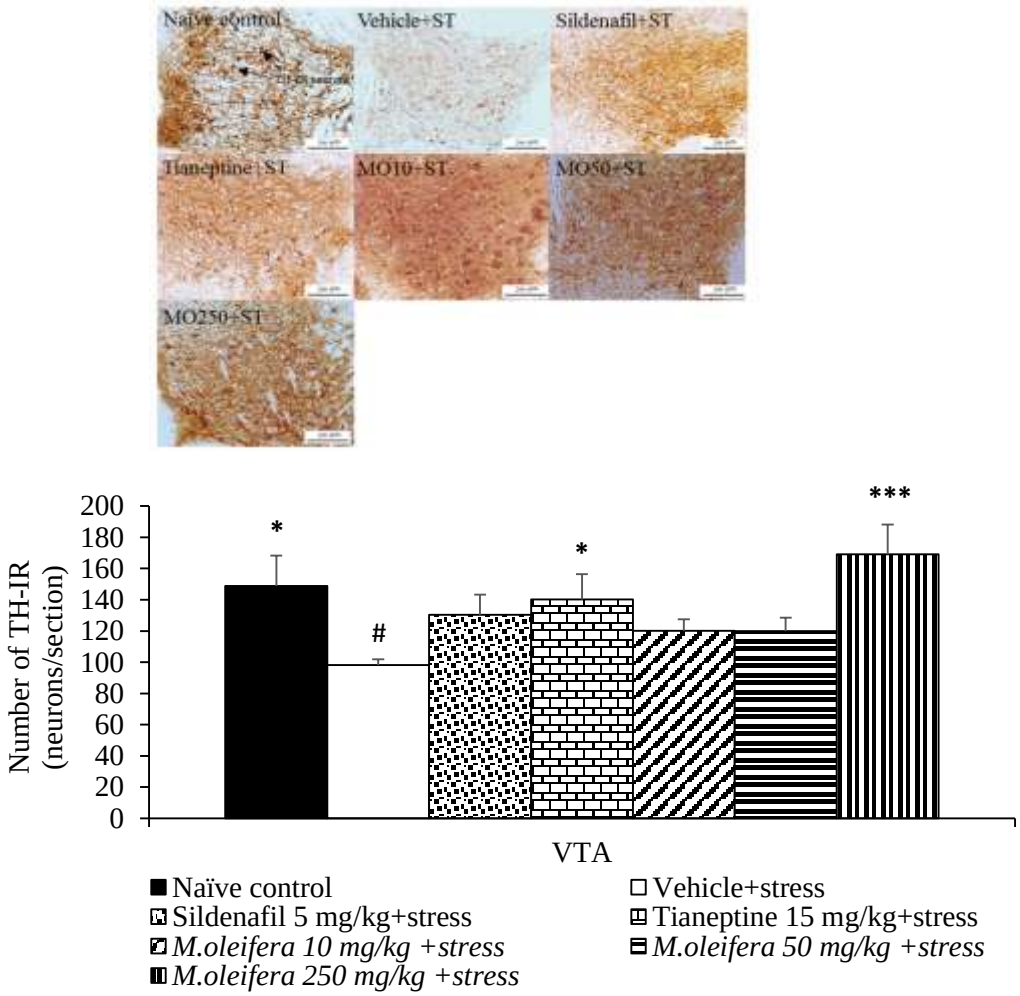


Figure 4-17 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on tyrosine hydroxylase immunoreactive neurons in ventral tegmental area of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,*** *P*-value <0.05 and 0.001; compared with vehicle plus stress. $^{\#}$ *P*-value <0.05; compared with control group.

4.3.1.7 Effect of *M.oleifera* leaves extract on endothelial nitric oxide synthase in penis

Since nitric oxide (NO) essential for penile erection, the effect of the extract on endothelial nitric oxide synthase (eNOS), an enzyme playing a crucial role on NO synthesis, in penis was investigated and results were shown in Figure 4-18. It was found that stress exposure decreased the expression of eNOS in penis of rats (P-value<.05; compared to control group). Interestingly, the extract at

doses of 10 and 250 mg.kg-1 BW could restore this change to normal level (P-value<.05 all; compared to vehicle+stress treated group)

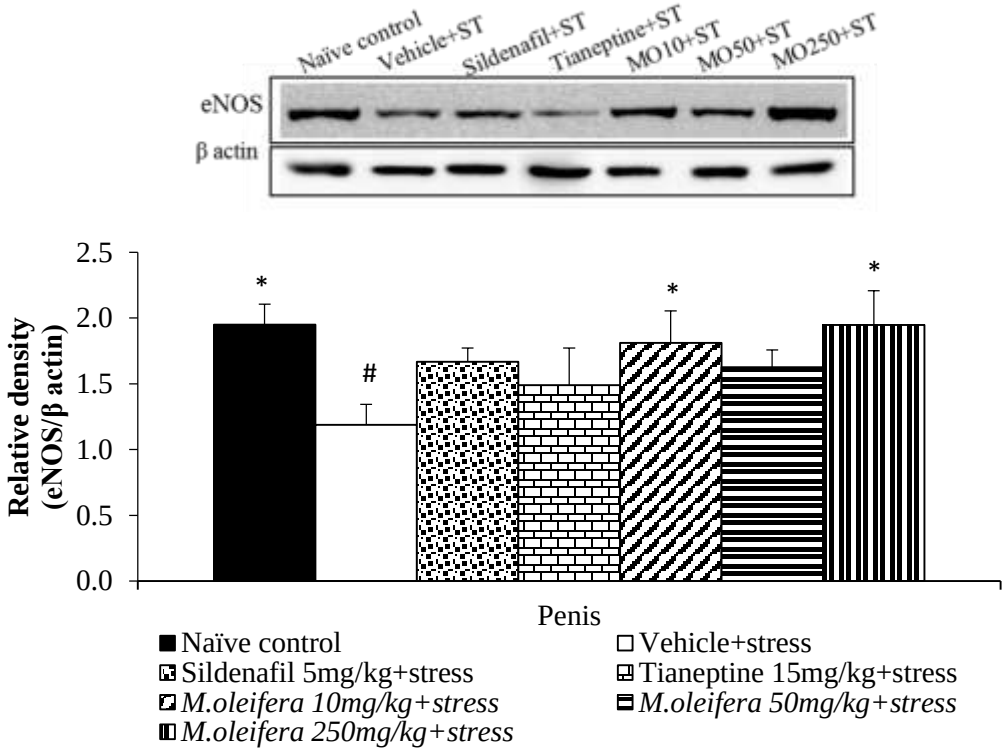


Figure 4-18 The effect of hydro-alcoholic extracts of *M.oleifera* leaves extract on endothelial nitric oxide synthase in penis of stress-exposed rats. Data were expressed as mean±S.E.M. (n=4-5/group). * *P*-value <0.05; compared with vehicle plus stress. # *P*-value <0,05; compared with control group.

4.3.2 Effect and possible underlying mechanism of *A.occidentale* leaves extract on sexual dysfunction in immobilization rats

4.3.2.1 Effect of *A.occidentale* leaves extract on male sexual behaviors

The effects of *A.occidentale* leaves extract on sexual behaviors were presented in Figure 4-19 – 4-24. Stress-exposed rats which received vehicle significantly increased mounting latency and intromission number after the

single dose of administration (P-value<.001 and .01 respectively; compared to control group), and at 7 (P-value<.05 and .01 respectively; compared to control group) and 14 days of treatment (P-value<.001 and .05 respectively; compared to control group). In addition, the increased ejaculation latency was also observed in stress-exposed rats at 14 days of treatment (P-value<.05; compared to control group). The elevation of mounting latency in stress-exposed rats was attenuated by Sildenefil citrate, Tianeptine and *A.occidentale* at doses of 5, 100 and 200 $mg.kg^{-1}$ BW after the single administration (P-value<.001, .001, .01, .001 and .001 respectively; compared to vehicle+stress treated group) and at 14-day study period (P-value<.001, .001, .01, .01 and .01 respectively; compared to vehicle+stress treated group). Stress-exposed rats which received *A.occidentale* leaves extract at all doses used in this study also increased mounting number in stress exposed rats at 7 days of treatment (P-value<.05 all; compared to vehicle+stress treated group). When the treatment was prolonged to 14 days, only stress-exposed rats which subjected to the treatment of low dose of extract showed the enhanced mounting number (P-value<.05; compared to vehicle+stress treated group). The increased intromission latency induced by stress exposure was also attenuated by a single dose of administration of Sildenefil citrate, Tianeptine and *A.occidentale* at all doses used in this study (P-value<.01, .001, .05, .01 and .01 respectively; compared to vehicle+stress treated group). The decreased intromission latency in stress exposed rats which received Sildenefil citrate still presented at 7 days of treatment (P-value<.05; compared to vehicle+stress treated group). Sildenefil citrate, Tianeptine and *A.occidentale* at doses of 100 and 200 $mg.kg^{-1}$ BW significantly enhanced the intromission number in stress exposed rats (P-value<.05, .05, .01 and .05 respectively; compared to vehicle+stress treated group) after the single administration. The enhanced intromission numbers in stress-exposed rats at 7 days of treatments were observed in Sidenefil citrate and both low and high doses of *A.occidentale* leaves extract treated groups. At 14 days of treatment, the increased intromission numbers were observed in stress-exposed rats which treated with Sildenefil citrate, Tianeptine and both medium and high doses of extract (P-value<.01, .01, .05 and .05 respectively; compared to vehicle+stress treated group). Sildenefil citrate also decreased ejaculation latency in stress exposed rats (P-

value<.01; compared to vehicle+stress treated group) whereas both medium and high doses of extract decreased ejaculation latency (P-value<.05 and .01; compared to vehicle+stress treated group) but increased ejaculation number (P-value<.05 and .01; compared to vehicle+stress treated group) in stress-exposed rats.

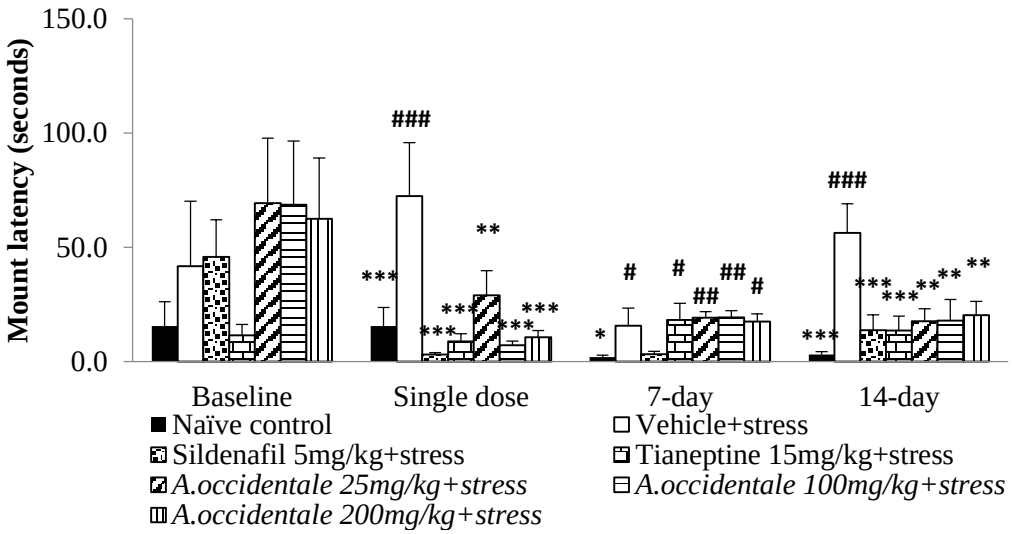


Figure 4-19 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on mounting latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,**,****P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#,\#\#\#}$ *P*-value <0.05, 0.01 and 0.001; compared with control group

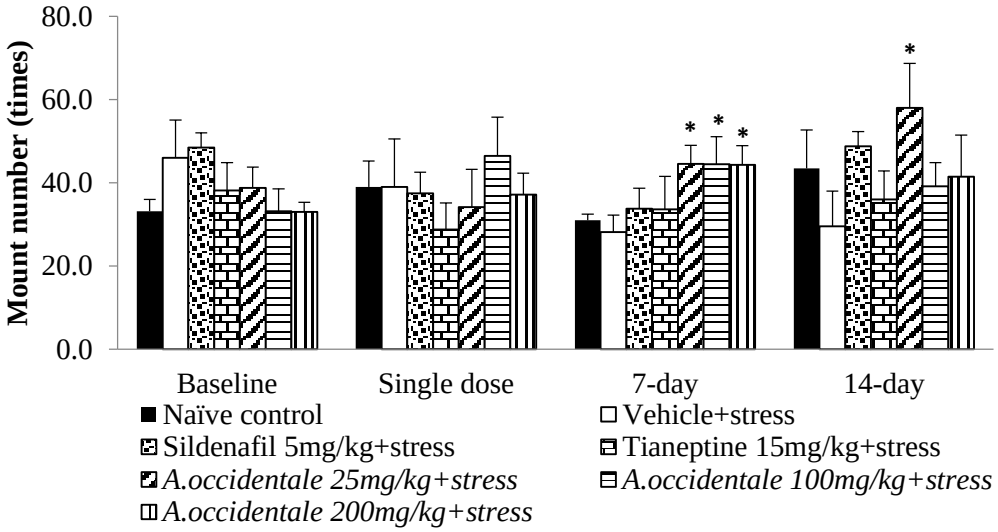


Figure 4-20 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on mounting number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). * *P*-value <0.05; compared with vehicle plus stress

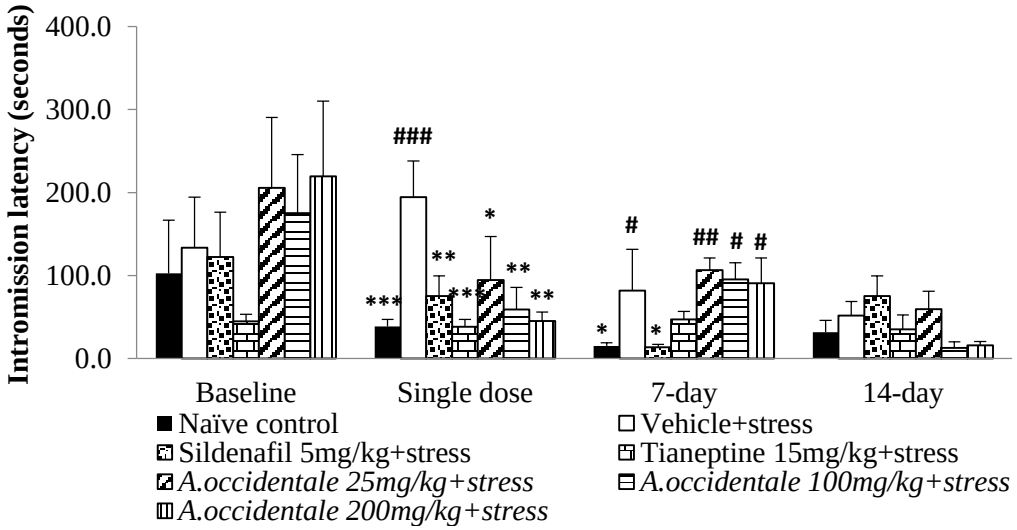


Figure 4-21 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on intromission latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *, **,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. #,##,### *P*-value <0.05, 0.01 and 0.001; compared with control group

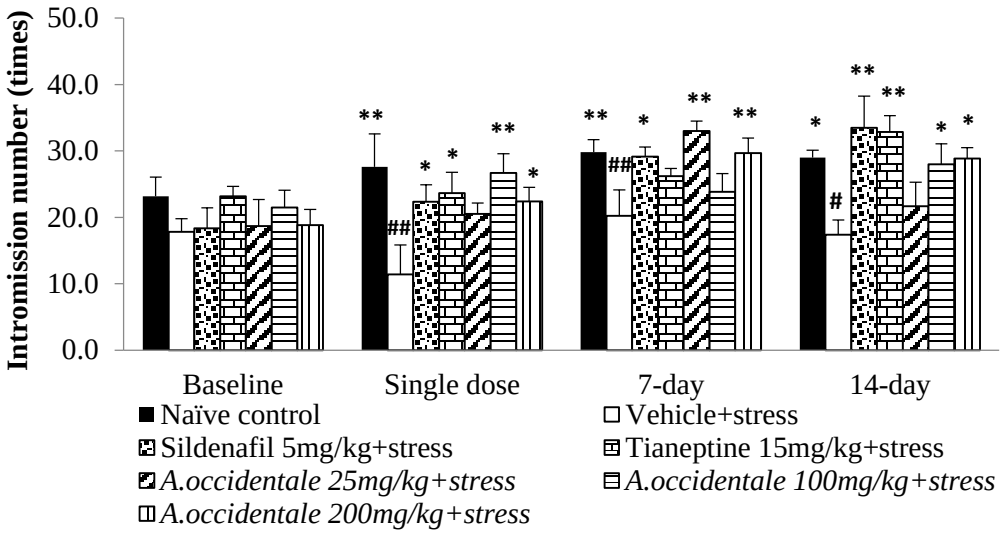


Figure 4-22 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on intromission number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **}$ *P*-value <0.05 and 0.01; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0.05 and 0.01; compared with control group

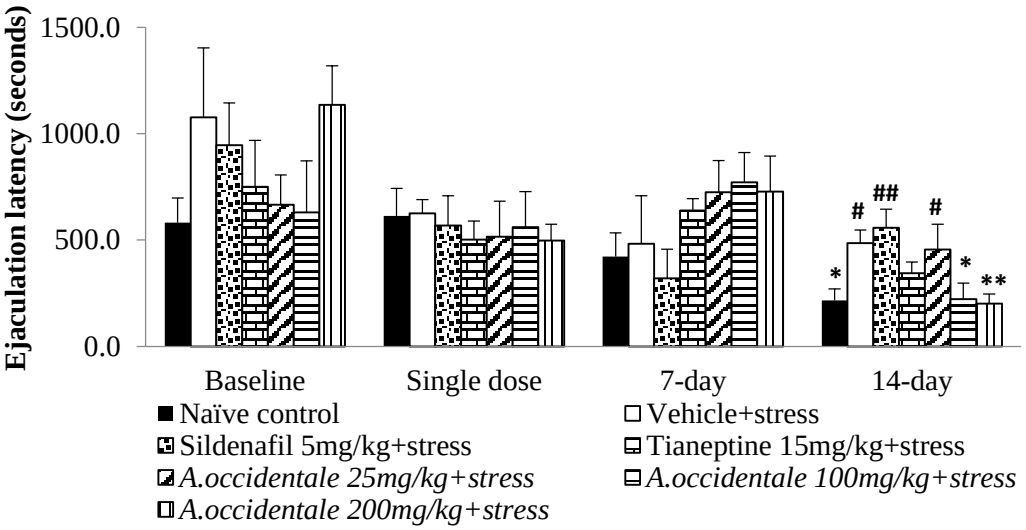


Figure 4-23 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on ejaculation latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). * *P*-value <0.05; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0.05 and 0.01; compared with control group

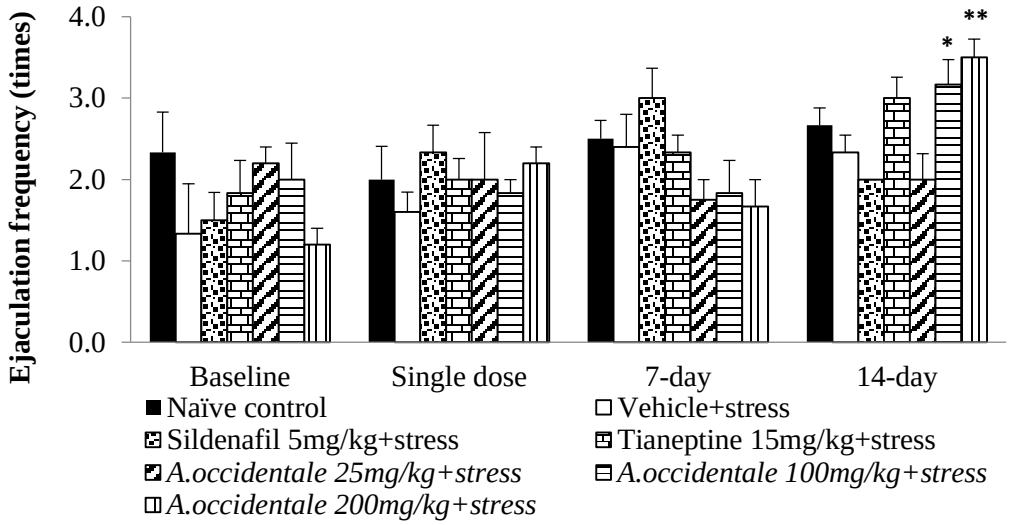


Figure 4-24 The effect of hydro-alcoholic extract of *A.occidentale* leaves extract on ejaculation frequency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,** *P*-value <0.05 and 0.01; compared with vehicle plus stress.

4.3.2.2 Effect of *A.occidentale* leaves extract on serum testosterone and corticosterone levels

The effect of *A.occidentale* leaves extract on serum testosterone level was shown in Figure 4-25. Stress exposure significantly decreased testosterone levels at 7 and 14 days of intervention (P-value<.001 all; compared to control group) Sildenefil citrate and A.occidentale leaves extract at dose of 200 mg.kg-1 BW increased serum testosterone levels only at 1-day study period (P-value<.05 all; compared to vehicle+stress treated group) while the extract at doses of 100 and 250 mg.kg-1 BW produced the significant elevation of serum testosterone both at 7 and 14 days of treatment (P-value<.001 all; compared to vehicle+stress treated group).

Figure 4-27 showed the effect of *A.occidentale* leaves extract on serum corticosterone level. It was found that only stress-exposed rats which received the medium dose of extract significantly decreased serum corticosterone level (P-value<.05; compared to vehicle+stress treated group).

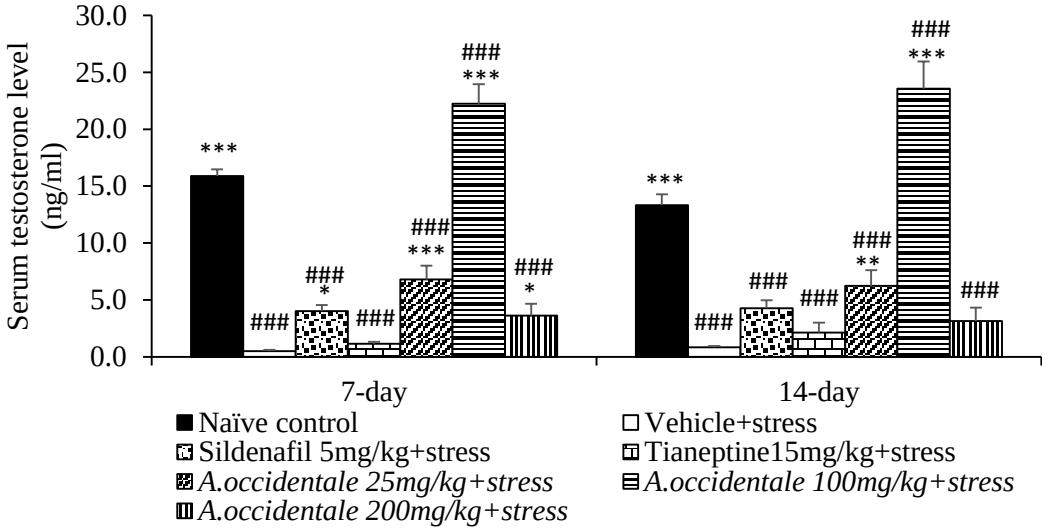


Figure 4-25 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on serum testosterone level of stress-exposed rats at 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{###}$ *P*-value <0.001; compared with control group.

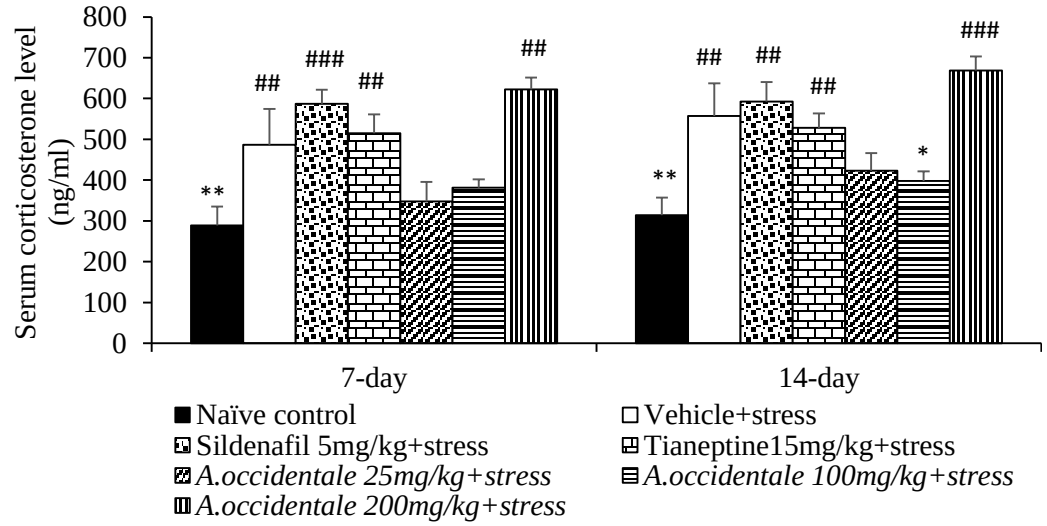


Figure 4-26 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on serum corticosterone level of stress-exposed rats at 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,** *P*-value <0.05 and 0.01; compared with vehicle plus stress. $^{##,###}$ *P*-value <0.01 and 0.001; compared with control group

4.3.2.3 Effect of *A.occidentale* leaves extract on phosphodiesterase-5 (PDE-5) activity

Figure 4-27 showed the effect of *A.occidentale* leaves extract on phosphodiesterase-5 activity in penis. Rats which received vehicle and subjected to immobilization stress showed the increased PDE-5 activity in penis (P-value<.05; compared to control group). However, this change was mitigated by Sildenefil citrate, Tianeptine and *A.occidentale* leaves extract at doses of 25, 100 and 200 mg.kg^{-1} BW (P-value<.05, .05, .05, .001 and .01 respectively; compared to vehicle+stress treated group)

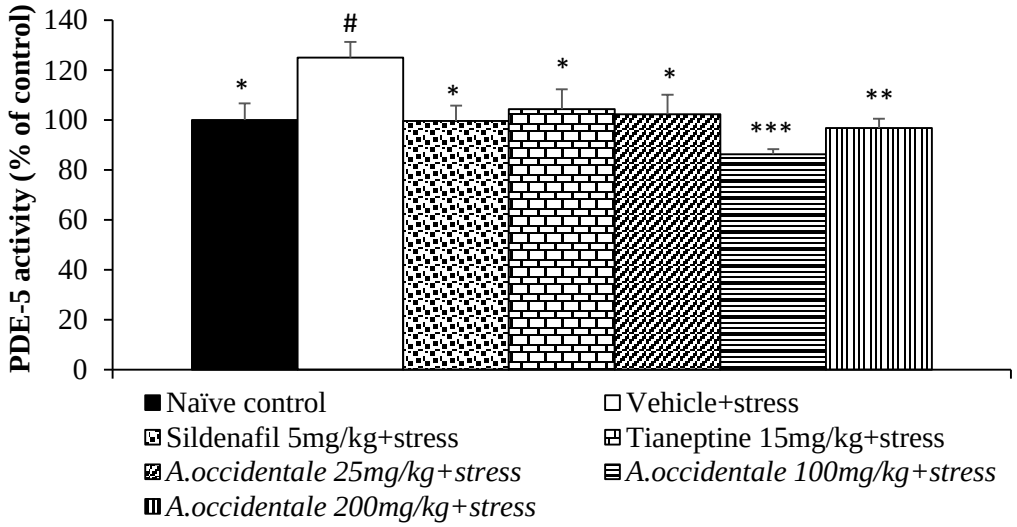


Figure 4-27 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on phosphodiesterase-5 activity in penis of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.01; compared with vehicle plus stress. $^{\#}$ *P*-value <0,05; compared with control group

4.3.2.4 Effect of *A.occidentale* leaves extract on monoamine oxidase B activity

The effects of *A.occidentale* leaves extract on MAO-B activity in MPOA and NAc were shown in Figure 4-28. The current data showed that stress exposure significantly increased MAO-B activity in NAc (P-value<.001;

compared to control group) but not in MPOA. Sildenefil citrate and the extract at doses of 100 and 200 mg.kg^{-1} BW produced significant reduction of MAOB both in MPOA (P-value<.001, .05 and .05; compared to vehicle+stress) and NAC (P-value<.001 all; compared to vehicle+stress) of stress-exposed rats. Tianeptine and *A.occidentale* leaves extract at dose of 25 mg.kg^{-1} BW also significantly decreased MAO-B in NAC (P-value<.001 all; compared to vehicle+stress).

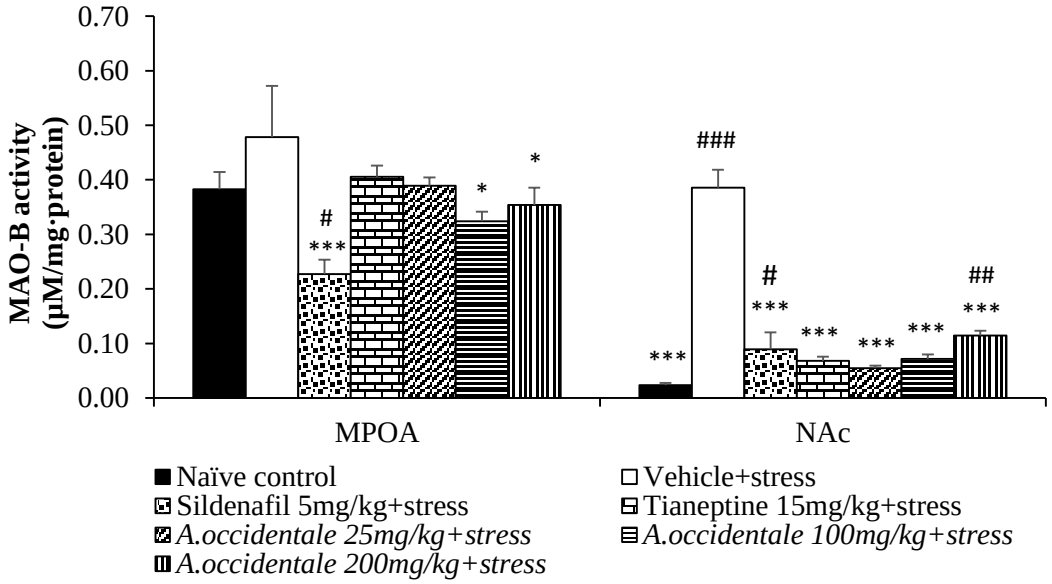
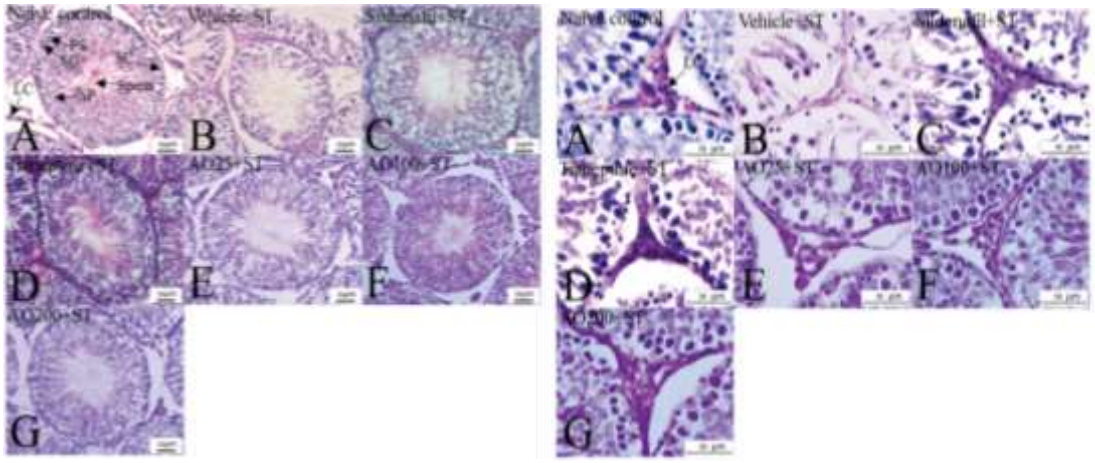


Figure 4-28 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on monoamine oxidase-B in medial preoptic area and nucleus accumbens of stress-exposed rats. Data were expressed as mean± S.E.M. (n=6/group). *,*** *P*-value <0.05 and 0.001; compared with vehicle plus stress. $^{\#,\#\#\#}$ *P*-value <0,05 and 0.001; compared with control group

4.3.2.5 Effect of *A.occidentale* leaves extract on testicular histomorphology

The results were shown in Figure 4-29. In the vehicle+stress treated group, the seminiferous epithelium was disorganized and fewer interstitial cells of Leydig were observed. Rats subjected to stress and sildenafil, or stress plus

Tianeptine, or stress plus *A.occidentale* extract at all doses used in this study showed a more organized seminiferous epithelium than those treated with the vehicle plus stress. In addition, rats in all these groups appeared to have more interstitial cells of Leydig and more spermatozoa in the lumen of the seminiferous tubules than those in the vehicle+stress treated group.



1) Seminiferous tubules 2) Interstitial cells of Leydig

Figure 4-29 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on histomorphology of rat testis stained with Haematoxylin and eosin (H&E). The cross section photographs showed the 1) seminiferous tubules (40x magnification) and 2) interstitial cells of Leydig (100x magnification), Sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS), spermatids (SP), sperm and Leydig cells (LC) in the following treatment groups: A) naïve control B) vehicle+stress C) Sildenafil citrate 5mg/kg+stress D) Tianeptine 15mg/kg +stress E) -G) *A.occidentale* at doses of 25, 100 and 200 mg/kg+stress, respectively.

4.3.2.6 Effect of *A.occidentale* leaves extract on tyrosine hydroxylase immunoreactive neurons

The present data revealed that rats which received vehicle+stress significantly decreased the density of tyrosine hydroxylase immunopositved cells in both NAcC and NAcS (P-value<.05 and .01 respectively;

compared to control group). However, these changes were mitigated by Sildenefil citrate (P-value<.01 and .001 respectively; compared to vehicle+stress treated group), Tianeptine (P-value<.01 and .05 respectively; compared to vehicle+stress treated group) ans *A.occidentale* leaves extract at doses of 25, 100 and 200 $mg.kg^{-1}$ BW (.01 all; .001 all; .001, .01 respectively; compared to vehicle+stress treated group) as shown in Figure 4-30.

The effect of *A.occidentale* leaves extract on the density of tyrosine hydroxylase immunopositved cells in VTA was also investigated and results were shown in Figure 4-31. Rats which exposed to vehicle and immobilization stress showed the significant decrease in density of tyrosine hydroxylase immunopositved cells in VTA (P-value<.05; compared to control group). This change was attenuated by Tianeptine and both medium and high doses of extract (P-value<.05, .05 and .001 respectively; compared to vehicle+stress treated group).

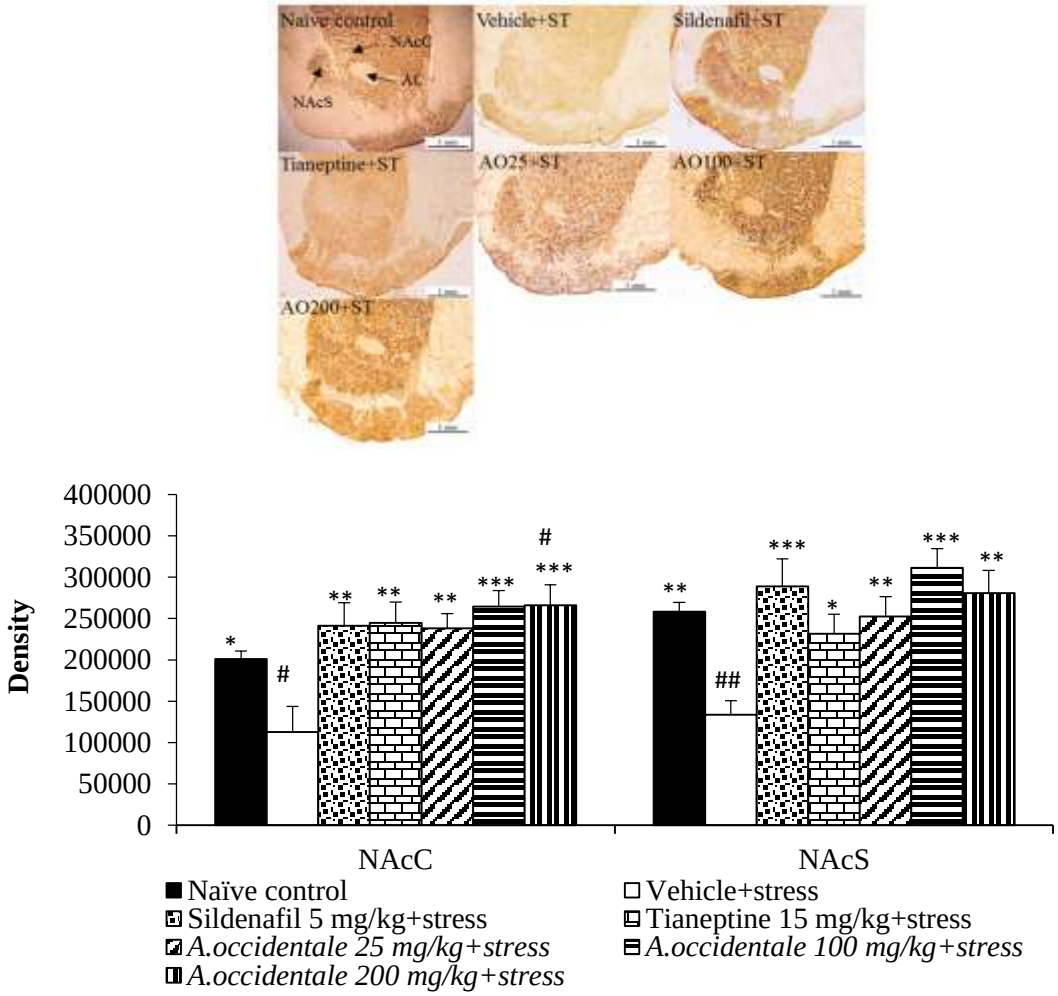


Figure 4-30 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on tyrosine hydroxylase immunoreactive neurons in core and shell of nucleus accumbens of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0,05 and 0.01; compared with control group

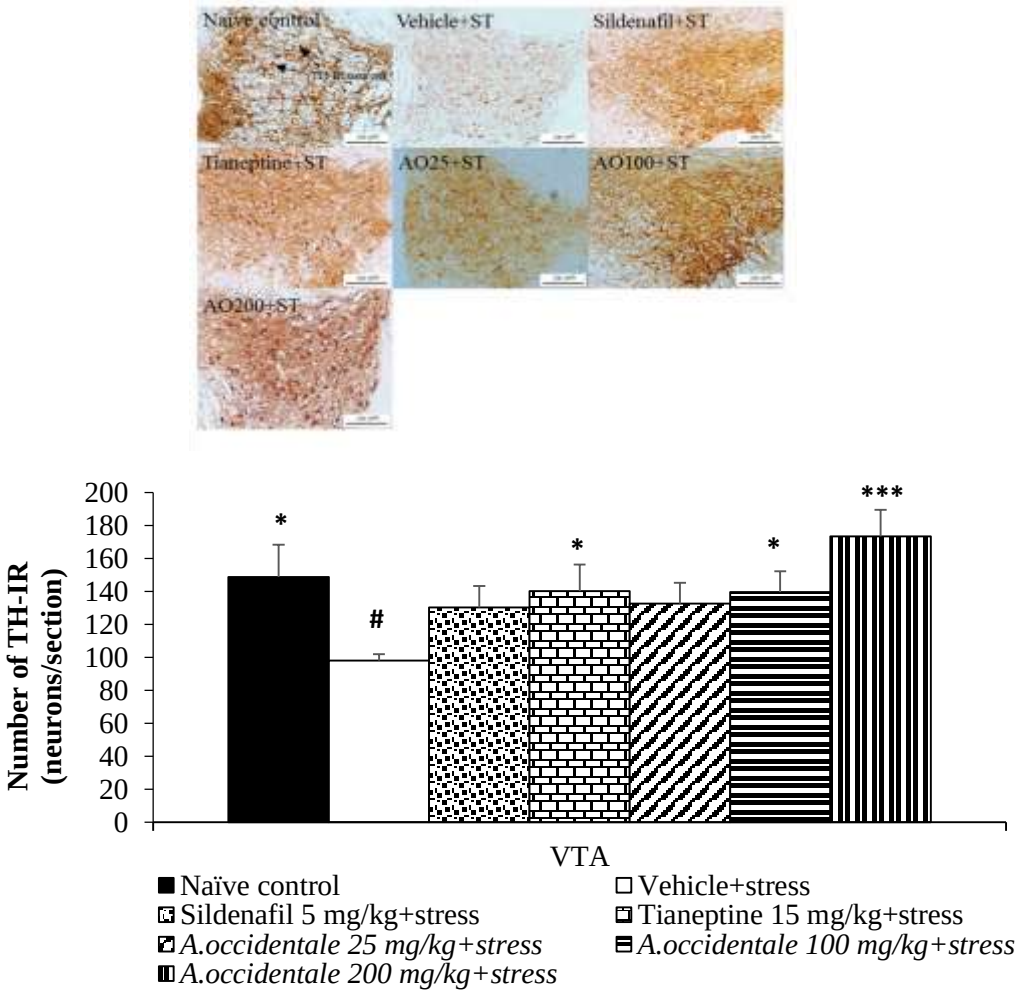


Figure 4-31 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on tyrosine hydroxylase immunoreactive neurons in ventral tegmental area of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,*** *P*-value <0.05 and 0.001; compared with vehicle plus stress. $^{\#}$ *P*-value <0,05; compared with control group.

4.3.2.7 Effect of *Anacardium occidentale* leaves extract on endothelial nitric oxide synthase (eNOS) in penis

Figure 4-32 showed that stress significantly decreased the expression of eNOS in penis (P-value<.05; compared to control group). Interestingly, this change was mitigated by the high dose of *A.occidentale* leaves extract (P-value<.05; compared to vehicle+stress treated group).

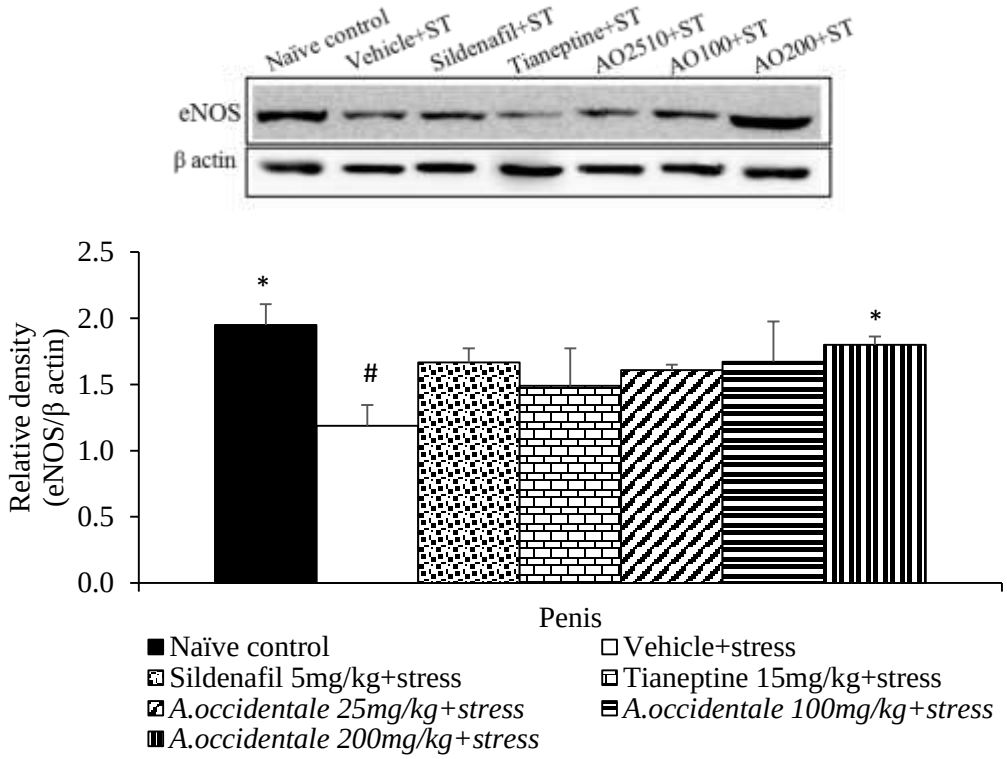


Figure 4-32 The effect of hydro-alcoholic extracts of *A.occidentale* leaves extract on endothelial nitric oxide synthase in penis of stress-exposed rats. Data were expressed as mean±S.E.M. (n=4-5/group). * *P*-value <0.05; compared with vehicle plus stress. # *P*-value <0,05; compared with control group

4.3.3 Effect and possible underlying mechanism of *N.nucifera* flowers extract on sexual dysfunction in restraint rats

4.3.3.1 Effect of *N.nucifera* flowers extract on sexual behaviors

Figure 4-33 –Figure 4-38 demonstrated the effect of *N.nucifera* flowers extract on male sexual behaviors of stress-exposed rats. An immobilization stress produced significant increase in mounting latency after the single administration and at 7 and 14 days of treatment (P-value<.01, .05 and .001 respectively; compared to control group) but produced no change on mounting frequency. The elevation of mounting latency both after the single administration and at 14 days of treatment were observed in stress-exposed rats which were treated with

Sildenefil cirate (P-value<.001 and .01 respectively; compared to vehicle+stress treated group), Tianeptine (P-value<.01 and .001 respectively; compared to vehicle+stress treated group) and *N.nucifera* at doses of 100 (P-value<.05 and .01 respectively; compared to vehicle+stress treated group) and 200 mg.kg-1 BW (P-value<.05 all; compared to vehicle+stress treated group). Immobilization stress also increased intromission latency after the single dose of administration and at 7 days of intervention (P-value<.01 and .05 respectively; compared to control group) but no change was observed at 14 days of treatment. The decreased intromission number after the single dose of administration and at 7 and 14 days of intervention (P-value<.01, .05 and .05 respectively; compared to control group). After the single dose of treatment, the increased intromission numbers were observed in Sildenefil citrate, Tianeptine and *N.nucifera* flowers extract at doses of 10, 100 and 200 $mg.kg^{-1}$ BW (.01, .01, .01, .001 and .01 respectively; compared to vehcle+stress treated group). At 7 days of treatment, the increased intromission number was observed in stress-exposed rats which received the low and medium dose of extract (P-value<.01 and .0; respectively; compared to vehicle+stress treated group). When the treatment was prolonged to 14 days, the elevation of intromission numbers were observed in Sildenefil citrate, Tianeptine and *N.nucifera* flowers extract at doses of 100 and 200 $mg.kg^{-1}$ BW (P-value< .01, .01,.05 and .05 respectively; compared to vehicle+stress treated group). It was found that immobilization stress could increase ejaculation latency at 4 days of treatment (P-value<.01; compared to control group) but produced no change on ejaculation number. At 14-day study period, Tianeptine could enhance ejaculation number. The present data clearly demonstrated that *N.nucifera* flowers extract decreased ejaculation latency (P-value<.01, .05 and .05 respectively; compared to vehicle+stress treated group) but increased ejaculation number (P-value<.05 all; compared to vehicle+stress treated group).

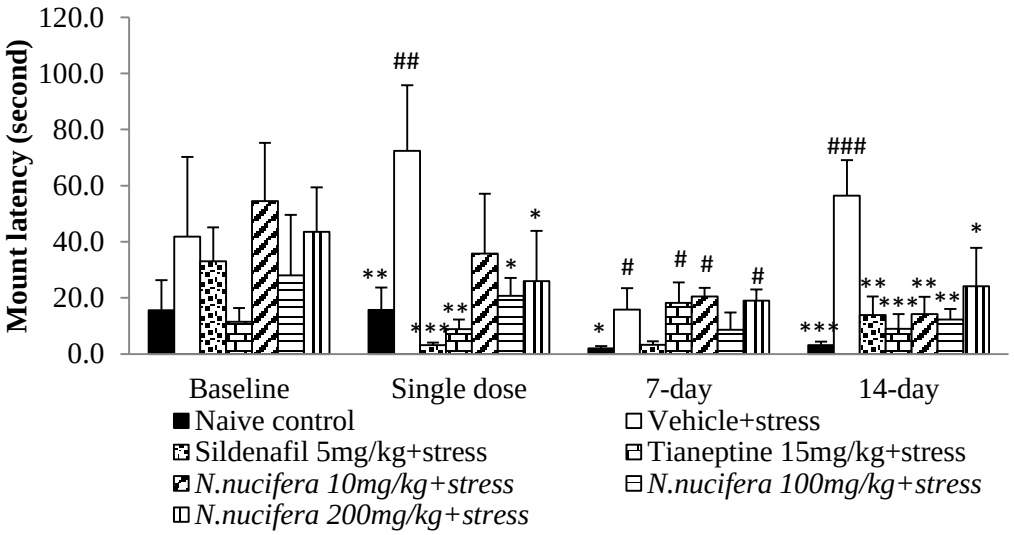


Figure 4-33 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on mounting latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **, ***}$*P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#,\#\#\#}$ *P*-value <0.05, 0.01 and 0.001; compared with control group

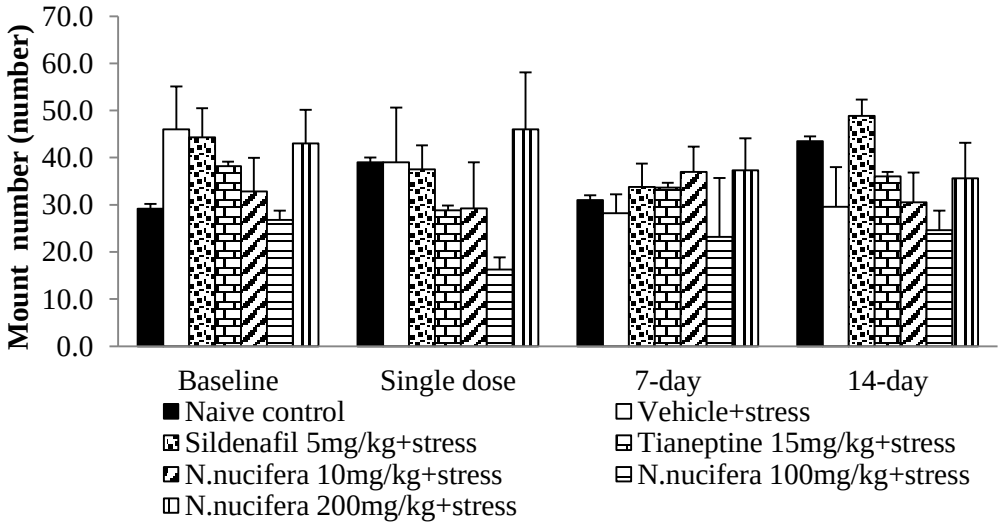


Figure 4-34 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on mounting number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group)

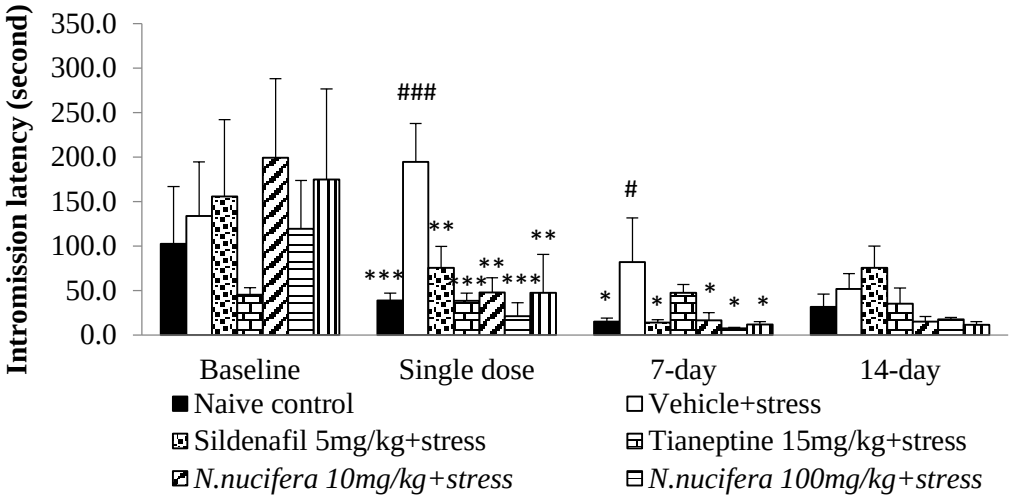


Figure 4-35 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on intromission latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **, ***}$*P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#, \#\#\#}$ *P*-value <0.05 and 0.001; compared with control group

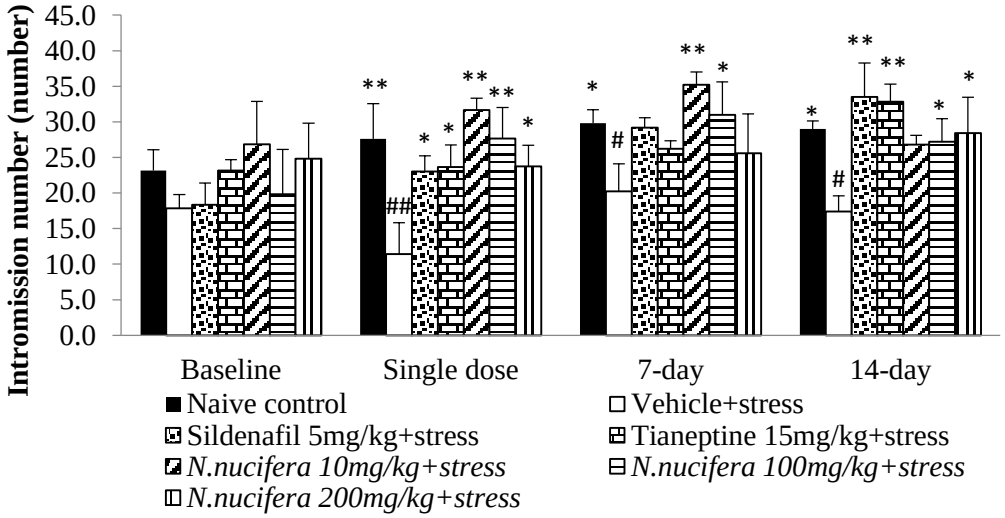


Figure 4-36 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on intromission number of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **}$ *P*-value <0.05 and 0.01; compared with vehicle plus stress. $^{\#, \#\#}$ *P*-value <0.05 and 0.01; compared with control group

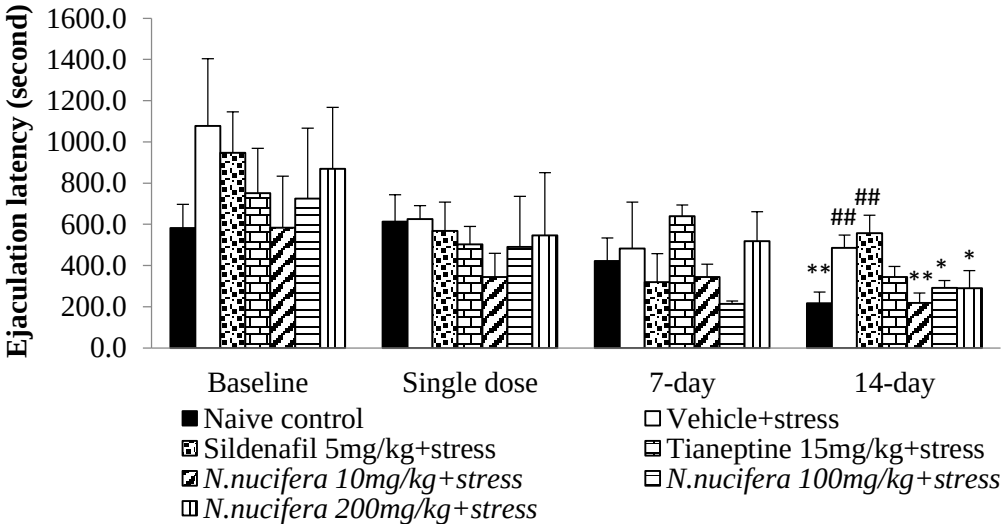


Figure 4-37 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on ejaculation latency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). $^{*, **}$*P*-value <0.05 and 0.01; compared with vehicle plus stress. $^{\#\#}$ *P*-value <0.01; compared with control group

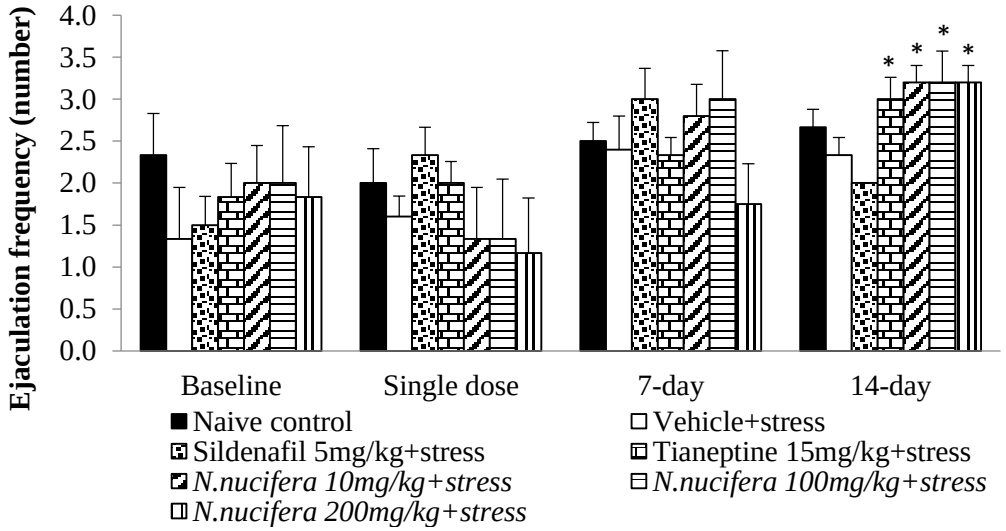


Figure 4-38 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on ejaculation frequency of stress-exposed rats at baseline, after a single dose, 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). * *P*-value <0.05; compared with vehicle plus stress

4.3.3.2 Effect of *N.nucifera* flowers extract on serum testosterone and corticosterone levels

The current data showed that immobilization stress significantly decreased serum testosterone level both at 7 and 14 days of treatment (P-value<.001 all; compared to control group). Sildenefil citrate mitigated the reduction of testosterone in stress-exposed rats both at 7 and 14 days of treatment (P-value<.001 all; compared to vehicle+stress treated group) whereas Tianeptine showed the significant modification effect on serum testosterone level induced by immobilization stress at 14 days of treatment (P-value<.05; compared to vehicle+stress treated group). It was found that *N.nucifera* flowers extract failed to produce the significant change on serum testosterone of stress-exposed rats as shown in Figure 4-39.

The effect of *N.nucifera* flower extract on serum corticosterone level was also investigated and data were shown in Figure 4-40. The elevation of serum corticosterone levels were observed both at 7 and 14 days of intervention (P-value<.05 all; compared to control group). The low dose of extract could produce the significant reduction of serum corticosterone level in stress-exposed rats both at 7 and 14 days of treatment (P-value<.05 and .001 respectively; compared to vehicle+stress treated group) while the medium and high doses of extract produced the significant decreased serum corticosterone level at 14 days of treatment (P-value<.01 and .05 respectively; compared to vehicle+stress treated group).

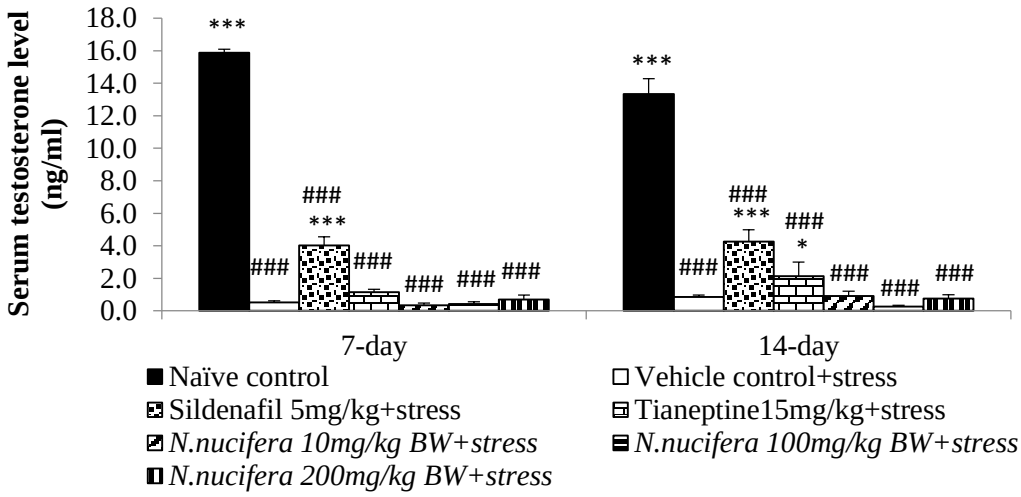


Figure 4-39 The effect of hydro-alcoholic extracts of *N.nucifera* flower extract on serum testosterone level of stress-exposed rats at 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,*** *P*-value <0.05 and 0.001; compared with vehicle plus stress. $^{###}$ *P*-value <0.001; compared with control group

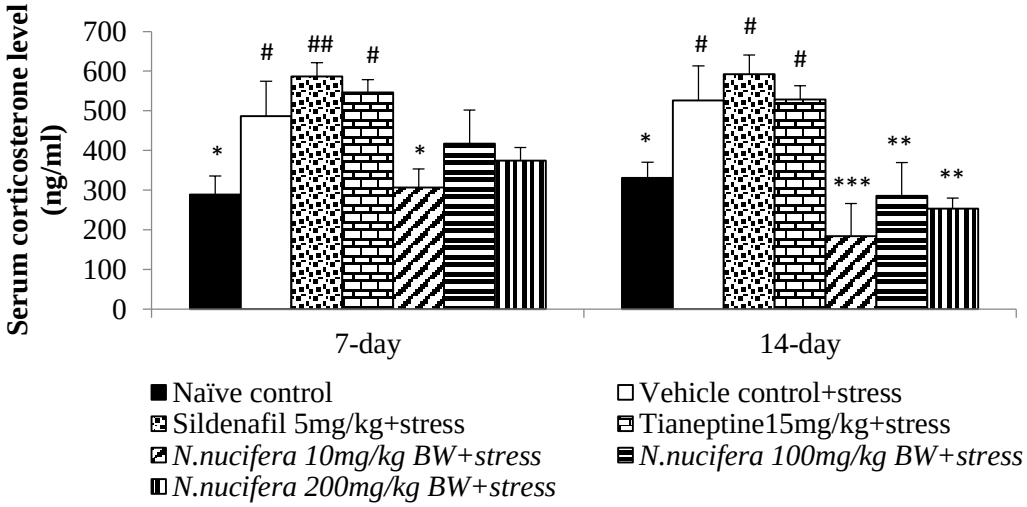


Figure 4-40 The effect of hydro-alcoholic extracts of *N.nucifera* flower extract on serum testosterone level of stress-exposed rats at 7 days and 14 days of treatment. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#}$ *P*-value <0.05 and 0.01; compared with control group

4.3.3.3 Effect of *N.nucifera* flowers extract on phosphodiesterase-5 (PDE-5) activity

Figure 4-41 showed that PDE-5 activities in penis of stress exposed rats were increased (P-value<.01; compared to control group). Sildenefil citrate, Tiaeptine and the extract at all doses used in this study could decrease PDE-5 activity in stress-exposed rats (P-value<.05, .05, .05, .05 and .001 respectively; compared to vehicle+stress treated group).

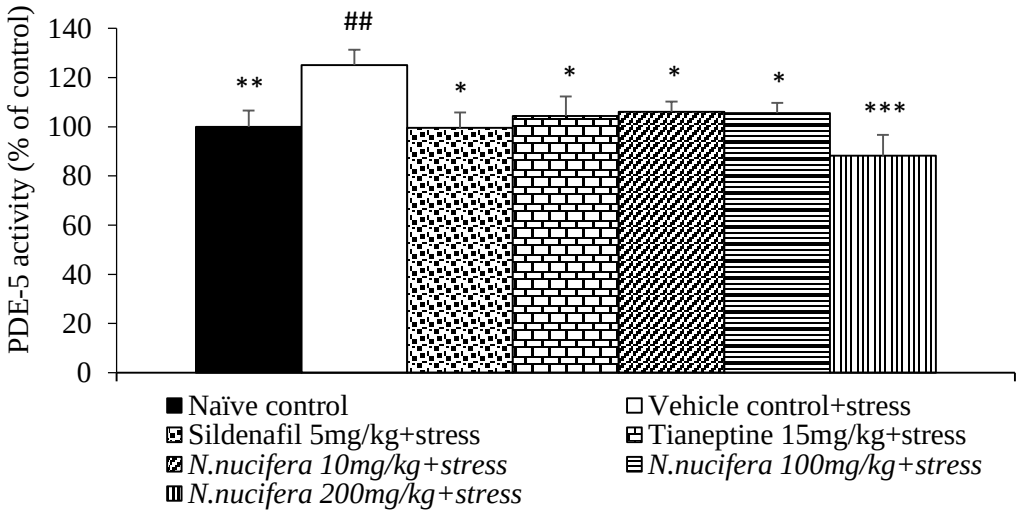


Figure 4-41 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on phosphodiesterase-5 activity in penis of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#\#}$ *P*-value <0.01; compared with control group

4.3.3.4 Effect of *N.nucifera* flowers extract on monoamine oxidase B activity

Figure 4-42 revealed that MAO-B activity in NAc of stress exposed rats which received vehicle (P-value<.001; compared to control group) but not in MPOA. Sildenefil citrate and *N.nucifera* flowers extract could produce significant reduction of MAO-B in MPOA and NAc of rats which subjected to immobilization stress (P-value<.001 all; compared to vehicle+stress reated group).

However, Tianeptine produced a significant decreased MAO-B activity in NAc (P-value<.001; compared to vehicle+stress treated group) but not in MPOA.

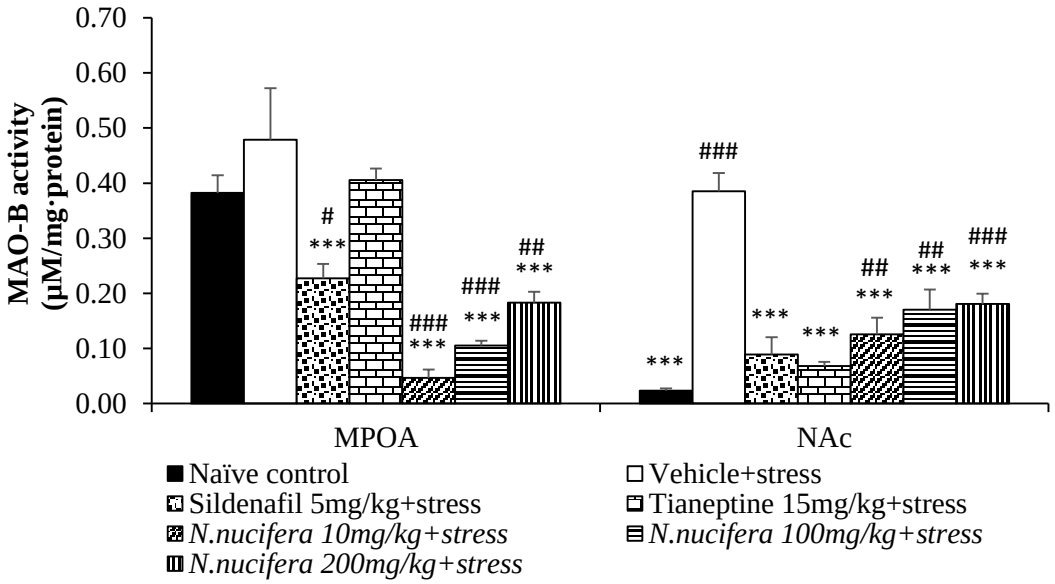
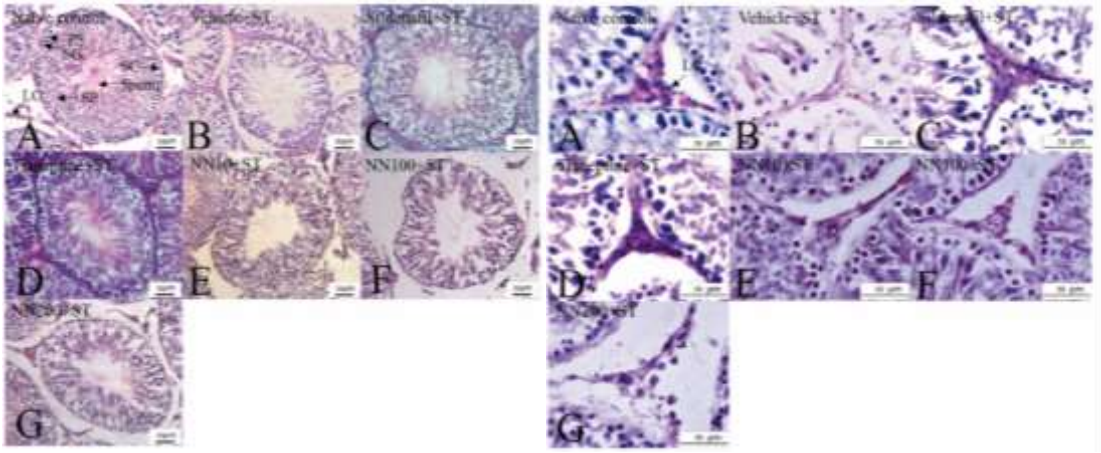


Figure 4-42 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on monoamine oxidase-B in medial preoptic area and nucleus accumbens of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *** *P*-value <0.001; compared with vehicle plus stress. #,##,### *P*-value <0,05, 0.01 and 0.001; compared with control group

4.3.3.5 Effect of *N.nucifera* flowers extract on testicular histomorphology

The results were shown in Figure 4-43. In the vehicle+stress treated group, the seminiferous epithelium was disorganized and fewer interstitial cells of Leydig were observed. Rats subjected to stress and sildenafil, or stress plus Tianeptine, or stress plus *N.nucifera* extract at all doses used in this study showed a more organized seminiferous epithelium than those treated with the vehicle plus stress. Stress-exposed rats which received *N.nucifera* extract at a dosage range used in this study didn't show significant difference of interstitial cells of Leydig and spermatozoa in the lumen of the seminiferous tubules when compared to vehicle+stress treated group.



1) Seminiferous tubules **2)** Interstitial cells of Leydig

Figure 4-43 The effect of hydro-alcoholic extracts of *N. nucifera* flowers extract on histomorphology of rat testis stained with Haematoxylin and eosin (H&E). The cross section photographs showed the 1) seminiferous tubules (40x magnification) and interstitial cells of Leydig (100x magnification), Sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS), spermatids (SP), sperm and Leydig cells (LC) in the following treatment groups: A) naïve control B) vehicle+stress C) Sildenafil citrate 5mg/kg +stress D) Tianeptine 15mg/kg +stress E) - G) *N. nucifera* at doses of 10, 100 and 200 mg/kg+stress, respectively.

4.3.3.6 Effect of *Nelumbo nucifera* flowers extract on the density of tyrosine hydroxylase immunoreactive neurons

It was found that stress rats which received vehicle showed the significant reduction of densities of tyrosine hydroxylase immunoreactive neurons in both NAcC and NAcS (P-value<.05 and .001 respectively; compared to control group)However, these changes were attenuated by Sildenefil citrate ((P-value<.001 all; compared to vehicle+stress treated group), Tianeptine (P-value<.001, .01 respectively; compared to vehicle+stress treated group) and the high dose of extract (P-value<.05, .01 respectively; compared to vehicle+stress treated group) as shown in Figure 4-44.

The reduction of tyrosine hydroxylase immunoreactive neurons density in VTA was also observed in stress-exposed rats (P-value<.05; compared to control group). This change was mitigated by Tianeptine and all doses of *N.nucifera* flowers extract (P-value<.05, .01, .001 and .001 respectively; compared to vehicle+stress treated group) as shown in Figure 4-45.

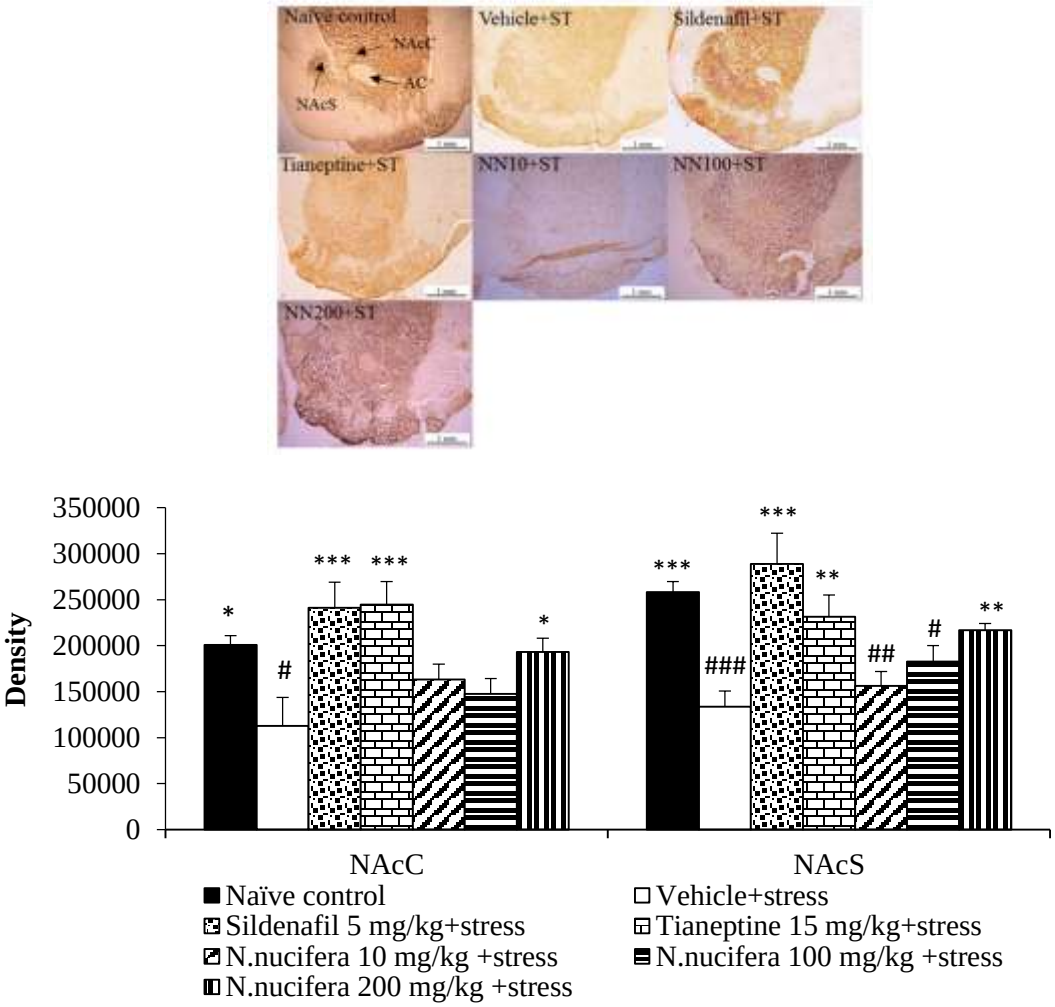


Figure 4-44 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on tyrosine hydroxylase immunoreactive neurons in core and shell of nucleus accumbens of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05, 0.01 and 0.001; compared with vehicle plus stress. $^{\#,\#\#,\#\#\#}$ *P*-value <0,05 0.01 and 0.001; compared with control group

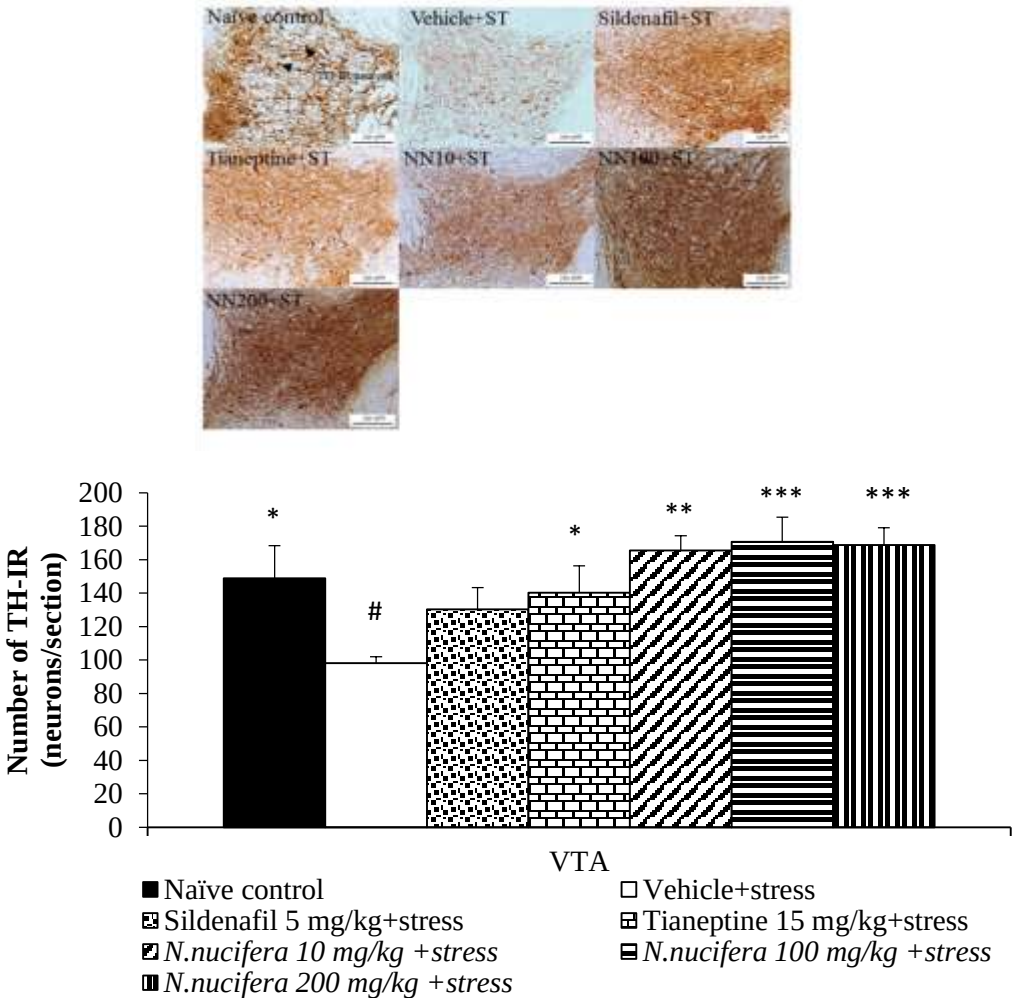


Figure 4-45 The effect of hydro-alcoholic extracts of *N.nucifera* flowers extract on tyrosine hydroxylase immunoreactive neurons in ventral tegmental area of stress-exposed rats. Data were expressed as mean±S.E.M. (n=6/group). *,**,*** *P*-value <0.05,0.01 and 0.0001; compared with vehicle plus stress. $^{\#}$ *P*-value <0,05; compared with control group

4.3.3.7 Effect of *Nelumbo nucifera* flowers extract on endothelial nitric oxide synthase in penis

Figure 4-46 showed that the expression of eNOS in penis of rats which received vehicle and subjected to immobilization stress were markedly decreased (P-value<.05; compared to control group). This change was mitigated by the medium dose of extract (P-value<.05; compared to vehicle+stress treated group).

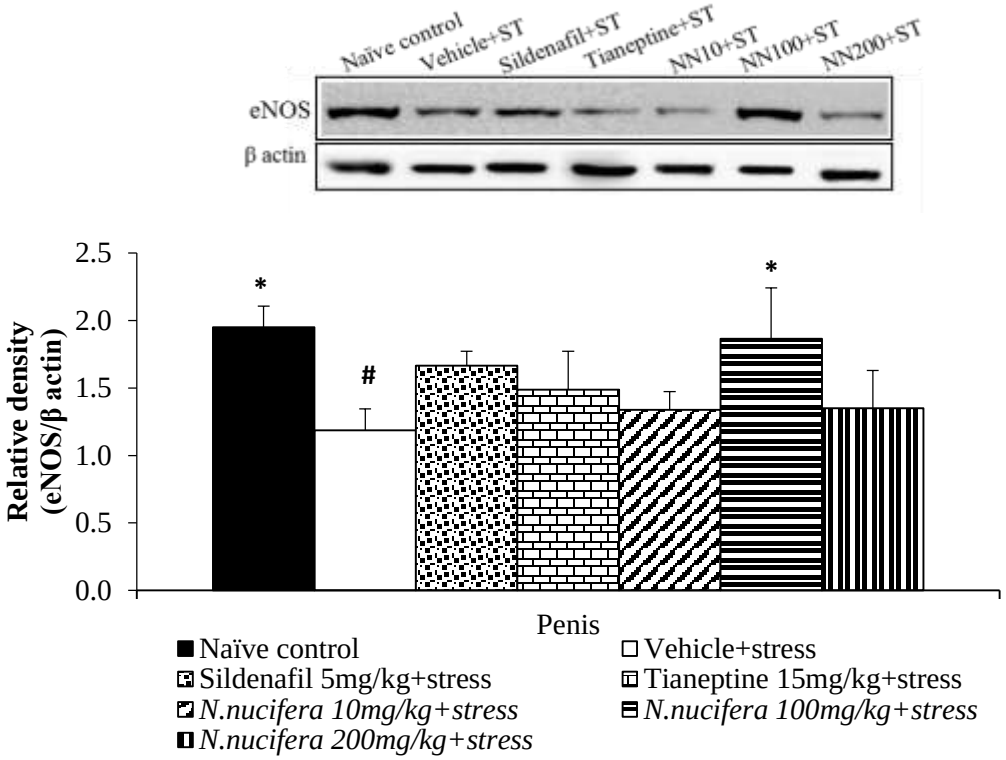


Figure 4-46 The effect of hydro-alcoholic extracts of *N. nucifera* flowers extract on endothelial nitric oxide synthase in penis of stress-exposed rats. Data were expressed as mean±S.E.M. (n=4-5/group). * *P*-value <0.05; compared with vehicle plus stress. # *P*-value <0,05; compared with control group.

4.4 Discussion

4.4.1 Effect of *M.oleifera* leaves extract on sexual dysfunction in restraint rats

The current study has revealed that *M. oleifera* leaves extract showed a single consumption increased libido. When the treatment was prolonged to 7 days, the low dose of extract enhanced penile erection capacity by decreased intromission latency and increased intromission number. At the 14 days, rats were received *M. oleifera* extract enhanced libido via inhibited MAO-B, decreased corticosterone, and increased tyrosine hydroxylase dopaminergic neurons. While, penile erection was evaluated by the inhibition of PDE-5 and increased eNOS levels.

Male sexual behavior is governed by a complex interaction between different systems in the brain that process sensory inputs, regulate reward and motivation, and integrate hormonal signals (Hull *et al.*, 2004). The regulation of sexual behavior can occur both at the brain and at peripheral sites. It has been reported that testosterone plays an important role in priming brain neural circuits for sexual behavior. The priming effect is achieved partly by the modification of regulating enzymes, receptors, or other proteins which affect neurotransmitter function. Testosterone can increase local dopamine synthesis and metabolism of dopamine (Purves-Tyson *et al.*, 2012), which in turn exerts an influence various aspects of male sexual function including sexual motivation, copulatory proficiency, and genital reflexes (Hull *et al.*, 2004).

Accumulated evidence has shown that dopamine plays a crucial role in male sexual behavior via the enhanced release of oxytocin from the paraventricular nucleus (PVN) of the hypothalamus, which in turn increases the release of nitric oxide from the carvernosal nerve. Nitric oxide penetrates the cytoplasm of smooth muscle cells and interacts with guanylylcyclase, catalyzing conformational changes which in turn induce the conversion of guanosine 5'-triphosphate (cGMP) to 3'-5'–cyclic guanosine monophosphate. Cyclic GMP in turn phosphorylates several proteins, resulting in decreased intracellular calcium levels causing the relaxation of arterial and trabecular smooth muscle, resulting in arterial dilatation, venous constriction, and the rigidity of penile erection. The inactivation of cGMP via PDE5 decreases the dilation of arterial vessels and the constriction of venous blood vessels and increases penile tumescence (Andersson, 2001).

In this study, stress exposure induced a reduction in the number of interstitial cells of Leydig and in serum testosterone levels. These changes may possibly have been due to enhanced oxidative stress (Bitgul *et al.*, 2013; Rai *et al.*, 2004). Plants extract attenuated the reduction in the interstitial cells of Leydig and serum testosterone levels induced by stress exposure. Based on the results of this study, we suggested that the antioxidant effect of the extract alleviated oxidative stress-related cell toxicity, resulting in increased numbers of interstitial cells of Leydig and serum testosterone levels. It has been reported that the elevation of glucocorticoid levels induced by stress can also inhibit the synthesis of testosterone in

interstitial cells of Leydig (Gao *et al.*, 1996). Therefore, the mitigating effect of plant extract on the reduction in testosterone induced by stress might occur partly via decreased corticosterone levels.

It has been shown that plant extract also inhibited MAO-B in MPAO and NAc. MPOA of the hypothalamus is one location where dopamine may act to regulate male sexual behavior including copulation, genital reflexes and sexual motivation (Dominguez and Hull, 2005). Many studies have shown that sexual motivation and copulation in male rats are associated with dopamine release in the nucleus accumbens (NAc) (Wang *et al.*, 1995). Due to the crucial role of dopamine in male sexual behavior mentioned earlier, VTA is origin of dopaminergic cells of the mesocorticolimbic dopamine system and is associated with reward circuitry, cognition, motivation, orgasm and drug addiction, available dopamine facilitate male sexual behavior. Moreover, the increasing level of dopamine might increases oxytocin release from the PVN and induces the release of nitric oxide from the carvernosal nerve, triggering the elevation of cGMP in the penis. The elevation of cGMP induced by plant extract and the suppression effect of the extract on PDE-5 may enhance the cGMP-dependent vasodilation effect of nitric oxide, leading to enhanced penile blood flow and erection. In addition, it has been reported that the elevation of testosterone can regulate PDE5 function (Aversa *et al.*, 2009) and modify dopamine inactivation via MAO-B, leading to increased penile erection (Sanna *et al.*, 2011). Moreover, NO is produced in endothelial cells by eNOS via the activation the cholinergic neurons or shear stress, can also induce penile erection (Lasker *et al.*, 2013). Therefore, the enhanced intromission phase observed in this study may have been related to MAO-B suppression activity, the ability to increase the testosterone level, PDE-5 suppression effect and increase eNOS.

In this study, an increased number of spermatozoa was also observed in stress exposed rats subjected to plants extract treatment. It has been reported that spermatozoa are vulnerable to oxidative stress because of the high concentration of polyunsaturated fatty acids involved in the regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually membrane fusion, and their low antioxidant capacity. An attack of oxidative stress may lead to structural damage and decreased viability. Therefore, the increased spermatozoa density

induced by the plant extract might possibly be due to the antioxidant effect of the extract.

The current data also suggested that the enhanced dopaminergic function induced by the plant extract might occur not only via the MAO-B inhibition effect but also increased tyrosine hydroxylase dopaminergic neurons. Moreover, the plant extract used in this study also contained abundant phenolics and flavonoids. Therefore, the antioxidant effect of the extract might have been due to these ingredients. Quercetin was the main ingredient in *M.olifera* leaves, a potent bioflavonoid, has been reported to have the antioxidant effect and ameliorated erectile dysfunction (Zhang *et al.*, 2011).

This study was the first to demonstrate the possible benefit of plant extract on dopaminergic and sexual function besides the possible role of phenolics and flavonoids in the extract, and the biochemical and histological changes related to stress and sexual function. However, the determination of the effect of the plant extract on serum testosterone, PDE-5 activity and eNOS in the penis at only a single time point and the lack of vascular blood flow measurement, were limitations of this study. The effect of the plant extract on serum testosterone before and during sexual performance should be determined to confirm the modulating role of the extract on the sexual enhancement effect of testosterone. The effect of the extract on PDE-5 in testis both before and during the sexual intromission phase should also be determined together with the levels of cGMP and penile blood flow, to confirm that the suppression of PDE-5 induced by the plant extract can enhance the cGMP-dependent vasodilation effect of nitric oxide, penile blood flow and penile erection.

Therefore, further investigations of the effect of plant extract on the alterations of testosterone and dopamine levels before and during sexual performance, together with evaluation of the cGMP-dependent vasodilation effect of nitric oxide, penile blood flow and penile erection, were required to improve understanding of the precise underlying mechanisms of the sexual enhancing effect of plant extracts.

In conclusions, the use of plant extract might provide a cheap and simple approach for improving sexual function under stressful conditions. Therefore, the extract appears to be a potential aphrodisiac, particularly for people with stressful daily lifestyles. However, more research is needed concerning the possible underlying

mechanisms of action of plant extracts, such as the determination of testosterone levels before and during sexual performance, and the evaluation of the cGMP-dependent vasodilation effect of nitric oxide, penile blood flow and penile erection.

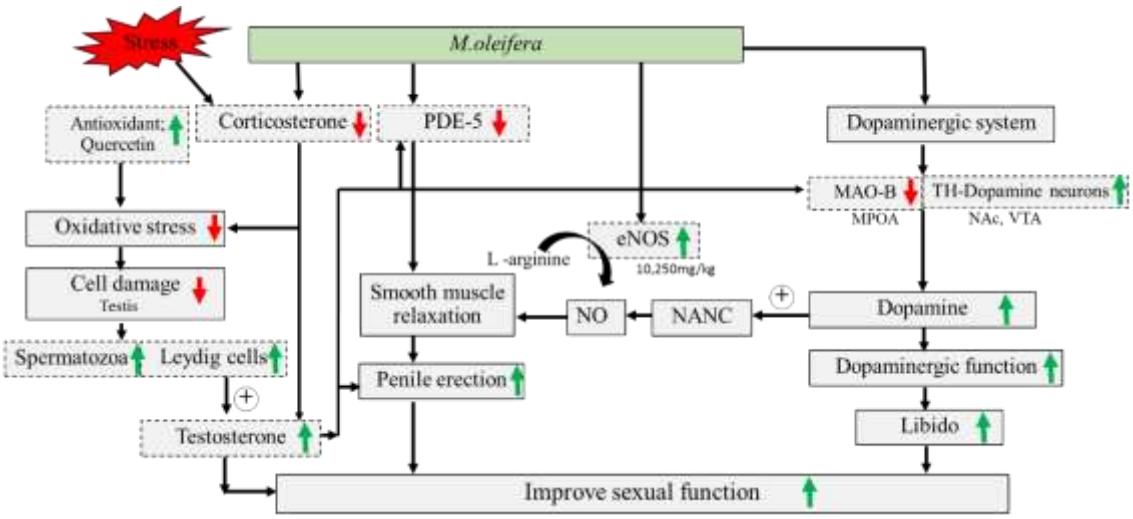


Figure 4-47 Schematic diagram showed the possible underlying mechanism of *M.oleifera* on sexual performance in stressed rats

4.4.2 Effect of *A.occidentale* leaves extract on sexual dysfunction in restraint rats

The current data is the first study which demonstrated that the modulation effect of neuroendocrine control circuit of male sexual behavior is modulated by *A.occidentale* leaves extract. It has clearly demonstrated that *A.occidentale* leaves extract enhanced sexual behaviors in male stress-exposed rats together with the suppression of MAO-B in both MPOA and NAc and the suppression of serum corticosterone level. In addition, the elevation of serum testosterone, the enhanced density of dopaminergic neurons in NAc and VTA and the increased eNOS were also observed in stress-exposed rats which received *A.occidentale* leaves extract.

It has well known that the increased mounting frequency together with the decreased mounting latency indicate the enhanced libido. The regulation of libido or sexual desire is associated with the functions of medial preoptic area of hypothalamus (MPOA), nucleus accumbens (NAc) and ventral tegmental area (VTA)

(Kim *et al.*, 2013). The increased dopaminergic function in VTA gives rise to the dopaminergic function in MPOA and nucleus NAc respectively (Stahl, 2010). Since the core area of NAc is linked to the motor circuit whereas the shell areas is linked to limbic circuit (Zahm, 1999), it has been proposed that the increased dopaminergic function in NAcS might enhance the glutamatergic function within the core of the nucleus accumbens (NAcC) resulting in the enhanced seeking female partner whereas the increased dopaminergic in NAcS might be responsible for the increased desire giving rise to the engaging in the behavior to seek the targeted partner and copulatory behavior. It is also reported that the function of dopaminergic neurons in MPOA is required the priming effect of testosterone via the enhanced nitric oxide synthase (Hull *et al.*, 1997). In addition to the regulation of libido, MPOA also projects the signals to the periaqueductal gray; pass through retroambiguus nucleus to reach the lumbosacral motor neurons that lead to perineal striated muscles that then augment penile erection (Nieuwenhuys *et al.*, 2007).

Penile erection is regulated not only via the central mechanism but also via peripheral mechanism. Endothelial nitric oxide synthase (eNOS) shows an indispensable role in the erectile response. eNOs in endothelial cells of penis corpus cavernosum may release nitric oxide which in turn increases cGMP giving rise to the relaxation of smooth muscle and penile erection. cGMP can be converted to inactive form of 5'-GMP by PDE-5. Therefore, the suppression of PDE-5 may increase the available cGMP which in turn increase smooth muscle relaxation and penile blood flow and finally results in penile erection (Musicki and Burnett, 2006).

Since the increased density of Interstitial cells of Leydig and the elevation of serum testosterone were also observed in stress-exposed rats which received *A.occidentale* leaves extract, it was suggested that *A.occidentale* leaves extract might increase the density of Interstitial cells of Leydig which in turn enhanced testosterone synthesis and serum testosterone level. This hormone can pass through blood brain barrier and priming the function of dopaminergic neurons in MPOA and sequentially increases the dopaminergic function of MPOA. In addition, the extract also enhanced the dopaminergic function in VTA by enhancing the density of tyrosine hydroxylase immunopositive neurons in this area. The enhanced dopaminergic function of VTA might possibly enhance the stimulation of MPOA

resulting in the increased dopaminergic function of MPOA which has been primed by testosterone and induced the increased density of dopaminergic neurons in the mentioned area. In addition, the suppression of MAO-B in MPOA also gave rise to the increased dopaminergic function. The increased dopaminergic function in VTA also enhanced the dopaminergic function of NAc both by increasing the density of core and shell compartments of NAc and by decreasing MAO-B activity in NAc. The increased dopaminergic activity in NAc might enhance the seeking behaviors for female partner and the engagement in the copulatory behavior. Moreover, the enhanced dopaminergic function in MPOA induced by *A.occidentale* leaves extract could increase the male sexual function including penile erection and ejaculation via the enhanced lumbosacral activity (Kim *et al.,* 2013). In addition the extract also suppressed PDE-5 and enhanced the expression of eNOS in penis which in turn may be responsible for the increased penile erection. However, the effect on the enhanced eNOS expression was observed only at high dose of extract whereas the decreased corticosterone, an important hormone which can decrease serum testosterone level (MacADAMS *et al.,* 1986), was observed only at medium dose treatment. Thus, the main mechanisms of *A.occidentale* leaves extract to enhance male sexual behaviors appeared depends on the application doses. The sexual enhancing effect of all doses of the extract appeared to occur via the enhanced neuroendocrine response via the increased density of interstitial cells of Leydig and the serum testosterone level and the increased dopaminergic function in the key regulatory areas of male sexual behaviors and the suppression of PDE-5.

No dose dependent manner was observed in this study because the extract which was used in this study was the crude extract and contained numerous ingredients which may possibly to produce the masking effect inactive constituents. In addition, the regulation of male sexual behaviors is very complex and is under numerous effects so no single linear relationship was observed.

Although the identification of active ingredient which is responsible for the sexual enhancing effect of *A.occidentale* leaves extract in stress exposed rats was beyond the scope of this study, it was suggested that quercetin, one of the important flavonoids in the extract might play some roles based on the its role on the enhanced dopaminergic function (Haleagrahara *et al.,* 2011; Sriraksa *et al.,* 2011).

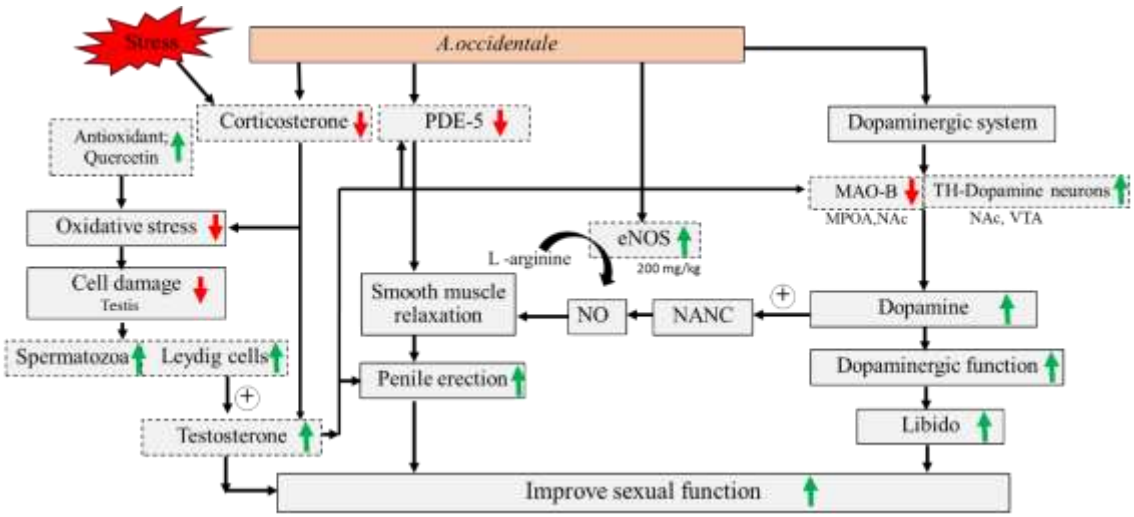


Figure 4-48 Schematic diagram showed the possible underlying mechanism of *A.occidentale* on sexual performance in stressed rats

4.4.3 Effect of *N.nucifera* flowers extract on sexual dysfunction in restraint rats

The present study clearly demonstrated that *N.nucifera* flowers extract improved male sexual behaviors decreased serum corticosterone level while it produced no change in serum testosterone level. It also suppressed MAO-B in both MPOA and NA cans suppressed PDE-5 in penis but increased the density of tyrosine hydroxylase immunopositived neurons mainly in VTA. The increased density of tyrosine hydroxylase immunopositived neurons in NAc was observed only at high dose treatment. The increased expression of eNOS was also presented but only in the medium dose treatment.

The previous findings have demonstrated that that dopaminergic system in VTA, MPOA and NAc contribute the pivotal roles on the regulation of male sexual behaviors (Kim *et al.*, 2013). In this study, the increased male sexual behaviors was observed in accompany with the increased suppression effect of MAO-B both in MPOA and NAc and the increased density of tyrosine hydroxylase immunopositived neurons in VTA at all dosage range. Therefore, *N.nucifera* flowers extract might exert sexual enhancing effect principally via the enhanced tyrosine hydroxylase immunopositived neurons in VTA which in turn increased the activities

of both NAc and MPOA. The increased in dopaminergic activity in MPOA and NAc in turn increased the sexual desire indicating by the decreased mounting latency but increased mounting frequency. In addition, the increased MPOA activity might also increase penile erection and ejaculation by increasing the output from MPOA to lumbrosacral motor neurons which facilitate penile erection via the increased function of periaqueductal gray and retroambiguus nucleus (Kim *et al.*, 2013). The increased dopaminergic function in NAc via the suppression of MAO-B might also contribute the important role on the enhanced male sexual behaviors induced by *N.nucifera* flowers extract. The increased dopaminergic function in the mentioned area by increasing density of tyrosine hydroxylase immunopositived neurons in NAc might play the role only at the high dose level of extract.

It was found that the extract also suppress PDE-5, en enzyme playing the critical role on conversion of cGMP to 5' GMP which in turn decreased the relaxation of smooth muscle of penis corpus cavernosum leading to the decreased penile blood flow and penile tumescence. Therefore the increase behaviors in intromission phase of sexual cycle observed in this study may not depend only on the central effect via the enhanced dopaminergic function of MPOA and NAc but also via the suppression of PDE-5 in penis and finally resulting in the increased penile erection. At medium dose level, the increased eNOS expression in penis was also observed. Thus, eNOs may also play the role on sexual enhancing activity induced by *N.nucifera* flowers extract at the medium dose level. No tight association between the expression of eNOS and the concentration of *N.nucifera* was observed because the peripheral regulation of penile erection via nitric oxide induced vasodilation was not depended only on eNOS. The neuronal or nNOS which is released from nonadrenergic noncholinergic (NANC) nerve at penis also plays the crucial role on penile erection (Burnett *et al.*, 1996). Therefore, no simple linear relationship between both parameters existed.

The current data has revealed that *N.nucifera* flower extract showed the improvement of sexual function by increased libido via inhibited MAO-B, decreased corticosterone and increased tyrosine hydroxylase dopaminergic neurons including increased interstitial cells of Ledig and spermatozoa. In addition, *N.nucifera* could enhance penile erection by inhibited PDE-5 and increased eNOS level in penis.

According to previous discussion part of *M.oleifera* leaves, our data demonstrated that stress could suppress the sexual function in male rats. Rats were received *N.nucifera* flower showed the increased libido by decreasing mount latency at medium and high doses and increased penile erection by decreasing intromission latency at all doses and increasing intromission number at medium and high doses. When the treatment was prolonged to 7 days, all doses of extract enhanced penile erection by decreasing intromission latency at all doses and increasing intromission number at low and medium doses. At the 14 days of experiment, rats were received *N.nucifera* extract enhanced libido via decreasing mount latency at all doses and enhanced penile erection by increasing intromission number at medium and high doses. Moreover, all doses of *N.nucifera* increased ejaculation number which indicated that rat can quickly recover and begin to next sexual behavior cycle.

It was found that *N.nucifera* enhanced sexual function via inhibition of MAO-B in NAc and MPOA at all doses. In addition, all doses of *N.nucifera* also showed the increasing TH-dopaminergic neurons in VTA whereas high dose of *N.nucifera* showed the increasing TH-dopaminergic neurons in NAc. Moreover, medium dose of *N.nucifera* increased penile erection by increasing eNOS level and inhibition PDE-5 in penis at all doses. Therefore, *N.nucifera* flowers extract might exert sexual enhancing effect principally via the enhanced dopaminergic

Although testosterone has been shown to exert the important role on male sexual behavior, no changes of this hormone were observed in stress-exposed rats which received *N.nucifera* flowers extract. Therefore, the sexual enhancing effect of this plant may not be related with testosterone changes.

N.nucifera flowers extract also failed to show dose dependent response due to the same reason as mentioned in the effect of *A.occidentale* leaves extract.

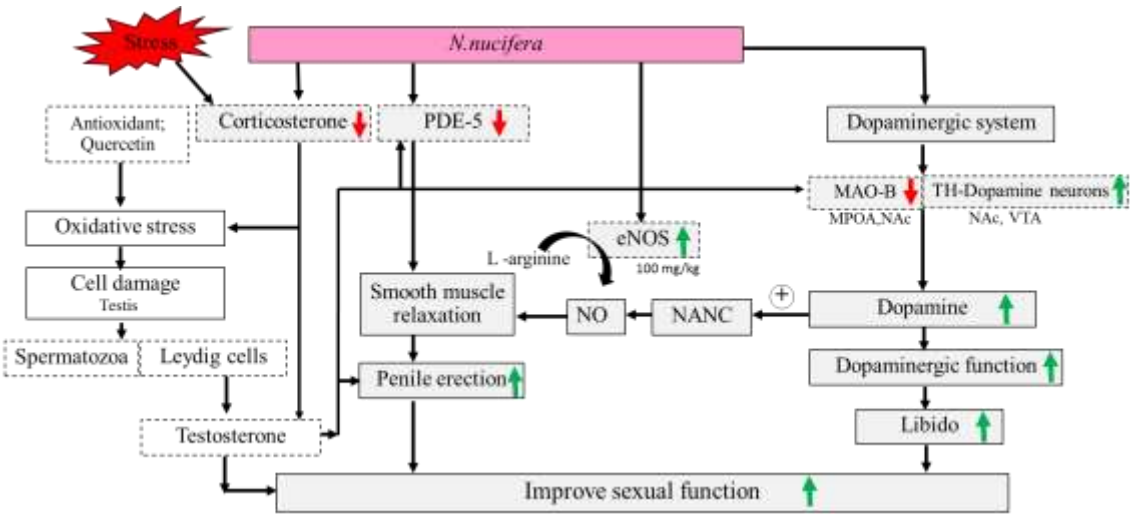


Figure 4-49 Schematic diagram showed the possible underlying mechanism of *N.nucifera* on sexual performance in stressed rats

4.5 Conclusion

This study is the first study to demonstrate the scientific evidence concerning the sexual enhancing effect of the *M.oleifera*, *A.occidentale* and *N.nucifera*, the medicinal plants reputed for aphrodisiac property in traditional folklore and their underlying mechanisms. Interestingly, these plants can exert sexual enhancing effect both via the central effect by enhancing the dopaminergic function in the key areas which regulate male sexual behaviors and via peripheral effect at penis by suppressing PDE-5 activity. They can also provide benefits in many phases of sexual cycle in sexual dysfunction induced by stress exposure. In addition, most of them are not expensive easy to approach. Therefore, they may be served as the potential aphrodisiac agent and the potential candidate for the sexual enhancing regimen for sexual dysfunction. However, further researches concerning the active ingredient, pharmacokinetic and drug interaction are essential before moving to the clinical trial.