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Evaluation of CNS stimulating activity of hydroalcoholic extract of *Brassica oleracea* L.var. *italica* in laboratory animals

G. V. Sutar, S. J. Sajane, S. T. Taralekar, Prakash I. Nargatti◆ and A. A. Jadhav*

Department of Pharmacology, Annasaheb Dange College of Pharmacy, Ashta, Walwa, Sangli-416301, Maharashtra, India

**Department of Pharmacology, YSPMs, Yashoda Technical Campus, Faculty of Pharmacy, Satara, Satara-415011, Maharashtra, India*

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Abstract

Brassica oleracea L. var. *italica* Plenck (BOVLI) belongs to the family Brassicaceae, is a very famous vegetable with high content of antioxidant bioactive compounds and having versatile therapeutic applications. In the present study, the CNS stimulating activity of hydroalcoholic extract of BOVLI florets was investigated. Preliminary phytochemical screening of BOVLI was carried out. Thereafter, hydroalcoholic extract of BOVLI was evaluated for CNS stimulant activity, *i.e.*, by locomotor activity using actophotometer apparatus and spontaneous motor activity by using Y maze apparatus test was used as parameter for evaluation of CNS stimulating activity. The phytochemical screening through chemical test revealed the presence of alkaloids, saponins, glycosides, anthraquinones, steroids, terpenoids and flavonoids. Dopamine and serotonin level from rat brain were estimated to evaluate CNS stimulant activity and resulted significant ($p<0.001$) CNS stimulation activity when compared with negative control group. Finally, the result concluded that, due to presence of high content of flavonoids in BOVLI, the hydroalcoholic extract resulted as potent CNS stimulant.

1. Introduction

Depression is a chronic mental disorder that causes changes in mood, thoughts, behavior and physical health. It is a common but serious disease that can take away a person's ability to enjoy life and cause decline in capacity to undertake even the simplest daily tasks (Fekadu *et al*., 2017). As per WHO (2017) estimation, around clinical depression affects 121 million people globally. Suicide will be the second largest cause of death by 2020, owing to the high prevalence of suicide in depressed patients (up to 15%), as well as complications originating from stress and its impact on the cardiovascular system (Agarwal *et al*., 2015). Patients who are having major depression may have effect on level of dopamine, norepinephrine and serotonin (Jayanthi *et al*., 2012; Patil *et al*., 2021; Nargatti *et al*., 2021). Therefore, CNS stimulants are essential drugs whose primary action is to stimulate the CNS activity or to improve the specific physical and mental brain functions (Sonpetkar *et al*., 2012; Tripathi, 2013). CNS stimulants are useful for reducing drowsiness and fatigue and increasing mental alertness (Saha and Banerjee, 2013). From the past, few decades herbal medicines have been used not only for treatment but also for cure of diseases and its disorders (Bishwo *et al*., 2016). Ethnomedicinally, herbs are used in the treatment of pain relief, wound healing and abolishing fever that bring useful information to identify a wide range of compounds to develop new therapies for cancer, hypertension, diabetes and antiinfective medicines (Zaman *et al*., 2015). There are numerous synthetic antidepressant and CNS stimulant medicines available in the market today; however, their efficacy does not extend to the full population suffering from this illness. Further more, side effects and drug interactions are significant limitations in their clinical use. Therefore, herbal medicines are utilized all over the world to prevent the negative effects of synthetic pharmaceuticals because of its wide application and therapeutic efficacy with little side effects. Mahuang (*Ephedra vulgaris* Rich.) in China, Khat (*Catha edulis* Forsk) in Africa, and Coca (*Erythroxylum coca* Lam.) in South America are examples of the drugs which have been used as CNS stimulant from ancient times (Mestry *et al*., 2016).

Of late, *Brassica oleracea* L.var. *italica* Plenck (BOVLI) provides many health-promoting properties with its high antioxidant nature. Not only that, it also claims essential therapeutic benefits with its bioactive compounds. Phytochemical analysis of Broccoli has been showed presence of phenolic compounds, particularly flavonoids, vitamin C and E, amino acids, flavonols like quercetin and kaempferol, the carotenoids b-carotene, lutein, and the glucosinolates (Leja *et al*., 2001). Various parts of the plant have been scientifically proved for many activities, *viz*., antianemic activity (Vamsee *et al*., 2015), antibacterial activity (Sibi *et al*., 2013), anticancer activity (Talreja and Moon, 2014), antidementic activity (Ashwlayan, 2017), antidiabetic activity (Shah *et al*., 2016), antigenotoxic effects (Kumari *et al*., 2012), and antioxidant (Bhagat *et al*., 2012), but scanty information or no such scientific evidences were established on CNS stimulant activity on BOVLI florets hydroalcoholic extract. Therefore, it was worthwhile to investigate BOVLI florets or Broccoli heads for CNS stimulant activity in laboratory animals.

Corresponding author: Mr. Prakash I. Nargatti

Department of Pharmacology, Annasaheb Dange College of Pharmacy, Ashta, Walwa, Sangli-416301, Maharashtra, India.

E-mail: prakashnargatti@gmail.com

Tel.: +91-7709884468

2. Materials and Methods

2.1 Plant material and extraction

The plant was collected from Satara region of Maharashtra in the month of October-November 2017 and was authenticated by Department of Botany, Yashwantrao Chavan Institute of Science, Satara. The voucher specimen of BOVLI was stored as herbarium in Pharmacognosy Department (Herbarium No: BOVLI-104/PCOG-2019-20), Annasaheb Dange College of Pharmacy, Sangli, Maharashtra. The florets parts of BOVLI were dried under shade and coarsely powdered by using grinder mixer. 500 g of dried material was used for the extract preparation. The hydroalcoholic extract (2:1) was prepared by Soxhlet extraction for 9 h at 40° C temp. Further, the extract was dried using rotary flash evaporator at 45° C to get viscous semisolid crude extract. The final semisolid mass of crude extract was stored in 4°C in refrigerator using 100 ml glass bottles for retaining the potency of the bioactive compounds for the said experimentation.

2.2 Yield and phytochemical screening

The yield of the crude extract followed by phytochemical screening of the stored florets parts of BOVLI hydroalcoholic extract was performed for presence of group of phytochemicals.

2.3 Animals

Albino Wistar rats (150-200 gm) of either sex were used. The temperature in the experimental animal room was 22°C (± 3°C). Artificial lighting the sequence being 12 h light, 12 h dark was maintained. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water. Total 66 animals were used for the study. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) (Protocol No. YSPM/YTC/PHARM/18/2017) and were conducted according to the principles and guidelines of the committee for the purpose of control and supervision of experimentation on animals, India.

2.4 Chemicals and drugs

For the conduction of the said experimentation, phenobarbitone (Phenobarbitone injection, Nitin Lifesciences Ltd.) and caffeine (Cafneon injection, Neon Laboratories Ltd. Mumbai) were procured.

2.5 Acute oral toxicity

The acute oral toxicity (AOT) study of hydroalcoholic extract of BOVLI was carried out as per OECD guidelines No. 423. Female Albino rats (n = 6) were used for toxicity study. The oral dose (2000 and 4 000 mg/kg) of tested plant extract was administered to two groups in single dose and general behavior, adverse effects and mortality were determined up to 72 h and compared to normal group (Kifayatullah *et al*., 2015).

2.6 Experimental models

Locomotor activity using actophotometer apparatus and Y maze test were followed for the study as *in vivo* models and thereby, dopamine and serotonin were estimated from brain tissues of experimental rats.

2.6.1 Locomotor activity

The locomotor activity can be an index of wakefulness (alertness) of mental activity. Actophotometer is used for measuring locomotor activity which photoelectric cells are connected in circuit with a counter. When the beam of light falling on the photocell was cut off by the animal, a count was recorded. Animals were divided in five groups each comprising six animals. The dose was selected as per acute toxicity studies based on safety dose.

- Control group was treated with normal saline solution.
- Negative control group was treated with phenobarbitone (50 mg/kg) dose.
- Standard was treated with phenobarbitone (50 mg/kg) dose and caffeine (10 mg/kg) dose.
- Test I group was treated with phenobarbitone (50 mg/kg) dose and hydroalcoholic extract of BOVLI (200 mg/kg) low dose.
- Test II group was treated with phenobarbitone (50 mg/kg) dose and hydroalcoholic extract of BOVLI (400 mg/kg) high dose.

Each rat was separately placed in an actophotometer for duration of 10 min 1 h after the treatments. The count was recorded on the display when the beam of light landing on the photocells was cut by the moving animal (Kulkarni, 2011; Bora *et al*., 2015).

2.6.2 Y maze test

Y maze test has been shown to be reliable and effective in rodents for evaluation of the spontaneous alternation behavior (Sibi *et al*., 2013). Y maze composed of three equally spaced arms (120°, 50 cm long and 18 cm height, 15 cm width). Animals were divided in five groups each comprising six animals. It was performed for all groups of the rats at 30, 60, 90 and 120 min by placed individually in a symmetrical Y-shaped runway for 3 min and the number of the maze with all 4 ft (an 'entry') were counted.

- Control group was treated with normal saline solution.
- Negative control group was treated with phenobarbitone (50 mg/kg) dose.
- Standard was treated with phenobarbitone (50 mg/kg) dose and caffeine (10 mg/kg) dose.
- Test I group was treated with phenobarbitone (50 mg/kg) dose and hydroalcoholic extract of BOVLI (200 mg/kg) dose.
- Test II group was treated with phenobarbitone (50 mg/kg) dose and hydroalcoholic extract of BOVLI (400 mg/kg) dose.

Each rat was placed in one of the arm compartments and was allowed to move for 10 min freely until its tail completely enters another arm. The sequence of arm entries was manually recorded, the arms being labeled A, B, or C. The number of times a rat entered in the arm of the maze with all four feet was counted as a single entry and used for the comparison of control and drug treated groups (Avijit *et al*., 2010).

3. *In vitro* models

3.1 Estimation of dopamine and serotonin from rat brain tissue

3.1.1 Preparation of tissue extracts

The rats were sacrificed after completion of Y maze experiment; the entire brain was taken out and the forebrain was separated. The tissue was weighed and homogenized in 0.1 ml hydrochloric acid and n-butanol (0.85 ml of 37 per cent hydrochloric acid in one-liter n- butanol by spectroscopy for 1 min in a cool environment). After

that, the sample was centrifuged for 10 min at 2,000 rpm. Separate 0.08 ml of supernatant to an eppendorf tube containing 0.2 ml heptane (for spectroscopy) and 0.025 ml 0.1 M hydrochloric acid. The tube was centrifuged under the same settings after 10 min of vigorous shaking to separate the two phases. Upper organic phase was discarded and lower the aqueous phase (0.02 ml) was used for estimation of serotonin, and dopamine assay.

3.1.2 Estimation of dopamine

The assay represents a miniaturization of the trihydroxide method. To 0.02 ml of HCl phase, 0.05 ml 0.4 M and 0.01 ml EDTA/sodium acetate buffer were added in 0.02 ml of HCl and pH maintained at 9. Then, 0.01 ml of iodine solution (prepared with 0.1 M ethanol) added and kept for 10 min for oxidation. The reaction was stored after 2 min by addition of 0.01 ml Na_2SO_3 in 5 M NaOH. Acetic acid was added 1.5 min later. The solution was then heated to 100ºC for 6 min. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette as with 5-HT by the photofluorometer at 330-375 nm (Das *et al*., 2008) and the dopamine level was estimated.

3.1.3 Estimation of serotonin

To estimate serotonin level, reduced volume for 5-HT determination, the O-pthaldialdehyde (OPT) method was followed. From the OPT reagent, 0.025 ml were added to 0.02 ml of the HCl extract. The mixture was heated at 100°C for 10 min to form fluorophore. After the samples reached equilibrium with the ambient temperature, excitation/estimation spectra or intensity reading at 360-470 nm by using the photofluorometer (Das *et al*., 2008).

3.1.4 Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis of difference between groups was evaluated by ANOVA, followed by Tukey's multiple comparison test. *p* values less than 0.05 were considered significant.

4. Results

4.1 Yield and preliminary phytochemical screening

The yield of the extract was calculated and reveled the amount of the crude extract was 189.56 g (w/w) or 37.91%. Further, preliminary phytochemical screening for the same extract was carried out and revealed the presence of some active principles which was tabulated in Table 1.

4.2 Acute toxicity

The acute toxic effect of hydroalcoholic BOVLI extract was determined as per the OECD guideline, 423 at higher dose of 4000 mg/kg was used. No treatment related toxic symptom or mortality was observed after oral administration of the hydroalcoholic BOVLI extract at a dose of 2000 and 4000 mg/kg. The general behavioral of the extract treated animals and control group was observed for initially 4 h followed by long period 72 h. It was revealed that no abnormalities in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature or any death occurred for the all 6 animals. Therefore, the extract may be safe at a dose level of 4000 mg/kg, and the LD_{50} dose for the extract was considered be more than 4000 mg/kg.

Table 1: Preliminary phytochemical screening of hydroalcoholic extracts of BOVLI

Sr. No.	Plant constituents	Hydroalcoholic extract
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Carbohydrates	+
5	Glycosides	+
5	Saponins	+
6	Terpenoids	+
7	Phenols	+
8	Resins	–
9	Protein	–
10.	Sterols	–

(+) = present; (–) = Absent

4.3 Evaluation of locomotor activity using actophotometer apparatus

The locomotor activity of the animals was determined and compared with the normal control, negative control and with the standard. The result revealed that hydroalcoholic BOVLI extract in low dose (304.5 sec) and high dose (321.8 sec), both showed significantly higher activity than phenobarbitone alone, but the result was lesser than standard drugs (576.2 sec). The dose of 400 mg/kg has shown significant increase in locomotor activity ($p<0.001$) (Figure 1).

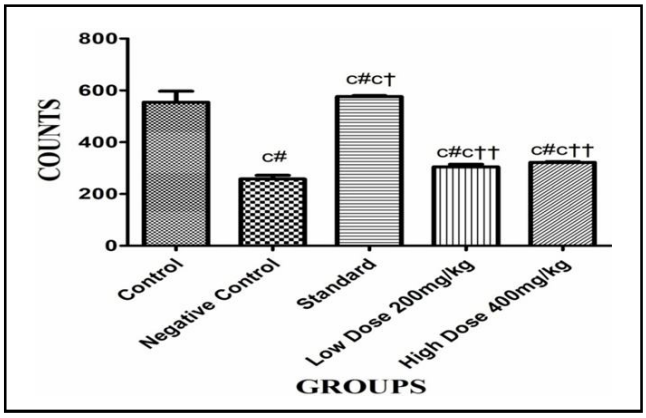

Figure 1: Effect of hydroalcoholic extracts of BOVLI on locomotor activity.

Values represent mean ± SEM; n= 6; analysis was performed using one-way ANOVA, followed by Tukey's multiple comparison test. $p<0.05$ was considered as statistically significant. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p< 0.001$. #Data compared with normal control. *Data compared with negative control. **Data compared with standard treatment.

4.4 Evaluation of Y maze apparatus test

The neuropharmacological study was evaluated through Y maze apparatus. The extract showed significant increase in exploratory behavior in Y maze test as compared to negative control, showed its CNS stimulating activity (Table 2).

Table 2: Effect of hydroalcoholic extracts of BOVLI on Y maze

Treatment	Total number of counts (min)			
	30	60	90	120
Control	12.66 ± 0.917	12.64 ± 0.915	12.63 ± 0.910	12.68 ± 0.918
Negative control (50 mg/kg)	7.656 ± 0.421c#	7.665 ± 0.432c#	7.657 ± 0.421c#	7.667 ± 0.426c#
Standard (10 mg/kg)	21.63 ± 1.26 c#c†	21.66 ± 1.25 c#c†	21.62 ± 1.22 c#c†	21.67 ± 1.24 c#c†
Low dose (200 mg/kg)	12.12 ± 0.741c#c††	12.14 ± 0.742#c††	12.15 ± 0.731c#c††	12.17 ± 0.721c#c††
High dose (400 mg/kg)	15.83 ± 0.741c#c††	15.84 ± 0.743c#c††	15.82 ± 0.744c#c††	15.86 ± 0.749c#c††

Values represent mean ± SEM; n= 6; analysis was performed using one-way ANOVA, followed by Tukey's multiple comparison test. $p<0.05$ was considered as statistically significant. [a] $p<0.05$, [b] $p<0.01$, [c] $p< 0.001$. [#]Data compared with normal control. [†]Data compared with negative control. [††]Data compared with standard treatment.

4.5 Estimation of dopamine level from brain tissue

Dopamine level for each group of animals was calculated from the homogenized brain tissue and result revealed increased level of dopamine concentration as increased in dose of the hydroalcoholic extracts of BOVLI. It has also shown that extract of 400 mg/kg dose was more significantly higher (457.3 pg/ mg) than negative control and lesser than standard drug, caffeine (Table 3).

Table 3: Effect of hydroalcoholic extracts of BOVLI on dopamine level from rat brain tissue

Treatment	Dopamine level (pg/ mg)
Control	407.3 ± 4.402
Negative control (50 mg/kg)	89.17 ± 1.701c#
Standard (10 mg/kg)	518.5 ± 4.752c#c†
Low dose (200 mg/kg)	421.3 ± 2.906c†c††
High dose (400 mg/kg)	457.3 ± 3.818c#c†c††

Values represent mean ± SEM; n= 6; analysis was performed using one-way ANOVA, followed by Tukey's multiple comparison test. $p<0.05$ was considered as statistically significant. [a] $p<0.05$, [b] $p<0.01$, [c] $p< 0.001$. [#]Data compared with normal control. [†]Data compared with negative control. [††]Data compared with standard treatment.

4.6 Estimation of serotonin level from rat brain tissue

The serotonin level similarly evaluated for each group of animals from the homogenized brain tissue and result revealed increased level of serotonin concentration as increased in dose of the hydroalcoholic extracts of BOVLI. The result was depicted in Table 4.

Table 4: Effect of hydroalcoholic extracts of BOVLI on serotonin level from rat brain tissue

Treatment	Serotonin level (pg/ mg)
Control	87.33 ± 2.848
Negative control (50 mg/kg)	97.00 ± 2.978
Standard (10 mg/kg)	198.3 ± 5.364c#c†
Low dose (200 mg/kg)	132.8 ± 2.272 c#c†c††
High dose (400 mg/kg)	142.3 ± 1.626 c#c†c††

Values represent mean ± SEM; n= 6; analysis was performed using one-way ANOVA, followed by Tukey's multiple comparison test. p value less than 0.05 was considered as statistically significant. [a] $p<0.05$, [b] $p<0.01$, [c] $p< 0.001$. [#]Data compared with normal control. [†]Data compared with negative control. [††]Data compared with standard treatment.

5. Discussion

Depression is a common mental disorder and one of the most important causes of disability in the world, especially during COVID-19 pandemic. The rate of the same drastically increased due to lock down effect. A vast number of medicinal plants are effectively used for the treatment of the same from the ancient period. Many are scientifically proved, and some are still in process to establish their efficacy. After the isolation of active constituents, such as morphine from opium poppies, scientific understanding of psychoactive plants has significantly advanced over the last two centuries (Sarris *et al.*, 2011). Established classes of CNS stimulants have shown side effects like euphoria dizziness, tremor, convulsions circulatory collapse, *etc.*

To overcome the side effects of synthetic drugs hydroalcoholic extract of *B. oleracea* was investigated for CNS stimulant activity. Preliminary the phytochemical screening of the hydroalcoholic extract of BOVLI florets showed the presence of many essential phytochemicals which are significantly contributes in many therapeutic efficacies. The yield of the extract was more in hydroalcoholic extract because many of the phytoconstituents are soluble in the same and resulted higher yield and also the presence of many bioactive compounds. The same result was also revealed by the earlier scientific data (Kaneria *et al.*, 2012).

In the present study, caffeine is used as standard drug for the CNS stimulating activity because caffeine is competitive antagonist at adenosine receptor and produces range of central and physiological effects that are opposing those of adenosine. Caffeine mobilizes intracellular calcium in neurons by reducing calcium uptake in microsomal vesicles and stimulates calcium release from the endoplasmic reticulum. Increase in intracellular calcium concentrations causes the release of neurotransmitters such as norepinephrine (NE) and dopamine (Alasmari, 2020). In negative control, phenobarbitone was used as CNS depressant agent that acts on GABA Benzodiazepine (BZD) receptor-Cl channel complex and potentiates GABA ergic inhibition by prolongation of opening of –Cl channel (Tripathi, 2013). BOVLI is a rich source of flavonoids. Flavonoids acts on different systems of brain. Flavanols, flavanones and anthocyanins may act in protective ways, increasing the cerebral

blood flow and protecting the neurons against inflammatory processes leading to cell injury (Jäger and Saaby, 2011).

Acute oral toxicity study was performed at the dose of 4000 mg/kg and showed the extract was safe, and hence any two doses, *i.e.*, 200 mg/kg and 400 mg/kg were selected as low dose and high dose, respectively. The assessment of CNS activity of any drug depends on the locomotor activity of animals which was carried out by using actophotometer. The locomotor activity of animal was determined by the estimation of excitability of the CNS level. An increase in alertness referred to as locomotor activity whereas the same indicates sedative effect when it decreases. There is a close relationship between increased locomotor activity and stimulation which is derived from CNS stimulation (Aziz and Sarkar, 2016). In the present investigation, hydroalcoholic extract of BOVLI at the dose of 400 mg/kg of dose have shown highly significant increase (p<0.001) in locomotor activity. This indicates that BOVLI might have CNS stimulant activity. This result indicates that the extract might possesses CNS stimulant activity which probably act *via* competitive antagonism at adenosine receptors leading to increase in nor-epinephrine secretions and enhance neural activity in numerous brain areas since extract's effect was compared to caffeine (Owolabi *et al*., 2008). Assessment of learning, memory function and exploratory behaviors in rodents are broadly conducted by Y maze test (Aziz and Sarkar, 2016). In the present investigation, hydroalcoholic extract of BOVLI at the dose of 400 mg/kg of dose have shown highly significant increase (p<0.001) in total number of entries in each arm. In negative control, rats have shown reduction in total number of entries in each arm might be due to decrease in dopamine, nor-epinephrine and serotonin level. After treatment with the extract, the total number of entries in each arm has increased. This showed that possibility of central monoaminergic system could be involved in the mode of action of extract (Thakur *et al*., 2014).

Depression symptoms are associated with changes in monoamine neurotransmitter (*i.e.*, NE, DA, and 5-HT) levels in the CNS. The prefrontal cortex and hippocampus, which regulate emotion, motivation, learning, and memory, are essential in depression. NE is related to alertness, energy, and attention, and that DA is linked to pleasure, reward, and motivation in life. The 5-HT transmitter is related to compulsion, obsession, and anxiety (Du *et al*., 2014). In the present investigation, chronic administration of hydroalcoholic extract of BOVLI at the 400 mg/kg of dose have shown highly significant increase (p<0.001) in dopamine and serotonin level. Extract of BOVLI probably act through restoration of neuro transmitters by increasing 5-HT and dopamine level at synaptic area of these monoaminergic neurons. These scientific evidences support the present investigation.

6. Conclusion

Hydroalcoholic extract of BOVLI florets was evaluated for CNS stimulant effect using locomotor activity and spontaneous motor activity through Y maze test apparatus. Initially were prepared. Preliminary phytochemical screening of hydroalcoholic extracts of *B. oleracea* revealed that presence of some active ingredients such as alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, terpenoids and phenols. Acute oral toxicity study revealed the safety of the extract and further CNS stimulating activity observed by increased in locomotor and spontaneous motor activity in rats. Thereafter, the extract at higher dose shown significant increase in dopamine and serotonin level and affirmed its dose dependent potentiality as CNS stimulant.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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