Research Article

Effect of Standardized Extract of *Commiphora* Mukul Engl. (*Burseraceae*) on Bulbectomy Induced Sexual and Immune Dysfunction in Rats.

Padmaja Kalshetti[a*], Prasad Thakurdesai[b], Ramesh Alluri[c]
[a]Department of Pharmacology, MAEER'S Maharashtra Institute of Pharmacy, Paud Road, Kothrud, Pune, India.
[b]Indus Biotech Private Limited, 1, Rahul Residency, Off Salunke Vihar Road, Kondhwa, Pune, India.
[c]Dept of Pharmacology, Vishnu Institute of Pharmaceutical Education & Research, Narsapur, Medak District, Andhra Pradesh, India.
***Corresponding author's E-mail:** padmaja.kalshetti@gmail.com

Accepted on: 07-05-2015; **Finalized on:** 30-06-2015.

ABSTRACT

The main objective of this study was to evaluate the standardized extract of *Commiphora mukul* Engl. (Burseraceae) in olfactory bulbectomy induced sexual and immune dysfunction in rats. Olfactory bulbectomy (OBX) was performed in male Sprague dawley rats (250-270gms). After recovery period of 14 days, 8 groups of animals were made (6 per group) as, Group-I rats served as SHAM control (Without surgery, burr holes drilled), Group-II rats served as OBX control group, Group-III, IV, V served as standard treatment groups i.e. imipramine, fluoxetine and desipramine (20mg/kg p.o., 30mg/kg p.o., 15mg/kg p.o.) treated group, Groups VI, VII and VIII served as drug treatment groups (HECM 50, 100 and 200 mg/kg p.o.). All the treatments were continued for 14 days after recovery period. Sexual behaviour parameters were taken on 29th day and animals sacrificed at the end for brain dopamine estimation. All three standards as well as all three doses of hydro alcoholic extract of *Commiphora mukul* (HECM) does dependently normalized OBX induced sexual and immune abnormalities. There was a significant improvement in all the parameters of sexual behavior, altered differential leukocyte count, weight of spleen and thymus and brain DA level by the treatment with standards as well as two higher doses of HECM. *Commiphora mukul* at all the doses significantly improved sexual and immune dysfunction induced by OBX, which is comparable with standard drugs.

Keywords: stress, depression, olfactory bulbectomy, dopamine, sexual dysfunction

INTRODUCTION

Depression is a major disorder affecting nearly 13% - 20% of the world's population.[1] Roughly, 90% of depressive patients suffer with mild to moderate depression, while only 10% are suffering with severely depression. In case of mild to moderate depression, in particular, some of the physicians and patients are reluctant for use of standard antidepressants like selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants because of their potential and toxic side effects. Therefore, the additional treatment strategies with fewer side effects profile, moderate costs and credible benefits are of particular interest.[2,3]

So, in search of new molecules really useful for the treatment of certain neurological disorders, medicinal plant research is progressed worldwide by demonstrating pharmacological effectiveness of various plant species in a different variety of animal models.[4]

Commiphora mukul plant belongs to Burseraceae family and has a resinous secretion known as guggul. Guggul is one of the most valuable remedies in ayurvedic system of medicine. Its usage backs to over 2500 years. Guggul has been used as antihyperlipidemic, antidiabetic, anti-inflammatory, antiosteoarthritic, sedative, antispasmodic, astringent, antiseptic, carminative, expectorant, emmenagogue, thyroid stimulant, diaphoretic, antiobesity, diuretic, anthelminitic and demulcent in traditional medicine. *Commiphora mukul* has been reported to contain flavonoids, terpenes, and phytosterols.[5] However, the steroid guggulsterone has been purified from the ethylacetate extract of resin.[6] Guggulsterone is the active steroid present in plant and acts as antagonist of the bile acid farnesoid X receptor, by demonstrating the hypolipidemic effect of the resin.

The olfactory bulbectomized (OBX) rat is a validated animal model that meets most of the criteria's of a clinical depression. Bilateral removal of the olfactory bulbs results in a phenotype modeling. Several of the symptoms seen in major depression, including many of the behavioral, neurochemical, neuroendocrine and immune alterations.[7-9]

Recently we have reported the antidepressant effects of standardized extract of *commiphora mukul* (HECM) in olfactory bulbectomy induced depression model. It was found that, HECM represents restorative effects in OBX induced depression model in rats probably due to stress reliving mechanisms.[10] Therefore it was found interesting to study the effect of sub-acute administration of HECM in olfactory bulbectomy induced sexual dysfunction in rats.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (250–270 g) were purchased from National Toxicology Centre (NTC), Pune. The animals were housed at a temperature of 25±1 °C and relative humidity of 45–55% under 12:12 light: dark cycle. The animals had free access to feed pellets (Chakan Oil Mills

Ltd., Sangli, and Maharashtra, India) and tap water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Maharashtra Institute of Pharmacy, Pune (Approval number-No: MIP/IAEC/2013-14/M1/0012). All observations were recorded between 8:00 and 15:00 h and each animal was used only once. To avoid subjective bias, the observer was kept unaware about the given treatment (blind study). Each experimental group consisted of six animals. Rats were transported from the animal house to the testing area in their housing cages and were allowed to adapt to the new environment before testing.

Drugs and Chemicals

Imipramine hydrochloride (imipramine), Fluoxetine hydrochloride (fluoxetine), Desipramine, Dopamine (DA) was purchased from Sigma-Aldrich, USA. The other chemicals were purchased from local vendors. The standardized extract was obtained as a gift sample from from Konark herbals, Mumbai. (Batch number-KH/CM/001/12).

Preparation of Drug Solutions

The test drug (HECM) and standard drugs were dissolved in distilled water immediately before use and administered once daily for 14 days, orally 1 h before the experiments.

Bilateral Olfactory Bulbectomy Surgery[11]

The male Sprague Dawley rat was anesthetized with ketamine (80 mg/kg i.p.). The animal was placed in streotaxic frame (Inco, India). Head was shaven and midline scalp sagittal incision (1cm) was made and bilateral burr holes (2mm diameter) were drilled 8mm anterior from bregma and 2mm lateral from midline. Both main and accessory olfactory bulbs were aspirated through both burr holes using a blunt hypodermic needle attached to water pump without damaging frontal cortex. The burr holes were then plugged with a haemostatic sponge to control bleeding. Povidone iodine solution was applied to the wounds and the rat was allowed to recover for 14 days.

Treatment Schedule in Olfactory Bulbectomized (OBX) rats[10]

Group I rats were treated as sham control (had surgery but no OBX) and were administered saline. After recovery period of 14 days, OBX rats were divided into following groups of six rats each and received oral drug treatments as follows.

Group II was OBX control and received distilled water. The OBX rats in Group III, IV, and V were administered with imipramine (20 mg/kg), fluoxetine (30 mg/kg), and desipramine (15 mg/kg) respectively. Group VI, VII and VIII OBX rats were treated with HECM (50, 100, 200 mg/kg). All the treatments were administrated orally once daily for 14 days.

Effect on Sexual Behavior Parameters

Female Wistar rats (200-250 g) previously experienced with sexual behavior were used for the study. The rectangular wooden box with Plexiglas front and wire mesh top was used as an apparatus. One male rat was placed 5 minutes before introduction of female. The parameters of copulatory behaviour such as mounting latency (ML), Mounting frequency (MF), intromission latency (IL) and intromission frequency (IF) were observed. All the observations were made for 15 minutes. The study was carried out at 22.00 – 24.00 h under dim white light. Sexual behavior in male rats was studied by the methods described by Aswar.[12]

Effect on Immune Parameters

On day 20 of surgery, the blood was collected by retro orbital plexus after anesthesia to rats. Blood was collected in the EDTA-coated tubes and mixed well. The samples were sent to the Astha Laboratory, (Pune, India) for some hematological parameters.[41]

The weight of spleen and thymus was checked at the end of study.[13]

Effect on Brain DA levels[14]

The whole rat brain was homogenized in acidified cold n-butanol solution and 2-5 ml of the supernatant was pipetted into a 25 ml glass stoppered tube and mechanically shaken for 5 min with n-heptane and HCl containing L-cysteine.

The two phases were separated by centrifugation. To 0.02 ml of the HCl phase, HCl and EDTA/sodium acetate buffer (pH 6.9) were added, followed by 0.01 ml of iodine solution for oxidation.

The reaction was then stopped by addition of Na_2SO_3. Acetic acid was added 15 min later. The solution was then heated and the sample again reached room temperature, excitation and emission spectra were read in 395-485 nm for NA, 330-375 nm for DA.

Statistical Analysis

The data was expressed as Mean ± SEM analyzed with the help of software prism version 5.0.3 (Graph pad Inc., California, USA). All the data was analyzed by One way ANOVA followed by Dunnett's test.

RESULTS

Effect on Mounting Latency (ML)

Mounting latency (ML) in SHAM operated animals was found to be 75.30 ± 6.63 seconds. OBX significantly ($P < 0.001$) increased the ML to 88.82% as compared to SHAM operated group. Moclobemide, HECM 50,100 and 200 exhibited significant ($P < 0.001$, $P < 0.05$) reduction in ML.

Effect on Mounting Frequency (MF)

Mounting frequency (MF) in SHAM operated animals was found to be 191.4 ± 34.55 seconds. OBX rats showed

significant (P < 0.001) increase in MF by 76.23% as compared to SHAM animals.

Treatment with moclobemide and all three doses of HECM showed significant (P < 0.001, P < 0.01) reduction in MF by 280%, 126.8%, 180% and 295.2% as compared to OBX rats.

Effect on Intromission Latency (IL)

Intromission latency (IL) in SHAM group was found to be 135.0 ± 15.81 seconds. OBX rats showed significant (P < 0.001) increase in IL to 517% as compared to SHAM operated group.

Treatment with imipramine and fluoxetine did not show any effect on IL. Moclobemide and all the doses of HECM significantly (P < 0.001) reduced IL by 73.26%, 23.51%, 29.99% and 61.78% as compared to OBX rats.

Effect on Intromission Frequency (IF)

Intromission frequency (IF) in SHAM operated group was found to be 7.00 ± 1.00. OBX significantly (P < 0.001) reduced IF by 85.72% as compared to SHAM group.

Treatment with imipramine and fluoxetine had no effect in improvement IF.

Treatment with moclobemide and all three doses of HECM significantly (P < 0.001) increased IF by 366%, 233%, 216%, 316% as compared to OBX group.

Effect on Neutrophil Count

Neutrophils/mm^3 in SHAM operated group was found to be 23.00 ± 2.02. OBX animals showed significant (P < 0.001) increase in Neutrophils/mm^3 i.e to 114.4% as compared to SHAM group.

All the standards like imipramine, fluoxetine, moclobemide and highest dose of HECM significantly (P < 0.001) reduced the count to 64.19%, 49.93%, 58.99% and 58.1% as compared to OBX rats.

Effect on Lymphocyte Count

Lymphocytes/mm^3 in SHAM operated group was found to be 69.17 ± 2.38. OBX animals showed significant (P < 0.001) reduction in Lymphocytes/mm^3 i.e to 43.38% as compared to SHAM group.

Treatment with all the standards like imipramine, fluoxetine, moclobemide and highest dose of HECM significantly (P < 0.001) increased count by 101.6%, 73.16%, 101.68% and 93.59% as compared to OBX rats.

Effect on Weight of Spleen

Weight of spleen in SHAM operated animals was found to be 661.8 ± 2.63 mg. OBX significantly (P < 0.001) reduced weight of spleen by 39.93% as compared to SHAM operated group.

Treatment with all standards significantly (P < 0.001) increased the spleen weight by 16.27%, 30.47% and 23.86% as compared to OBX rats. HECM at all the dose levels significantly (P < 0.001) increased the weight of spleen by 4.17%, 25.14% and 43.66% as compared to OBX group.

Effect on Weight of Thymus

Weight of thymus in SHAM operated animals was found to be 653.5 ± 2.93 mg. OBX significantly (P < 0.001) reduced weight of spleen by 45.18% as compared to SHAM group.

Treatment with all standards and all doses of HECM significantly (P < 0.001) increased the weight of thymus by 16.94%, 65.58%, 26.23%, 4.57%, 41.92% and 81.04% as compared to OBX group.

Effect on Brain Dopamine (Schlumpf)

Brain dopamine level (ng/g) in SHAM operated animals was found to be 108.4 ± 2.70 ng/g. OBX significantly (P < 0.001) reduced brain dopamine levels by 53.98% as compared to SHAM group.

Treatment with standards like fluoxetine (30) and desipramine (15) significantly (P < 0.001) increased brain dopamine levels i.e. 48.84%, 54.15% respectively. HECM at higher dose (200mg/kg p.o.) significantly (P < 0.001).

Table 1: Effect of HECM on sexual behavior parameters in OBX rats

	Mounting Latency (ML)	Mounting Frequency (MF)	Intromission Latency (IL)	Intromission Frequency (IF)
Sham Control	75.3 ± 6.62	10.67 ± 0.71	135.0 ± 15.81	7.00 ± 0.73
OBX control	673.3 ± 50.71###	2.50 ± 0.34###	833.8 ± 19.86###	1.00 ± 25###
OBX + IMP (20)	624.5 ± 50.07	3.16 ± 0.40	777.3 ± 34.72	2.00 ± 0.36
OBX + FLX (30)	634.0 ± 47.95	2.50 ± 0.34	704.8 ± 60.16*	2.33 ± 0.49
OBX + DSP(15)	182.2 ± 8.46***	9.50 ± 0.76***	223.0 ± 10.08***	4.66 ± 0.55***
OBX + HECM (50)	539.0 ± 20.87*	5.66 ± 0.61**	637.8 ± 12.87***	3.33 ± 0.42**
OBX + HECM (100)	515.3 ± 16.98*	7.00 ± 0.44***	583.8 ± 35.28***	3.16 ± 0.34**
OBX + HECM (200)	412.8 ± 15.81***	9.83 ± 0.54***	318.7 ± 30.16***	4.16 ± 0.30***

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the parenthesis indicate dose in mg/kg, p.o. Data was analyzed by one-way ANOVA followed by Dennett's test. #P < 0.05, ##P < 0.01, ###P < 0.001 as compared to sham control, *P < 0.05, **P < 0.01 and ***P < 0.001 when compared to the OBX control, n = 6 per group.

Table 2: Effect of HECM on immune parameters in OBX rats

	Weight spleen (gm)	Weight thymus (gm)	Neutrophils (cells/mm^3)	Lymphocytes (Cells/mm^3)
Sham Control	661.8 ± 2.63	653.5 ± 2.93	23.00 ± 2.01	69.17 ± 2.38
OBX control	450.5 ± 3.89###	358.3 ± 3.98###	49.23 ± 2.23###	39.17 ± 2.92###
OBX+IMP (20)	523.8 ± 12.16***	419.0 ± 4.06***	17.67 ± 1.74***	79.00 ± 1.63***
OBX + FLX (30)	587.8 ± 5.23***	593.3 ± 7.17***	27.17 ± .32***	67.83 ± 7.19***
OBX + DSP(15)	558.0 ± 7.73***	452.3 ± 9.33***	20.33 ± 2.10***	79.00 ± 2.40***
OBX + HECM (50)	460.5 ± 2.79	374.7 ± 6.19	15.83 ± 1.64	81.83 ± 1.70***
OBX + HECM (100)	563.8 ± 10.92***	508.5 ± 7.41***	20.67 ± 1.66	75.83 ± 0.30***
OBX+ HECM (200)	647.2 ± 4.42***	648.7 ± 2.89***	--	--

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the parenthesis indicate dose in mg/kg, p.o. Data was analyzed by one-way ANOVA followed by Dunnett's test. #P < 0.05, ##P < 0.01, ###P < 0.001 as compared to sham control, *P < 0.05, **P < 0.01 and ***P < 0.001 when compared to the OBX control, n = 6 per group.

Table 3: Effect of HECM on Brain DA

	Brain dopamine levels (ng/g wet weight tissue)
Sham Control	108.4 ± 2.70
OBX control	49.89 ± 10.87###
OBX + IMP (20)	44.12 ± 5.84
OBX + FLX (30)	97.5 ± 3.61***
OBX + DSP(15)	108.8 ± 1.62***
OBX+ HECM (50)	29.12 ± 5.55
OBX + HECM (100)	57.32 ± 2.14
OBX + HECM (200)	115.5 ± 5.82***

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the parenthesis indicate dose in mg/kg, p.o. Data was analyzed by one-way ANOVA followed by Dennett's test. #P < 0.05, ##P < 0.01, ###P < 0.001 as compared to sham control, *P < 0.05, **P < 0.01 and ***P < 0.001 when compared to the OBX control, n = 6 per group.

DISCUSSION

Sexual activity is a multifaceted activity, which involves a complex interaction between endocrine system, nervous system, vascular system and a variety of other structures that are instrumental in sexual excitement, intercourse and satisfaction. Sexual dysfunction can result from a wide variety of psychological and physiological causes including derangements in the levels of sex hormones and neurotrensmitters.[15]

Increased brain activities of catecholamines like noradrenaline and dopamine may improve some parameters of copulatory activity, indicating their facilitatory role in the process.[16,17] Although there are some previous reports regarding an opposite role for serotonin[17], more recent studies have proved that 5-HT1A and 5-HT2 receptor agonists may also stimulate sexual motivation.[18-20] The effects of both serotonin and dopamine on male sexual behavior seem to occur by interaction with testosterone.[17-20] All the four major dopaminergic pathways are proposed to play a role in sexual behavior. The incertohypothalamic dopaminergic pathway stimulation increases all the phases of male rat copulatory behaviour and induces penile erection. In female rats it induces sexually receptive posture named as lardosis. The mesolimbic pathway is involved in the anticipatory phase of sexual activity associated with motivation and sexual reward.[15] The nigrostriatal pathway is important for the motor behaviour required for the sexual activity in male rats. Lastly the tubero infudibular pathway plays a role in baseline sexual interest.[16,21,22] In present study, brain DA levels were improved by HECM at higher dose indicating possible dopaminergic mechanism in improving parameters of sexual dysfunction. HECM might be acting by either of the dopaminergic pathway. A proper androgenic status is also necessary for a normal sexual performance.

Hypercortisolism (Cushing syndrome) can produce a constellation of symptoms which includes severe depression, insomnia and also decreased libido in males and females. Cortisol levels were found higher in men with psychogenic erectile dysfunction[23]. Serotonin acts at both central as well as peripheral receptors in mediating sexual activity[21]. Centrally, serotonin was found to downregulate and diminish the levels of mesolimbic dopaminergic activity and also to elevate prolactin levels, resulting in decreased libido.[24,25] In addition, serotonergic activation at different receptor subtypes has differential effects on the sexual functioning. Activation of receptor subtype IA lowers the threshold for ejaculations, while activation of 2A, 1B or IC inhibits sexual performance and stimulation of 2C facilitates the behaviour in animal models.[26] Peripherally, at spinal receptors, serotonin has inhibitory effects on ejaculation in rodents.[27] Serotonin also acts on the smooth muscles of the genitals possibly inhibiting the muscular contraction that characterizes the orgasm.[21] Further, it also acts at peripheral nerves, where it affects the flow of genital sensory information. Lastly, serotonin also delays orgasm through pre-synaptic inhibition of adrenergic neurotransmission.[27]

In men, blood plasma NE levels were elevated during sexual activity and declined to baseline within 2 minutes of reaching orgasm.[28,29] GABA facilitating benzodiazepine's use has been implicated in some case

reports of decreased libido, erectile dysfunction and anorgasmia.[30] While mechanisms are very poorly understood, it is hypothesized that central sedation and peripheral muscle relaxation may be a responsible factor.[21] Apart from the above mentioned ones, many other putative neurotransmitters, including angiotensin II, argininevasopressin, substance P, a-MSH, neuropeptide-Y, Gn-RH are involved in regulation of sexual behaviour, but there is little research into their precise role.[31] Ablation of the olfactory lobes of the rat not only results in anosmia but also a loss in the detection of pheromones. Pheromones are chemical signals that carry information on the behavioral and physiological status of the animal.[32] In rats, pheromone plays a key role in sexual behavior, gender recognition, aggressive behavior and social dominance among male rodents and in the passive avoidance behavior.[33]

The role of pheromones is of importance for understanding the behavior of the OB rat as the input from the vomeronasal organ, which detects pheromones and is linked to the amygdala via the accessory olfactory lobe, is ablated during the surgical procedure.[32] In our study, OBX rats failed to copulate with sexually receptive female, the reason for this may be the difficulty in pheromone detection. Also, the signaling of several neurotransmitter systems are deregulated in OBX rats (serotonergic, dopaminergic, adrenergic, and gabaergic).[34] The neurotransmitter dopamine (DA) facilitates male sexual behavior in all studied species, including rodents and humans.[35] In the present study, HECM at all the dose levels significantly improved all the parameters of sexual behavior in male OBX rats. The reason for this may also be the elevation of brain dopamine levels. Testosterone facilitates the sexual behavior in rats.[36] But, it is reported that, OBX induced sexual dysfunction is not attributed to the impaired production of gonadal secretions.[37] In this context, there are reports on sexual dysfunction of OB in which the antidepressant treatment reverses these deficits following an OB.[38,39] After removal of olfactory bulbs, rats subsequently develop a series of changes in behavior, neurotransmitter, endocrine and immune aspects which are similar to those occurring in depressed patients. A significantly decreased thymus weight was also found which was coupled with increased weight of adrenals. Reduced weight of thymus in rodents has been linked to reduced serotonergic activity and increased emotional reactivity to social stress. In the present study weight of thymus was reduced significantly following surgery.[40] Treatment with both standards as well as test group significantly increased the thymus as well as spleen weight. Further studies are planned where HECM will be tested for its effects on serum testosterone levels.

CONCLUSION

Commiphora mukul at all the doses significantly improved sexual and immune dysfunction induced by OBX, which is comparable with standard drugs.

REFERENCES

1. Licinio J. and Wong M.L., The Role of Inflammatory Mediators in the Biology of Major Depression: Central Nervous System Cytokines Modulate the Biological Substrate of Depressive Symptoms, Regulate Stress-Responsive Systems, and Contribute to Neurotoxicity and Neuroprotection, *Molecular Psychiatry*, 4, 1999, 317-327.
2. Ernst, E. St. John's Wort, an Antidepressant? A Systematic, Criteria-Based Review. *Phytomedicine*, 2, 1995, 67-71.
3. Laakmann G., Dienel A. and Kieser M., Clinical Significance of Hyperforin for the Efficacy of Hypericum Extracts on Depressive Disorders of Different Severities, *Phytomedicine*, 5, 1998, 435-442.
4. Zhang Z.J., Therapeutic Effects of Herbal Extracts and Constituents in Animal Models of Psychiatric Disorders, *Life Sciences*, 75, 2004, 1659-1699.
5. Sultana N, Jahan S, Studies on the constituents of Commiphora mukul, Journal of Chemical Sciences, 60(11), 2005, 1202–1206.
6. Bellamkonda R, Rasineni K, Singareddy SR, Kasetti RB, Pasurla R, Chippada AR, Antihyperglycemic and antioxidant activities of alcoholic extract of Commiphora mukul gum resin in streptozotocin induced diabetic rats, *Pathophysiology,* 18(4), 2011, 255–261.
7. Leonard BE, Tuite M. Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat, International Review of Neurobiology, 22, 1981, 251-286.
8. Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther.*; 74, 1997, 299-316.
9. Harkin A, Kelly JP, Leonard BE, A review of the relevance and validity of olfactory bulbectomy as a model of depression, *Clinical Neuroscience Research*; 3, 2003, 253-262.
10. Kalshetti P, Alluri R., Thakurdesai PA., Antidepressant Effects of Standardized Extract of *Commiphora mukul* Engl. in Olfactory Bulbectomized Rats. *Brazilian Archives of Biology and Technology*, 58(1), 2015, 41-48.
11. Redmond A, Kelly J, Leonard B, Behavioural and neurochemical effects of dizocilpine in the olfactory bulbectomized rat model of depression. *Pharmacology Biochemistry and Behaviour*, 58(2), 1997, 355–359.
12. Aswar U.M, Kalshetti P.B, Shelke S.M, Bhosale S.H., Bodhankar S.L. Effect of newly synthesized 1,2,4-triazino[5,6-b]indole-3-thione derivatives on olfactory bulbectomy induced depression in rats. Asian Pacific Journal of Tropical Biomedicine, 2(12), 2012, 992-998.
13. Song C., Kelly J. and Leonard B. E, Is the state of leukocyte adhesiveness/aggregation an immune marker of stress in the olfactory bulbectomized rat? *Stress Medicine*, 9, 1993, 255-258.
14. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F, A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue, Biochemical Pharmacology, 23(17), 1974 , 2437-2446.

15. Nagaraj, Anil Kumar Pai, Nagesh Brahmavar Rao, Satheesh Rao, Telkar Srinvasarao and Goyal Nishant, Biology of Sexual Dysfunction, *Online Journal of Health and Allied Sciences*, 8(1), 2009, 1-7.
16. Melis MR & Argiolas A. Dopamine and sexual behavior. *Biobehav Reviews*, 19, 1995, 19-38.
17. Meisel RL, Sachs BD, The physiology of male sexual behavior, *The Physiology of Reproduction*, 2nd edition, Raven Press, New York, 1994, 3-105.
18. Padoin MJ & Lucion AB, The effect of testosterone and DOI on male sexual behavior of rats, *European Journal of Pharmacology*, 277, 1995, 1-6.
19. Rasia-Filho AA & Lucion AB, Effects of 8-OH-DPAT on sexual behavior of male castrated at different ages, *Hormones and Behavior*, 30, 1996, 251-258.
20. Rowland DL & Houtsmuller EJ, 8-OH-DPAT interacts with sexual experience and testosterone to affect ejaculatory response in rats. *Pharmacology Biochemistry and Behavior*, 60, 1998, 143-149.
21. Hallward A, Ellison JM, Sexual dysfunction. In: Antidepressants and Sexual Function. London: Harcourt Health Communications. 2001, 28-57.
22. Foreman MM, Moss RL., Role of hypothalamic dopaminergic receptors in the control of lordosis behaviour in the female rat, *Physiology and Behaviour, 22,* 1979, 283-289.
23. Granata A, Bancroft J, Del Rio G, Stress and the erectile response to intracavernosal prostaglandin E1 in men with erectile dysfunction, *Psychosomatic Medicine,* 57, 1995, 336-344.
24. Rosen RC, Lane RM, Menza M, Effects of SSRIs on sexual function: a critical review, *Journal of Clinical Psychopharmacology,* 19, 1999, 67-85.
25. Zajecka K, Strategies for the treatment of antidepressant related sexual dysfunction. *Journal of Clinical Psychiatry,* 62(13), 2001, 35-43.
26. Bitran D, Hull EM, Pharmacological analysis of male rat sexual behaviour. *Neuroscience and Biobehavioural Reviews,* 11, 1987, 365–389.
27. Zajecka J, Fawcett J, Schaff M, Jeffriess H, Guy C, The role of serotonin in sexual dysfunction: fluoxetine associated orgasm dysfunction, *Journal of Clinical Psychiatry,* 52(2), 1991, 66-68.
28. Kruger J, Exton MS, Pawlak C, Vonzur Muhlen A, Hartmann U, Shedlowski M, Neuroendocrine and Cardiovascular response to sexual arousal and orgasm in men, *Psychoneuroendocrinology*, 23, 1998, 401-411.
29. Wiedeking C, Ziegler MG, Lake CR. Plasma noradrenaline and dopamine beta-hydroxylase during human sexual activity. *Journal of Psychiatric Research,* 15, 1979, 139-145.
30. Gitlin MJ, Psychotropic Medications and their effects on sexual function: diagnosis, biology, and treatment approaches. *Journal of Clinical Psychiatry,* 55(9), 406-413.
31. Baldwin DS, Birtwistle J, Antidepressant drugs and sexual function: Improving the recognition and management of sexual dysfunction in depressed patients. In Briley M, Montgomery S (eds). Antidepressant therapy at the dawn of the third millennium. London: Martin Dunitz, 1998, 231-253.
32. Song C., and B. E. Leonard, The olfactory bulbectomised rat as a model of depression, Neuroscience and Biobehavioral Reviews, 29(4-5), 2005, 627-647.
33. R. Tirindelli, M. Dibattista, S. Pifferi, A. Menini, From pheromones to behavior, Physiological Reviews, 89, 2009, 921–956.
34. Skelin, Neurochemical and Behavioural Changes in Rat Models of Depression. *Croatica Chemica Acta,* 84 (2), 2011, 287–299.
35. Dennis F. Fiorino and Anthony G. Phillips. Facilitation of Sexual Behavior and Enhanced Dopamine Efflux in the Nucleus Accumbens of Male Rats after d-Amphetamine-Induced Behavioral Sensitization, The Journal of Neuroscience, 19(1), 1999, 456–463.
36. McCormick C.M., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, *Hormones and Behaviour*, 63(1), 2012, 5–12.
37. Larsson, K. Sexual impairment of inexperienced male rats following pre- and postpuberal olfactory bulbectomy, *Physiology & Behavior*, 14(2), 1975, 195-199.
38. Wang J. F. Defects of mitochondrial electron transport chain in bipolar disorder: implications for mood-stabilizing treatment. *Canadian Journal of Psychiatry,* 52, 2007, 753-762.
39. Chambliss H. O., J. D. Van Hoomissen, P. V. Holmes, B. N. Bunnell and R. K. Dishman, Effects of chronic activity wheel running and imipramine on masculine copulatory behavior after olfactory bulbectomy, *Physiology & Behavior*, 82(4), 2004, 593-600.
40. Song C., Kelly J. and Leonard B. E, Is the state of leukocyte adhesiveness/aggregation an immune marker of stress in olfactory bulbectomized rat? Stress Medicine, 9, 1993, 255-258.
41. Song C. and Leonard B. E., The effects of lithium chloride administration on some behavioral and immunological changes in bilaterally bulbectomized rat, Journal of Psychopharmacology, 8, 1994c, 440-447.

Source of Support: Nil, **Conflict of Interest:** None.