ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antidepressant Effect of*Brassica nigra* Seeds in Swiss Mice Using Tail Suspension Test.

R Parthiban[1], S Anand[2], R Vishnupriya[3], and Sangavai Mathiazhagan[3]*.

[1]Department of Pharmacology, Government Trichy Medical College and Hospital, Trichy,Tamil Nadu, India.
[2]Department of PharmacologySreeBalaji Medical College and Hospital, Chromepet, Chennai, Tamil Nadu, India.
[3]Department of PharmacologyGovernment Stanley Medical College and Hospital, Chennai, Tamil Nadu, India.

ABSTRACT

Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous . The complexity of daily life in modern society frequently leads to varying degree of anxiety and depression. Mood, depression and anxiety disorders havebeen found to be associated with chronic pain among medical patients in both developed and developing countries. These considerations implicate the search for new anxiolytic and antidepressant agents that havea fast onset of action present with less side effects and a wider safety margin. Mustard is among the oldest recorded spices as seen in sanskrit records dating back to about 3000BC (8) and was one of the first domesticated crops. *Brassica nigra* (Mustard) is used as remedy for the following few problems:-Bronchitis, Muscular and skeletal pains.It stimulates circulation in pain area and thus help to relieve pain.The plant is a folk remedy for arthritis, foot ache, lumbago, and rheumatism. The seed is used in the treatment of tumours in China. In Korea, the seeds are used in the treatment of abscesses, cold, lumbago, rheumatism, and stomach disorders. The root is used as a galactagogue in Africa. The present study has been undertaken with the following to evaluate the antidepressant action of ethanolic extract of *Brassica nigra* by forced swim test in albino rats and swiss mice.
Keywords: mustard, Brassica nigra, antidepressant, tail suspension test.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being (1).Depressed people may feel sad, anxious, empty, hopeless, worried, helpless, worthless, guilty, irritable, hurt, or restless. Depression is a common mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feeling of tiredness, and poor concentration. They may lose interest in activities that once were pleasurable, experience loss of appetite or overeating, have problems concentrating, remembering details, or making decisions, and may contemplate or attempt suicide(3). Insomnia, excessive sleeping, fatigue, loss of energy, tendencies to self-harm, or aches, pain, or digestive problems that are resistant to treatment may also be present (2). Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous (4). The complexity of daily life in modern society frequently leads to varying degree of anxiety and depression. Mood, depression and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries (5). These considerations implicate the search for new anxiolytic and antidepressant agents that havea fast onset of action present with less side effects and a wider safety margin. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for CNS disorders and related diseases (6).

Various plants are being used in complementary and alternative medicines for management of depression. The use of plant products is increasing in many segments of the population. Most of the synthetic drugs used at present for anti-depressant effect have many side and toxic effects. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (7).

Mustard is among the oldest recorded spices as seen in sanskrit records dating back to about 3000BC (8) and was one of the first domesticated crops.Originally it was the condiment that was known as mustardand word was derived from the Latin*mustum*. *Brassica nigra* (Mustard) is used as remedy for the following few problems,Bronchitis, Muscular and skeletal pains.It stimulates circulation in pain area and thus help to relieve pain.The plant is a folk remedy for arthritis, foot ache, lumbago, and rheumatism. The seed is used in the treatment of tumours in China. In Korea, the seeds are used in the treatment of abscesses, cold, lumbago, rheumatism, and stomach disorders. The root is used as a galactagogue in Africa. Ingestion may impart a body odour repellent to mosquitoes. Mustard oil is used in the treatment of skin eruptions and ulcers. Believed to be aperient and tonic, the volatile oil is used as a counterirritant and stimulant. In Java the plant is used as an antisyphilitic and emmenagogue (9). Leaves applied to the forehead are said to relieve headache. The Chinese eat the leaves in soups for bladder inflammation or haemorrhage.

Leaves

Antiscorbutic, diuretic, stimulant, relieve headache (10), muscular and skeletal pains, diaphoretic, liniment for rheumatic pain,antihelmintic,antidysentric, fever and cold , bladder inflammation or haemorrhage in china.

Seed

Rubefacientand stimulant in China. In Korea, these are used for abscesses, cold, lumbago, rheumatism, stomach disorders, hypoglycemia, and treatment of blisters in inflammatory neuralgic infections, obstinate vomiting and antioxidant activity.

Seed paste is used in backache, arthritis, paralysis, stye, oedema of the lungs and liver.

Powdered seed

Antibacterial activity, internally used for hiccup and augments the appetite.It relieves the phlegm in cough.

Oil

Aphrodisiac, lubricant,hair oil, preservative, counter-irritant, emetics in drunkenness and in poisoning, skin eruptions and ulcers (11), colic, andexternally applied for arthritis, antiseptic and anti-inflammatory. Seed oil with salt is an effective gargle in dental infections and pyorrhoea.

Seed residue

For cattle feed and also asfertilizer.

Roots

As galactagogue in Africa.

Dried leaf and flower

A body odour repellent to mosquitoes and for dengue fever.

Other parts

Diuretic, arthritis, foot ache, lumbago, rheumatism (12), and its decoction is useful in amenorrhea.

Further, it is widely believed that *Brassica nigra* is a potent antidepressant agent. There are no scientific studies to indicate the effect of*Brassicanigra*as antidepressant agent. The present study was undertaken to study antidepressanteffect of *Brassica nigra*in animal models using forced swim test and tail suspension test.

Aims and Objectives

The present study has been undertaken with the following aims & objectives.

- To evaluate the antidepressant action of ethanolic extract of *Brassica nigra* by Tail suspension test in swiss mice.
- To find the effective dose of *Brassica nigra*for antidepressant action.

MATERIALS AND METHODS

Plant material

Mustard seeds(*Brassica nigra*) was Purchased from the local market in Chennai and identified by The Director, National institute of herbal science, west tambaram, Chennai.

Experimental animals

Swiss mice and Albino rats of wistar strain of either sex weighing 30gm and 200gmrespectively, were used for the study. Animals were purchased from King Institute of Preventive Medicine, Guindy, Chennai and maintained in the Central Animal House, SreeBalaji Medical College and Hospital, Chennai, India. The animals were individually housed under controlled temperature and hygienic conditions. They all received a standard pellet diet and water ad libitum. Institutional Animal Ethics Committee approved the experimental protocol (002/01/IAEC/2013).

Preparation of *Brassica Nigra* Extract

Mustard seeds were obtained from the local market was dried and powdered using mechanical mixer. The plant extract was prepared using soxhlet apparatus, by maceration of 50 g of the dried seeds of *Brassica nigra*in a mixture of 200 ml ethanol and 200 ml distilled water by shaking them for 48 h and pressing the solution out of the material using a filter press. The extraction solvent was then removed under reduced

pressure until the extract was obtained as a dried gum. The final extracted material weighed 10g. Concentrations of the extract were prepared by dissolving final product in distilled water.All the other chemicals like fluoxetinewere of analytical grade and were procured from local commercial companies.

Selection of Dose

For the assessment of antidepressant activity, three dose levels were chosen i.e, 50 mg/kg, 100mg/kg and 200mg/kg respectively (13,14).

Screening Methods for Antidepressant Activity

Forced Swimming Test (FST)

The forced swimming test was used for the evaluation of antidepressant effect of *Brassica nigra*in rats. Animals were placed in pyrex cylinders (10 × 45 cm) which were filled with water at 24-25°C with a 30cmdepth and behaviors were monitored.. In two sessions separated by 24 hours, rats are forced to swim in the pyrex cylinder from which they cannot escape. The first 15-minutes session is conducted prior to drug administration and without behavioral recording. This prior habituation session ensures a stable and high duration of immobility during the 6-min test session, performed 24 hours later. Saline, fluoxetine and ethanolic extract of *Brassica nigra*were administered intraperitoneally 30 min prior to the test session. The duration of test was 6 min. After two min, immobility and swimming time was measured during the last 4 min(15). Immobility was assigned when no additional activity was observed other than that required to keep the animal's head above the water and swimming time assigned when animal did active movement of extremities and circling in the container.

Study Design

Number of groups: 12
Number of animals in each group: 6
Total number of animals
Forced swim test(Rats):36

Experimental Design

Tail Suspension Test

Group 1: Control (Normal saline 0.3ml, i.p)
Group 2: Fluoxetine (20 mg/kg, i.p)
Group 3: *Brassica nigra* (50mg/kg, i.p)
Group 4: *Brassica nigra* (100mg/kg, i.p)
Group 5: *Brassica nigra* (200mg/kg, i.p)
Group 6:Fluoxetine(20mg/kg, i.p) plus *Brassica nigra*(200 mg/kg, i.p)

Statistical analysis

All the data were expressed as mean ± SEM.The significance of differences among the groups was assessed using one way analysis of variance (ANOVA).For determining the significance of intergroup differences, t-test for independent samples were done . Significance was accepted at $p<0.05$.

RESULTS

This study was carried out with an attempt to evaluate the antidepressant activity of *Brassica nigra* in comparison to standard drug, Fluoxetine using tail suspension test. Results obtained from the study are summarized below.

ISSN: 0975-8585

Table1: The Antidepressant effect of *Brassica nigra* in comparison with control and standard drug Fluoxetine using tail suspension test

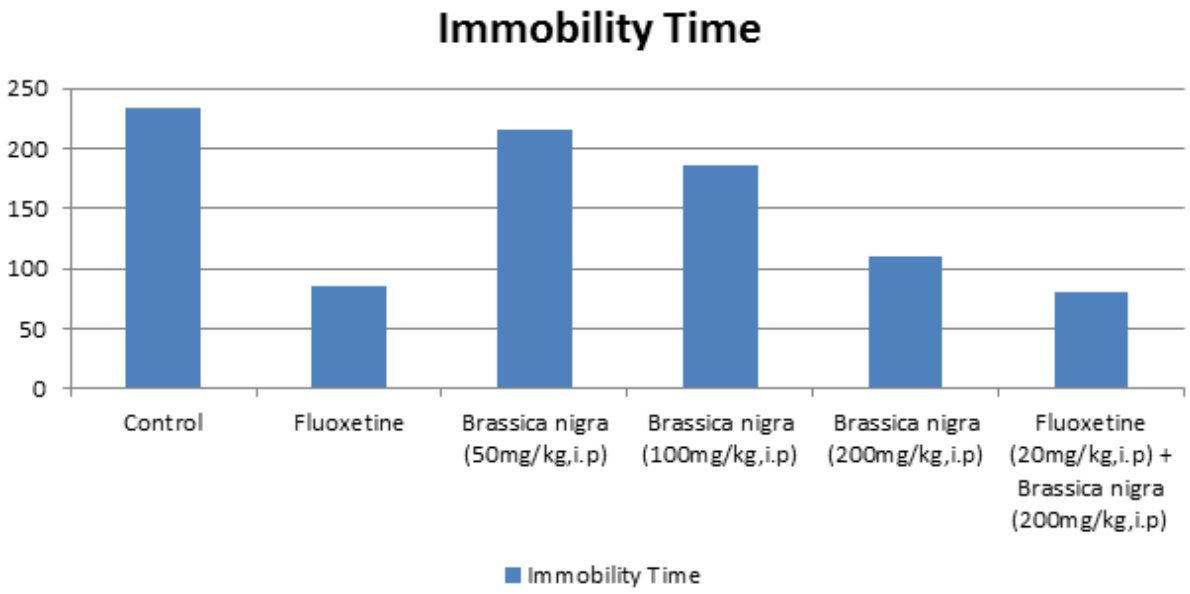
GROUPS	TAIL SUSPENSION TEST DURATION OF IMMOBILITY IN SECS (Mean±SEM)
Control(normal saline 0.3ml, i.p)	**233.00±3.17**
Fluoxetine (20mg/kg,i.p)	**86.00±1.41**
Brassica nigra(50mg/kg,i.p)	**216.00±3.90**
Brassica nigra(100mg/kg,i.p)	**185.33±3.93**
Brassica nigra(200mg/kg,i.p)	**109.16±3.80**
Fluoxetine(20mg/kg,i.p) plus *Brassica nigra*(200mg/kg,i.p)	**79.50±3.56**

The mean values of antidepressant effect between control, fluoxetine, *Brassica nigra* (50mg/kg,100mg/kg&200mg/kg), fluoxetine plus *Brassica nigra*(200mg/kg) groups were expressed as time in seconds as shown in the table.1. Results of estimations have been reported as mean of six animals in each group.

Identification of effective dose ofethanolic extract of *Brassica nigra*on Tail suspension test in mice

The ANOVA revealed significant effects of treatment on immobility, $F(5, 30)=397.63$, $p < 0.001$. Post hoc analysis demonstrated that *Brassica nigra*50, 100 and 200 mg/kg significantly shortened the immobility time in comparison to control values. Fluoxetine significantly decreased the immobility time during the test session. Moreover, higher dose of the extract (200 mg/kg) when compared with fluoxetine showed $p<0.01$.In addition, there was significant difference in immobility time between low (50 mg/kg) and high doses (200 mg/kg) of the extract ($p<0.001$). Hence, from the above results it can be seen that among the three doses of the extract used, the most effective dose for antidepressant action using tail suspension test is 200mg/kg of *Brassica nigra.*

Figure1: showing immobility time in comparison with control,standard and ethanolic extract of *Brassica nigra*in Tail suspension test.



The effect of Fluoxetine plus effective dose (200mg/kg) of *Brassica nigra* extract on immobility time in mice using tail suspension test

The effect of fluoxetine (20mg/kg)plus effective dose (200mg/kg) of *Brassica nigra*extracton immobility time in TST in mice has been shown in Figure.2. As shown, injection of fluoxetine plus effective dose (200mg/kg) of *Brassica nigra*extract significantly decreased immobility (**79.50±3.56** sec *vs***233.00±3.17** sec,

p<0.001) time compared to the control group. Also fluoxetine plus effective dose (200mg/kg) of *Brassica nigraextract*has no significant difference in immobility (**79.50±3.56** sec *vs.* **86.00±1.41** sec, p=0.758) when compared to fluoxetine alone group.These results show that *Brassica nigra* has a potential in reducing depression when combined with fluoxetine.

DISCUSSION

The present study was conducted to study the effect of *Brassica ni*gra to ameliorate depression in rats and mice and to see the effectiveness of *Brassica ni*gra,it is compared with fluoxetine group.

Depression, a widespread incapacitating psychiatric ailment, imposes a substantial health burden on society(16). The incidence of depression in the community is very high and is associated with lot of morbidity. Affective disorder are characterized by a disturbance of mood associated with alteration in behaviour, energy, appetite, sleep, and weight (17). Medications such as tricyclic antidepressants(TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), specific serotonin-norepinephrine reuptake inhibitors (SNRIs), 5-HT2 receptor antagonists, and other heterocyclics are clinically employed for drug therapy (18). However, these drugs can impose a variety of side-effects including sedation, apathy, fatigue, sleep disturbance, cognitive impairment, and sexual dysfunction, and so forth. Hence, there remains a pressing need for new effective and better-tolerated antidepressants (19). Hence, it is very important to address this problem and find effective remedies.

Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Despite the widely popular uses of *Brassica nigra*, there is an absence of scientific studies about the evaluation of its pharmacological effects as antidepressant.

In this work, it was demonstrated that the administration of different doses of the ethanol extract of *Brassica nigra*in rats and mice was able to induce antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviours.

The most widely used animal model for antidepressant screening is the forced swimming test. This test is quite sensitive and relatively specific to all major classes of antidepressants (20). In the FST, rats are forced to swim in restricted space from which they cannot escape. This induces a state of behavioural despair in animals, which is claimed to reproduce a condition similar to human depression (21). The forced swimming test demonstrated that the *Brassica nigra* extract acted like an antidepressant drug in the rats. Doses of 50 mg/kg,100 mg/kg and 200 mg/kg were able to reduce immobility time(48.33±0.90;43.83±1.30;36.83±2.70 vs. 21.83±0.79 of standard)and simultaneously, to enhance active behaviours, like swimming(17.83±0.70;20.83±1.24;30.33±1.56 vs 34.66±1.47 of standard). Reduction of immobility was comparable to that observed after the i.p administration of the reference antidepressant drug fluoxetine(p<0.01). Of this 200mg/kg is found to be the effective dose.

In agreement with previous reports (22), the decrease in immobility induced by fluoxetine was accompanied by an increase in swimming time(p<0.01). Results showed that the administration of the *Brassica nigra*produced a diminution of immobility time (a posture thought to reflect a state of ‘‘behaviour despair’’ in which animals have given up the hope to escape) of rats exposed to the forced swimming. In the present study, effective dose of *Brassica nigra*200 mg/kg, i.p administered to rats, produced significant antidepressant like effect in FST and their efficacies were found to be comparable to fluoxetine (20 mg/kg,i.p). Fluoxetine when combined with extract showed significant antidepressant effect compared to fluoxetine. Thus combining extract potentiates the antidepressant effect of Fluoxetine.

It has been established that the shortening of immobility time in the forced swimming test depends mainly on the enhancement of central 5-HT and catecholamine neurotransmission(23).The immobility is thought to reflect either a failure of persistence in escape-directed behaviour (i.e., despair behaviour) or the development of a passive behaviour, meaning the loss of the animal's ability to cope with stressful stimuli.*Brassicanigra*markedly showed a significant decrease in the time spent immobile by rodents. The antidepressant effects may be due to the flavonoid (23) content present in the extracts of*Brassicanigra*.It has

been demonstrated that swimming time is sensitive to serotoninergic compounds, such as the selective serotonin reuptake inhibitor fluoxetine (24,25,26,27).

Although other kind of studies is obviously necessary to elucidate the mechanism of action of *Brassica nigra* in the CNS, the pattern of effects observed in the FST suggests the involvement of both serotoninergic and catecholaminergic neurotransmitter systems on its antidepressant-like effect.Early evidence of a role for noradrenaline in depression came from the discovery that drugs, either causing or alleviating depression, acted to alter the noradrenaline metabolism.

Furthermore, depletion studies carried out in treated and untreated patients indicated a role for serotonin and noradrenaline in depression (26).Alkaloids present in *Brassica nigra*act as reversible monoamine oxidase inhibitors and in common with other beta carboline, binds to 5-hydroxy tryptamine (HT) receptors(27). MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amines in central nervous system. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines.

It has been reported that plants containing compounds such as flavonoids, and kaempefrol show antidepressant effect (15). It has also been shown that kaempefrol has antidepressant effect (29). In addition, it has been reported that flavonoids and kaempefrol show inhibitory effect on mono-amino oxidase enzyme (MAO) in vitro (15,28). Also the flavonoids components of *Brassica nigra* might be interacting with adrenergic and serotonergic systems in mediating the antidepressant effects of *Brassica nigra*.

The findings from the present investigation indicate that *Brassica nigra* possesses significant antidepressant activity as shown by its mitigating effects on differentexperimentally induced stress models in rats and mice.

However, further studies are required to identify the phytoconstituents responsible for the observed anti-depressant effect.

REFERENCES

[1] Sandra S ,*Depression: Questions You Have – Answers You Need*People's Medical Society. 1997;1:14
[2] "NIMH · Depression". *nimh.nih.gov*. October 2012.
[3] WHO,Mental Health,(www.who.int/depression/en/)2012-13
[4] Soulimani R, Younos C, Jarmouni S, Bousta D, Misslin R and Mortier F, Behavioraleffects of Passifloraincarnata L. and its indole alkaloid and flavonoid derivatives andmaltol in the mouse. J. Ethnopharmacol 1997; 57: 11-20.
[5] Dunham NM, Miya TS. A note on simple apparatus for detecting Neurological deficit inrats and mice. J.Am. pharm. 1957; 46: 208-9.
[6] Dhingra D and Sharma A. Antidepressant-like activity of Glycyrrhizaglabra. 2006 May;30(3):449-54.
[7] Ahmad F, Khan RA, Rasheed S.Study of analgesic and anti-inflammatory activity from plant extracts of Lactucascarliola and Artemsiaabsinthium. J IntAcadSci 1992;5: 111- 114.
[8] Mehra,K.L, History and ethiobotany of mustard in india.Adv.front,Pl,Sci, (1968),19:57
[9] Ouyang SW, Zhao KJ, Feng LX, Chye ML, Ram S. [BjCHI1 from Brassica juncea displays both chitinase and agglutination activity]. Sheng Wu Gong Cheng XueBao. 2002;18(5):572
[10] Burkill et al,Plant Breeding Reviews, 1996. Volume 35,55-57
[11] Joseph M. DiTomaso, Evelyn A. Healy,Perry,Weeds of California and Other Western States, Volume 1:19-80.
[12] Duke.J.A & Wain.K.K ,Medicinal plants of the world,1981.
[13] Moallem SA, Hosseinzadeh H, Ghoncheh H. 2007. Evaluation of Antidepressant Effectsof Aerial Parts of Echiumvulgare on Mice. Iranian Journal of Basic Medical Sciences,10: 189 - 196.
[14] Nemeroff C. B., "The burden of severe depression: a review of diagnostic challenges and treatment alternatives," J of Psychiatric Research, 2007, vol. 41, no. 3-4, pp. 189–206.
[15] Neal M. J.,Medical Pharmacology at a Glance, 2009.
[16] Anthony J.T., Bertram G. K., and Susan B. M., Pharmacology Examination and Board Review Ninth Edition, 2010. 45-63

[17] Lieh-Ching Hsu,1 Yu-Jen Ko,1 Hao-Yuan Cheng,2 Ching-Wen Chang,1 Yu-Chin Lin,3 Ying-Hui Cheng,1 Ming-Tsuen Hsieh,1 andWen Huang Peng1 Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2012, Article ID 497302, Antidepressant-Like Activity of the Ethanolic Extract from Uncaria lanosa Wallich var. appendiculataRidsd in the Forced Swimming Test and in the Tail Suspension Test in mice.

[18] Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl.) 1988; 94: 147–60

[19] Brunello N, Mendlewicz J, Kasper S, Leonard B, Montgomery S, et al. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. European Neuropsychopharmacology 2002; 12: 461–75

[20] Page ME, Detke MJ, Dalvi A, Kirby JG, Lucki I. Serotoninergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology 1999;147:162– 7.

[21] Abdel Fattah AFM, Matsumoto K, Gammaz and Watanabe H. PharmacolBiochemBehaviour 1995; 52: 421

[22] Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotoninergic and noradrenergic antidepressants.Psychopharmacology 1995;121:66– 72.

[23] Cryan JF, Lucki I. Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine2c receptors. J PharmacolExpTher 2000;295:1120– 6.

[24] Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future need. TIPS 2002;23:238– 45.

[25] Cryan JF, Page ME, Lucki I. Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swimming test. Eur J Pharmacol 2002;436:197–205.

[26] Setty BS, Kamboj VP and Khanna NM, Screening of Indian Plants for biological activityPart. VII. Spermicidal activity of Indian plants. Indian Journal of Expt. Biol, 15: 231–232, (1977).

[27] Ojha P, Maikhuri JP, Gupta G, Effect of spermicides on Lactobacillus acidophilus invitro - nonoxynol-9 vs. Sapindussaponins. Contraception, 68 (2): 135-138, (2003).

[28] Butterweck V, Nahrstedt A, Evans J, Hufeisen S, Rauser L, Savage J, et al. 2002. In vitro receptor screening of pure constituents of St.John'swort reveals novel interactions with anumber of GPCRs. Psychopharmacologia,162(2) :193-202

[29] Hosseinzadeh H, Motamedshariaty V, Hadizadeh F. 2007. Antidepressant effect of kaempferol, a constituent of saffron (Crocus sativus) petal, in mice and rats. Pharmacologyonline, 2: 367-370.