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Antidepressant Activity of Alcoholic Extract of the Fruits of *Osmanthus fragrans* Lour in Mice

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ABSTRACT

Majority of the antidepressant drugs improve depressive symptoms, but they exert multiple unwanted side effects. The search for more efficacious and well tolerated drugs is in progress. Owning to this, the present study was designed to evaluate the antidepressant activity of alcoholic extract of the fruits of *Osmanthus fragrans* (AEFOF) in mice. It was evaluated using the Tail Suspension Test (TST), Forced Swimming Test (FST) and influence on spontaneous locomotor activity (SLMA) in mice. The AEFOF (75 mg kg^{-1}, peroral (po) and 150 mg kg^{-1}, po) was administered orally in separate groups of Swiss mice for 14 days in TST and FST tests and for 7 successive days in SLMA test. The AEFOF showed a dose dependant reduction in duration of immobility in mice. The dose of 150 mg kg^{-1} of AEFOF significantly reduced the immobility time of mice in both FST and TST without any significance effect on locomotor activity of mice. The efficacy of extract was found to be comparable to fluoxetine (20 mg kg^{-1}, po). It was found to be toxicologically safe with no deaths of mice when administered orally at the dose of 2000 mg kg^{-1}. From the present study, it can be concluded that the AEFOF possess potent antidepressant activity as shown by the TST and FST tests and is toxicologically safe.

Key words: Depression, tail suspension test, forced swimming test, spontaneous locomotor activity

INTRODUCTION

Depression is a common mental disorder and one of the most important causes of disability in the world with a heavy social trouble and a substantial lifetime risk (Anonyms, 2010). It is frequently recurrent and chronic and has been associated with suicide risk and psychosocial dysfunction (Emslie *et al*., 2005). Antidepressant therapy includes drugs with exceptional structural chemical diversity; most of them increase monoaminergic neurotransmission (Elhwuegi, 2004). Although, the majority of the antidepressant drugs improve depressive symptoms, they exert multiple unwanted side effects. Moreover, 30% of depressive patients do not react appropriately to the first line treatment (Fava and Rush, 2006). Thus, the search for more efficacious and well tolerated drugs is in progress. The need for the discovery and development of new pharmaceuticals for the treatment of depression demands that all approaches to drug discovery be exploited. Among the possible approaches, the use of natural products has many distinctive and vital contributions to drug discovery (Newman *et al*., 2003; Cragg and Newman, 2013). In this regard, many medicinal plants have been used as a treatment for sadness, stress, anxiety and depression (Zhang, 2004).

Osmanthus fragrans, commonly known as sweet olive, fragrant olive or tea olive, belongs to the family Oleaceae and is native to Southwestern China (Larsen, 1995). It is widely cultivated as an ornamental plant for its fragrant flowers in Taiwan, Southern Japan, Southern China, Europe and North America. The flower of *O. fragrans* called *Kwai-fah* in China has been used as a beverage and as an additive for tea and foods such as cake, pastry, paste, vinegar and liqueurs (Larsen, 1995). It is popular because of its delicate fruity/floral aroma. Traditional Chinese medicine has also suggested the use of *O. fragrans* to treat weakened vision, halitosis, asthma, cough, panting, toothache, stomachache, diarrhoea and hepatitis (Hung *et al.*, 2013). The antidepressants can promote neurogenesis (Rajkowska *et al.*, 1999). The extract of dried flowers of *O. fragrans* showed neuroprotective, free-radical scavenging, antioxidative effects in *in vitro* assays and thus, can promote neurogenesis (Lee *et al.*, 2007).

The alcoholic extract of *O. fragrans* fruits contains nicotinamide, D-allitol, 5-hydroxymethyl-2-furancarboxaldehyde, acetyloleanolic acid, benzoic acid, ergosta-7,22-dien-3-one, β-sitosterol, borreriagenin, cerevistero, veratroylglycol, methyl-2-O-β-glucopyranosylbenzoate, 3',7-dihydroxy-4'-methoxyisoflavon, umbelliferone, caffeic acid methyl ester, oleanolic acid, (-)-chicanine, dillapiol, 3β,5α,9α-trihydroxyergosta-7-22-dien-6-one, 2α-hydroxy-oleanolic acid, betulinic acid, betulin, 3,3'-bisdemethylpinoresinol and lupeol (Yin *et al*., 2013).

Thus, the objective of the present study was to evaluate the antidepressant activity of alcoholic extract of the fruits of *O. fragrans* (AEFOF) in mice using the Tail Suspension Test (TST), Forced Swimming Test (FST) and their influence on spontaneous locomotor activity (SLMA) in mice.

MATERIALS AND METHODS

Collection and authentication of plant material: *Osmanthus fragrans* Lour fruits were collected from Delhi. It was authenticated by Drugs and Aromatics Plant Department of Narendra Dev University of Agriculture and Technology, Faizabad (Authentication Ref No: 350/nduat/horticulture/2012). The voucher specimen was deposited in the institutional herbarium for future reference.

Preparation of alcoholic extract of the fruits of *Osmanthus fragrans*: The raw materials of *O. fragrans* fruits were washed with running water at a volume ratio of 1:50 (raw materials/water) for 5 min followed by peeling and sequestering for the solid matter. The solid matter of the pulp was grounded (maximum particle size 0.4 mm) after drying in oven at 60±0.5°C. The ground sample of the dry pulp (0.5 kg) was extracted with 2000 mL of ethanol by a Soxhlet extractor for 6 h. Solvents were evaporated by rotary evaporator (Buchi Rotavapor-R, Labco, India) and the crude ethanolic extract (AEFOF) was obtained (Wang *et al.*, 2010).

Preliminary phytochemical screening: An attempt was made to observe the presence and absence of diverse phytochemical constituents in AEFOF viz., alkaloids (Maeyer's test), saponins (Foam test), flavonoids (Shinoda test), steroids and triterpenes (Lieberman-Burchard's test), carbohydrates (Benedict's test) and tannins (Ferric chloride test) according to standard methods (Trease and Evans, 1987).

Experimental animal: Adult male Swiss albino mice weighing between (25-35 g) were procured from the Central Drug Research Institute Lucknow, Uttar Pradesh. They were housed in polypropylene cages (22.5×37.5 cm) and maintained under standard laboratory environmental

conditions; temperature 25±2°C, 12 h light: 12 h dark cycle and 55±10% relative humidity with free access to standard pellet diet and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures [Hygia/M.Pharm./06/2011-12].

Experimental protocols: The mice were divided into four groups each consisting of six animals. Group 1 (control) was administered with normal saline 1 mL/100 g b.wt., po (per oral). Group 2 (standard) was treated with fluoxetine 20 mg kg^{-1} b.wt., po. Group 3 and group 4 (Drug-treated) were treated with AEFOF 75 and 150 mg kg^{-1} b.wt., po, respectively. In all these groups, respective drug treatment schedule was followed for seven successive days. Then, the immobility time was recorded after 60 min of the last dose.

Acute toxicity study: The procedure was followed as per the Organization for Economic Cooperation and Development (OECD) 423 guidelines. The extract was administered orally at a dose of 2000 mg kg^{-1} b.wt. to different groups of mice and observed for 14 days for signs of behavioral, neurological toxicity and mortality (Rivera *et al*., 2004).

Tail suspension test: Immobility study was performed by Tail Suspension Test (TST) (Steru *et al*., 1985). Mice were individually suspended by the tail with clamp (1 cm distant from the end) for 6 min in a box (35×23×53 cm) with the head 5 cm to the bottom. Testing was carried out in a darkened room with minimal background noise. The duration of immobility was observed during the final 4 min interval of the test (the animals initially tried to escape by making vigorous movements but became immobile when were unable to escape). The animal was considered immobile when it did not show any movement of body and hanged passively. The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress had been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility was noted during 6 min period. Each animal was used only once.

Forced swimming test: Antidepressant activity was performed by Forced Swimming Test (FST) which is the most widely used pharmacological *in vivo* model for assessing antidepressant activity (Porsolt *et al*., 1977). The apparatus utilized to perform the FST consisted of a clear glass cylinder (20 cm height×12 cm diameter) with water filled to a depth of 15 cm (24±1°C). Prior to the administration schedule, the mice were subjected to a pretest session in which every animal was individually placed into the cylinder for 15 min. A mouse was considered to be immobile when it remained floating in water without struggling making only minimum movements of its limbs necessary to keep its head above the water. The total duration of immobility was recorded during the next 4 min of the total test duration of 6 min. The changes in immobility duration were studied after administrating the drugs in separate groups of animals. Each animal was used only once. The development of immobility when mice were placed in an inescapable cylinder filled with water, reflects the cessation of persistent escape-directed behavior.

Spontaneous locomotor activity: Spontaneous Locomotor Activity (SLMA) of animals was measured to differentiate between sedative and central nervous system stimulant activity of drugs.

It was measured by using a digital photo-actometer (Sanmukhani *et al.*, 2011). Mice were placed in the photo-actometer covered with the fiber lid after two doses of drugs 24, 5 and 1 h before the test. Mice tried to explore the area and during their movement they intercepted the photobeams. The number of interceptions was counted by the photo-active cells. Locomotion of the animal was expressed in terms of total number of ambulation (total photobeam counts) during a 5 min test for each mouse.

Statistical analysis: Results were expressed as Mean±SEM. All the data was analyzed using one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test $p<0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical screening: The results of preliminary phytochemical screening tests revealed the presence of tannins, phenolic acids, alkaloids, flavonoids and glycosides in the crude extract (AEFOF).

Acute toxicity study: The extract AEFOF was studied for acute toxicity at doses of 2000 mg kg^{-1} b.wt., po. The extract was found devoid of mortality of all animals. So, the doses selected for the antidepressant activity were 75 and 150 mg kg^{-1}, po.

Tail suspension test: Animals treated with two doses of AEFOF (75 and 150 mg kg^{-1} b.wt., po) showed significant decrease in the immobility times in mice (98.16±1.64; $p<0.01$ and 86.33±3.67; $p<0.001$, respectively) when compared with control group (108.3±2.75). Similarly, animals treated with fluoxetine (20 mg kg^{-1} b.wt., po) showed a significant decrease in the immobility time (70.5±2.52; $p<0.001$) (Fig. 1).

Forced swimming test: After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. Animals treated with two doses of AEFOF (75 and 150 mg kg^{-1} b.wt., po) showed significant decrease in the immobility times (124±1.15; $p<0.01$ and 93.5±1.50; $p<0.001$, respectively) when compared with control group (132.33±1.89). Similarly, animals

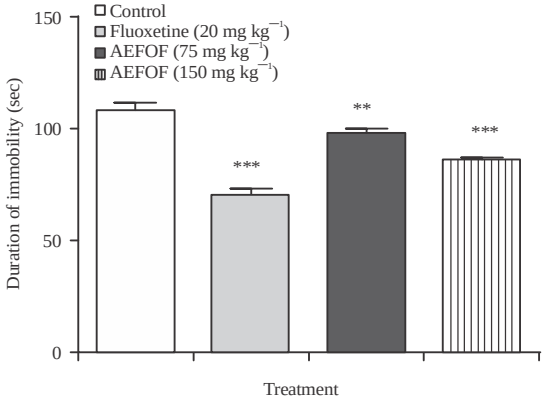

Fig. 1: Effects of AEFOF and fluoxetine on duration of immobility in the tail suspension test. Each value is expressed as Mean±SEM (n = 6). **p<0.01 and ***p<0.001 compared with normal group

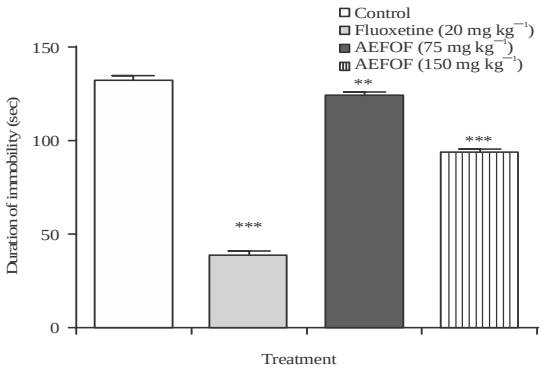

Fig. 2: Effects of AEFOF and fluoxetine on duration of immobility in the forced swimming test. Each value is expressed as Mean±SEM (n = 6). **p<0.01 and ***p<0.001 compared with normal group

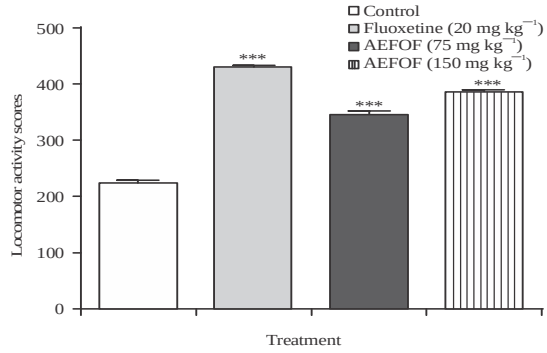

Fig. 3: Effects of AEFOF and fluoxetine on spontaneous locomotor activity. Each value is expressed as Mean±SEM (n = 6). ***p<0.001 compared with normal group

treated with fluoxetine (20 mg kg^{-1} b.wt., po) showed a significant decrease in the immobility time (38.5±1.66; p<0.001) (Fig. 2).

Spontaneous locomotor activity: Locomotor activity of mice as measured using digital photo-actometer was found to be significant and similar in all the groups (p<0.001) (Fig. 3).

DISCUSSION

The antidepressant drugs used in the health center today have heterogeneity in the therapeutically response, multiple side effects and high monetary cost. Furthermore, treatment of depression with conversional antidepressant drugs provides a complete diminution in 70% of the individuals treated (Fava and Rush, 2006). Therefore, the study of the antidepressant-like effects of herbs is an increasing attention (Newman *et al*., 2003). Medical therapies with herbs may be effective alternatives in the treatment of depression and the research of their effects has progressed significantly since the past decade (Hasrat *et al*., 1997a, b). In this regard, *Osmanthus fragrans* fruits have been studied. It was observed that AEFOF at doses of 75 and 150 mg kg^{-1} b.wt. exhibited significant reduction in immobility time in dose dependent manner when compared to control group in TST and FST tests. Similarly, the animals treated with Fluoxetine

(20 mg kg^{-1} b.wt.) as expected showed significant decrease in immobility time. Both the swimming and climbing behaviors in the FST are increased when the animals are treated by a drug which increases serotonin, norepinephrine and dopamine levels in the nerve terminals (Reneric and Lucki, 1998). An increase in all the three neurotransmitters could be by inhibition of monoamine oxidase (MAO) activity in the brain. A growing body of research indicates that besides depletion of serotonin and catechoamine neurotransmitters, depression could result from various other pathophysiological mechanisms as well. Researchers suggest that depression may inhibit neurogenesis in the hippocampus (Sapolsky, 2000; Henn and Vollmayr, 2004). This idea is supported by the finding that antidepressants can promote neurogenesis (Rajkowska *et al*., 1999). The alcoholic extract of the fruits of *Osmanthus fragrans* possesses potential antidepressant activity in mice as shown by the TST and FST tests and could be considered as toxicologically safe with no deaths of mice when administered orally at the dose of 2000 mg kg^{-1}. The AEFOF showed a dose dependant reduction in duration of immobility in mice. The efficacy of extract was found to be comparable to fluoxetine (20 mg kg^{-1}, po).

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CONCLUSION

From the present study, it can be concluded that the alcoholic extract of the fruits of *Osmanthus fragrans* (AEFOF) possess potent antidepressant activity as shown by the TST and FST tests and is toxicologically safe.

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