

Antidepressant effects of Dietary Supplements Garlic and Black Sesame Extracts in Ovariectomized Rats: Involving possible Estrogenic and Antioxidant Mechanism

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Abstract: Dietary supplements Garlic and Black Sesame consist of phytoestrogens; it has been clinically used for treating mild depressive disorders by possible Estrogenic and Antioxidant mechanism. The purpose of the study was to examine the effect of dietary supplements on depression in ovariectomized (OVX) rat by despair force swimming test and *In vivo*-lipid peroxidation study. After the treatment of Garlic 250 and 500 mg/kg and Black Sesame 500 mg/kg P.O. for three month in OVX rats, decrease the immobility period which was compare to OVX control group rats ($P < 0.0001$). Oxidative stress is a measure of steady state level of reactive oxygen species (ROS) in the biological system. Treatment of Garlic and Black Sesame can decrease the oxidative stress in OVX rats. In present study lipid peroxidation was measured in terms of TBARS and result indicate to significant increase in blood serum TBARS content in OVX control rats compared with Sham (SH) control rats ($P < 0.05$). However in oral administration of Estradiol Valerate 0.20mg/kg in standard group and treatment groups of Garlic-250mg/kg, Garlic-500 mg/kg and Black Sesame-500 mg/kg reduced the level of TBARS indicating a decrease rate of lipid peroxidation as compare to OVX control rats ($P < 0.05$). Thus present study concludes that dietary supplements Garlic and Black Sesame containing phytoestrogens can be useful for treating menopausal depression in patients.

Keywords: Antidepressant, Phytoestrogen, Ovariectomized, Dietary supplements, Garlic and Black Sesame.

Introduction:

Depressive disorders are among the most frequently occurring psychiatric diseases, and are prevalent in at least 15% of the population. However, the prevalence of depressive disorders is twice as high in women as in men.¹ The higher incidence and severity of depression is associated with the presence or absence of ovarian hormones.² The ovarian hormones related to the menopause-induced depression influence the hippocampal anatomy and physiology; thus they affect behavior in adult female rats.^{3,4,5} The hippocampus plays an important

role in the negative feedback mechanism of the limbic-hypothalamic-pituitary adrenal (LHPA) axis. It expresses high levels of adrenal steroid receptors and is especially susceptible to damage as a result of stress.^{6, 7} In the hippocampus stress hormones induce regression of the dendritic processes and cell death, inhibit neurogenesis and impair the ability of neurons to survive noxious stimuli such as seizure, hypoxia-ischemia, metabolic poisons, hypoglycemia and oxygen radical generators.⁸ The damage caused by a heightened stress response resulting from dysregulation of the LHPA axis is hypothesized to play a role in the reduced hippocampal volume often associated with major depression.^{9,10} Several neurotransmitter systems implicated in the regulation of the HPA axis have been hypothesized to be dysfunctional in major depression including acetylcholine (ACh) norepinephrine, serotonin and dopamine.¹¹ It may be used prophylaxis treatment against the postpartum depression.^{12,13} Estrogen may be useful as an antidepressant in perimenopause.^{14,15} Estrogen replacement therapy is however, effective in reducing mild depressive symptoms after menopause.¹⁶ Owing to its estrogenic nature it is possible that the plant may be useful in estrogen-related disorders like postmenopausal depression. The present study was therefore undertaken to evaluate the effect of alcoholic extract of *Allium sativum* (Garlic) and Pet. ether extract *Sesamum indicum* (Black Sesame) on depression induced by ovariectomy in young adult rats. The objective of the study was to assess the effect on estrogen deficiency induced depression and hormonal deficiency as in the case of aged ovariectomized (OVX) rats.

Material and Method:

Plant Material and reagent: Bulbs of *Allium sativum* and seeds of *Sesamum indicum* are collected commercially from Yavatmal district in Maharashtra, India. The collected bulbs of *Allium sativum* crushed in electric blender to form paste and subjected to maceration with ethanol for 30 days. The extract concentrated by evaporation at room temperature. Extract of *Sesamum indicum* seeds by using Soxhlet's extraction with petroleum ether (60 Grade) as solvent in the ratio of 1:4. The extraction continued till the solvent in the thimble becomes clear indicating the completion of extraction and evaporation of solvent to get semisolid extract.

Phytochemical Analysis: Qualitative phytochemical evaluation of Garlic and Black Sesame were performed according to Khandelwal¹⁷. Identification of steroid and flavanoid by thin layer chromatography by using mobile phase Chloroform: Methanol- 4:1 and Detecting reagent were Liebermann Burchardt reagent for steroid and Flavonoids having Mobile phase Toluene:Chloroform: Acetone-40:25:35 and Detecting reagent were Aluminium chloride reagent (10%).

Experimental Animals: Female wistar rat (150-170gm) housed in the animal house of Sudhakar Rao Naik Institute of Pharmacy, Pusa were used. The animals were housed at 25± 5° and relative humidity 60 ± 5% with 12h light-dark cycle. Food (standard rodent pellet diet) and water was provided ad libitum. Five groups were bilaterally OVX while one was subjected to sham operation. Ovariectomy was performed under ketamine + xylazine anaesthesia (80 mg/kg + 10 mg/kg; i.p.).¹⁸ Animals were housed individually for a week after the surgery and later on in a group of six. Body weight of the animals was recorded weekly. The experiment was conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSEA). The institution animal ethics committee approved the study (729/02/a/CPCSEA) after two weeks of adaptation, animals were used for study.

Chemicals and Instruments: Estradiol Valerate Tablet (Intas Pharmaceuticals Ltd) was purchased from local market. All the chemicals used for study were analytical grade. Swimming tank was used for despair swimming test.

Study Design: Sham control (SH) and OVX control animals were administered orally 0.5% w/v sodium methyl cellulose which was used as vehicle. Other four groups were administered Estradiol Valerate 0.20mg/kg with 0.5% w/v CMC + 0.2% v/v of Tween 20. Dose of Estradiol was selected on the basis of doses used by earlier researchers for the same activity.¹⁹ Dose of Garlic (250mg/kg and 500mg/kg) and Black sesame (500mg/kg) was decided on the basis of reported dose in research paper.^{20,21, 22, 23} Suspensions of Estradiol Valerate, Garlic and Black sesame were prepared in 0.5% of CMC and 0.2 % v/v of Tween 20. A drug treatment was started after one month of ovariectomy²⁴ and was divided doses with 2ml/kg dosing volume and continued for 3 months. On the last day of treatment animals were placed for despair force swimming test and *In vivo* lipid peroxidation test.

Despair Force Swimming Test: OVX female wistar rats weighing 160-180 g were used. They are brought to the laboratory at least one day before the experiment after receiving the three month of treatments and were housed separately in cages with free access to food and water. Rats were individually forced to swim inside a vertical cylinder (height: 60 cm; diameter: 30cm, containing 30 cm of water maintained at 25 °C). Rats placed in the cylinders for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. The frequency and total duration were calculated for each of the following category by two observers behavior score were 1) Struggling (With rhythmical simultaneous kicks and occasional pushes off legs) 2) Fast swimming (swimming with fast movement of legs) 3) Slow movement (swimming with slow movement of legs) 4) Floating (floating on the surface of water without any movement of body). Immobility behavior during this 6 min period was observed for each rat considering slow movement and Floating period. After 6 min in the water the rats were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. Experiment were conducted during the period of 9 a.m. to 5 p.m.^{25, 26}

Estimation of lipid peroxidation: Thiobarbituric acid reaction:²⁷

Animals were sacrificed after three month of treatment and bloods were collected from respective groups of animals and centrifuged serum was collected as a source of polyunsaturated fatty acid for determination of extent of thiobarbituric acid reactive substances (TBARS). 0.1 ml of blood serum collected in 10ml of test tube and mixed with 1.5 ml of to 20% Acetic acid, 0.2 ml of SLS, 1.5 ml of TBA and finally the volume adjusted to 4 ml with distilled water. The mixture was boiled for 60 min on water bath and cooled under tap water. 5 ml of n-butanol and pyridine (15:1) and 1 ml of distilled water was added and centrifuged. The absorbance of supernatant liquid measured at 532 nm.

Statistical Analysis: Group means were compared by ANOVA with GraphPad Prism (GraphPad Software, Inc.) and Using Unpaired student T tests.

Result and Discussion:

Phytochemical Analysis: Qualitative Phytochemical analysis of Ethanolic extract of Garlic and Pet. Ether extract of Black sesame attested presence of steroids and flavanoid. Rf values of Steroids and Flavanoid by TLC were found to be for ethanolic extract of Garlic- 0.91, 0.75, and petroleum extract of Black sesame- 0.95 0.70 respectively. Thus as comparative to previous literature steroids and flavanoid Rf value present in ethanolic extract of Garlic and Petroleum ether extract of Black Sesame have shown to be nearby Rf value of steroids- Diethyl stilbesterol, Estriol and Flavonoids- Kaempferol.²⁸

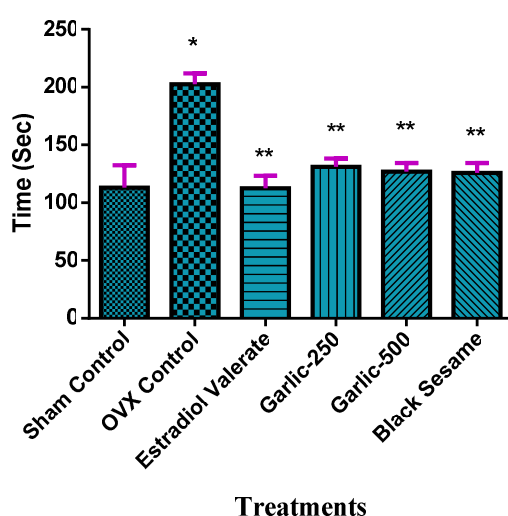
Despair force swimming Test: Animal when exposed to an aversive situation from which there is no probability of escaping eventually stop struggling and assume a typical immobile posture indicative of behavioral depression. Previous study reported that soyo-san containing phytoestrogen decrease the depression in OVX rats.²⁹

The effect of chronic treatment of Estradiol Valerate (0.20 mg/kg), Garlic (250 and 500 mg/kg), Black sesame (500mg/kg) for three month on the progressive of depression in OVX animals was evaluated by measuring the immobility behavior in FST model. The result shows significant increased in the immobility behavior i.e. slow movement and floating behavior in OVX control Group animals which was comparable to standard treatment of estrogen and Sham control group ($P < 0.0001$). However treatments group Garlic 250 and 500 mg/kg and Black sesame 500mg/kg also marked decrease in Immobility (slow movement and floating) as compared to OVX control rats ($P < 0.0001$). Thus by treatment of the Garlic and Black Sesame immobility period can be decrease in all OVX Rats.

Table No. 1 Effect of Estradiol Valerate (0.20mg/ml), Garlic 250mg/kg, Garlic 500mg/kg and Black Sesame 500mg/kg on immobility period by force swimming test in OVX rats.

Group	Sham Control	OVX control	Standard (Estradiol Valerate)	Garlic- 250	Garlic- 500	Black Sesame- 500
Immobility period	113±7.9	202.4±3.9*	112.4±4.4**	131±2.9**	126.8±3.1**	125.7±3.5**

Values are mean ± SEM, * P < 0.0001 vs. SH Control; ** P < 0.0001 vs. OVX Control, Significantly compared by using student unpaired T test, (n = 6/group), and one way annova test

Fig No. 1 Effect of Garlic and Black Sesame on Immobility period in FST in OVX Rats.**Table No. 2 Effect of Estradiol Valerate (0.20mg/ml), Garlic 250mg/kg, Garlic 500mg/kg and Black Sesame 500mg/kg on TBARS Levels in OVX Rats.**

Group	Sham Control	OVX control	Standard (Estradiol Valerate)	Garlic- 250	Garlic- 500	Sesame- 500
TBARS (abs)	0.2824±0.0870	0.5947±0.1838*	0.3118±0.1084**	0.4229±0.0770**	0.3357±0.0380**	0.3298±0.1090**

Data expressed as mean ±s.d. n=6. Values are statistically significant at * P < 0.05 vs. SH Control ** P < 0.05 vs. OVX Control, (Student unpaired t test)

Free radical and oxidative stress help in neurological disease.³⁰ Treatment of Garlic and Black sesame also decrease the oxidative stress in OVX rats. Study result indicated significant increase in blood serum TBARS content in OVX control rats compared with SH control rats (P<0.05). However oral administration of Estradiol Valerate and dietary treatment groups of Garlic-250, Garlic-500, and Black Sesame-500 reduced the level of TBARS indicating a decrease rate of lipid Peroxidation (P<0.05). These finding suggest that the Estradiol Valerate and ethanolic extract of Garlic and petroleum extract of black sesame prevent the formation of reactive oxygen species and induction of lipid Peroxidation by OVX rats. Thus ovarian failure and termination of reproductive female function can influence the mental and the cognitive activity of menopausal women. Although the mechanisms underlying menopausal mental disorders are unclear, the decline in ovarian steroids produces functional changes in the central nervous system.³¹

Clinical studies have indicated that estrogen replacement improves mood and it prevented some disorders in post-menopausal women who had no or mild depressive symptoms.^{32, 33} However, in postmenopausal women with major depression, estrogen alone is not sufficient, although it may be useful as an augmentation strategy. Thus, it is important to investigate this potential remedy for treating the major depression induced by menopause. We suggest that dietary supplements Garlic and Black sesame containing phytoestrogens can be useful for treating menopausal depression in patients.

Conclusion:

Thus on the basis of present study, it appears that dietary supplement Garlic and Black sesame can significantly show antidepressant activity through phytoestrogen by using force swimming test and antioxidant mechanism by lipid peroxidation method in OVX rats. Phytoestrogen may be used for prophylaxis treatment of perimenopause depression.

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