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BEHAVIORAL EVIDENCE OF ANTIDEPRESSANT-LIKE ACTIVITY OF *RAPHANUS SATIVUS* L. VAR. *CAUDATUS* IN MICE

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Abstract

Background: Currently-available antidepressant agents produce various adverse effects, and are expensive. At present, various plants are being evaluated for their possible role against numerous diseases, and no doubt, the role of traditional and complementary medicines in the development of effective therapy is incredible. The present study was designed to evaluate antidepressant-like activity of *Raphanus sativus* L. Var. *caudatus* at different doses in mice.
Materials and Methods: Antidepressant potential of ethanolic extract of *Raphanus caudatus* L. was evaluated at three different doses 250 mg/kg, 500 mg/kg and 1000 mg/kg by using forced swim test and tail suspension test on albino male mice. The results were compared with control and standard mice groups administered with normal saline and Fluoxetine respectively. In both parameters immobility period was recorded two times during 60 days dosing.
Results: The ethanol extract at all three tested doses (250 mg/kg, 500 mg/kg and 1000 mg /kg) and standard fluoxetine demonstrated notable antidepressant-like activity ($p<0.05$) in both FST and TST paradigms.
Conclusion: Our results clearly show that *Raphanus caudatus* ameliorate depression-like behavior in rodent model, and can be used to establish newer antidepressant approaches in future. However, mechanism-based studies are needed to establish the mechanistic action of *Raphanus caudatus* L.

Key Word: *Raphanus caudatus*, depression, FST, TST, immobility time, ethanol extract

List of Abbreviations: EERC Ethanol Extract of *Raphanus caudatus:* FST Forced swim test: TSTTail suspension test

Introduction

Depression is characterized by low mood, most commonly related to stress. The condition is the most common psychological disorder that can affect an individual's behavior and thoughts. It is postulated that depression is associated with depletion of the concentrations of monoamine neurotransmitters present in the brain, such as dopamine, or epinephrine and serotonin (Schildkraut, 1965). Depression is a global health problem affecting human health (Greden, 2001). The symptoms of this disorder include decreased energy level, apathy, despair, hopelessness, as well as suicidal ideation. Although various antidepressant agents are available such as tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors and selective noradrenaline reuptake inhibitors (SNRIs) but even then depression is still posing alarming situation to mankind (Yu, et al., 2002). According to World Health Organization (WHO), it has been estimated that around 450 million humans suffered behavioral problems (WHO, 2001) that approximately constitutes 12.3% of the global burden of disorder and this will lead to about 15% by 2020 (Reynolds, 2003).In this regard, the search for new antidepressant agents is going to increase day by day. Medicinal plants have been used successfully for various illnesses since time immemorial. The agents from plants are not only economical, but they also provide better alternatives with less adverse effects. *Raphanus sativus* L. belongs to family Brassicaceae. It is one of the very common plants consumed daily as diet worldwide, specially, in Asia and Europe (Gutiérrez and Perez, 2004). The vernacular name in Pakistan is "Mungra" and rat-tailed radish in English. This edible part of radish is available in Pakistan between the months of November and March, and is cooked in the form of delicious dishes. Taxonomic profile of plant is presented in Table 1.

Table 1: Taxonomical Classification of *Raphanus caudatus* L.

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super-division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order:	Capparales
Family	Brassicaceae/Cruciferae – Mustard family
Genus	*Raphanus* L. – radish
Species	*Raphanus caudatus* L. – rat tail radish / mougri / podding radish

Source: The Plants Database. (http://plants.usda.gov)

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The use of *Raphanus* has been documented in cardiovascular disorders, diabetes, cancer and other gastric ailments. It has been also reported as very effective hepatoprotective agent. Different parts such as leaves, roots, seeds have been documented to possesses antioxidant properties (Lugasi, et al., 1998, Papi, et al., 2008);(Barillari, et al., 2006, Beevi, et al., 2010, Takaya, et al., 2003, Vanitha Reddy, et al., 2010)

Raphanus caudatus is a rich source of phenolic, alkaloid, anthocynin and isothiocyanate type compounds (Sham, et al., 2013). These compounds are known for their neuro-pharmacological potentials (Wu, et al., 2016) . Although several pharmacological properties of plant have been reported, to the best of our knowledge, there has been no investigation on the possible antidepressant activity of *Raphanus caudatus*.

The present study, has, therefore, been designed to evaluate antidepressant-like activity of ethanolic extract from *Raphanus sativus* L. var. *caudatus* in mice, using forced swim test and tail suspension test.

Materials and Methods

Collection and Extraction of Plant material

Fresh pods (10 kg) of *Raphanus sativus* L. were purchased from local market of Karachi, Pakistan, and were identified by Dr. Mohtashim Associate Professor Department of Pharmacognosy, University of Karachi, Pakistan, and the specimen voucher number: RSP-01-14/17 was submitted to Department of Pharmacognosy. The plant material was air-dried and subjected to grinding to make powder. The powdered plant material of *Raphanus sativus* L. var. *caudatus* was subjected to extraction with ethanol, using Soxhlet apparatus (HMFT-5/63, Made in England). The extract was further subjected to Rotary Evaporation (R-200, Buchi) to obtain a semi-solid mass which was subsequently dried.

Animals Selection

A total of 50 healthy adult swiss albino male mice (25±2g) were purchased from the animal house of University of Karachi, Pakistan. Before the experiments the animals were placed in separate cages for 7 days. Standard iron cages (4 mice per cage) were used to keep the experimental animals at Animal House of Department of Pharmacology, University of Karachi. The animals were housed at 25±1^{o}C with humidity 50 to 60 % on 12/12 h light and dark cycle along with proper ventilation. All animals were allowed food and water ad libitum. University Board of Advanced Studies and Research of University of Karachi, Pakistan approved all the experimental protocol.

Protocol of the Experimental Study

Swiss albino adult male mice were randomly divided into five groups (I, II, III, IV and V) of ten animals in each group. Group I was kept as Control group administered with normal saline in equivalent volume of administered doses. Group II, III and IV were set as treated groups, administered orally with ethanolic extract of *Raphanus caudatus* (EERC) in doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. Group V was marked as standard kept on Fluoxetine (20 mg/kg). The dosing was done through oral route once daily (8.00 – 9.00 am) according to the body weight for successive 60 days.

Chemicals

Absolute Ethanol (Merck, Germany) and Fluoxetine (Hilton, Pharmaceutical, Pakistan).

Evaluation of antidepressant like activity

Forced swim test

The method of Porsolt et al., 1978 was employed to perform forced swimming test (Porsolt, et al., 1977). In brief, each mouse was subjected to forced swimming for a period of 6 min, in an open cylindrical container (diameter 10 cm, height 25 cm), containing 15 cm of water at 25 $\pm$ 1o C. The duration of immobility was observed during the last 4 minutes of the observation period. The mouse was considered immobile when it stopped struggling and floated motionlessly.

Tail Suspension Test

The test was performed as per the method described by (Steru, et al., 1985). In brief, each mouse was hung by the tail on a plastic string 50 cm above the surface with the help of an adhesive tape. The test was performed for a total duration of 6 minutes. The duration of immobility was observed during the last 4 minutes of test period. The mouse was considered to be immobile when it does not show any movement of body and remain hanging passively.

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Statistical analysis

Data are expressed as means ± standard error of means (SEM). Data were analyzed by One-way ANOVA followed by post hoc test. All statistical analyses were performed by using SPSS version -20. Cut off values were considered significant when p value is less than 0.05.

Results

The results of present investigation indicate that EERC possess noteworthy antidepressant-like activity at all tested doses (250 mg/kg, 500 mg/kg and 1000 mg/kg) as compared to control. The plant extract was administered for 30 days to mice and antidepressant activity was determined at day 30 as well as day 60 using FST and TST paradigms (Table 1 and 2). Results showed that immobility time was reduced significantly by EERC at the tested doses.

In FST, after 30 days of evaluation, group II that were administered 250 mg/kg of EERC exhibited non -significant ($p > 0.05$) diminution in immobility time when compared with control animal group. Whereas group III showed significant and group IV and standard fluoxetine exhibited highly significant reduction in immobility time when compared with control mice group. The effect on immobility time in all tested groups was highly significant at 60 days after continuous administration.

Mice treated with EERC at all three doses exhibited highly significant anti-immobility activity in comparison with control mice at 30th as well as 60th days of drug administration. In addition, antidepressant potential of standard drug fluoxetine was significant when contrasted with control group.

On comparison with fluoxetine, plant extract exhibit similar potential as insignificant differenced ($p > 0.05$) were observed between standard and test groups.

Table 2: Effect of *Raphanus caudatus* and fluoxetine on immobility period using FST in mice

Treatment	**Immobility time (seconds)**	
	on day 30	**on day 60**
Control	313.5±20.86##	303.2±15.11##
EERC 250 mg/kg	256.3±13.89#	230.1±8.98**
EERC 500 mg/kg	229.1±15.97*	184.1±12.28**
EERC 1000 mg/kg	219.6±7.72**	193.7±3.33**
Fluoxetine (20 mg/kg)	221.8±11.73**	180.5±7.53**

n= 10 in each group, values are mean ± S.E.M. Data was analyzed by one- way ANOVA followed by post hoc, *p<0.05, **p<0.001 vs. control mice group, #p<0.05, ##p<0.001 vs. standard mice group

Table 3: Effect of *Raphanus caudatus* and fluoxetine on immobility period using TST in mice

Treatment	**Immobility time (seconds)**	
	on day 30	**on day 60**
Control	268.5±19.96##	268.1±7.52##
EERC 250 mg/kg	220.6±25.25**	190.2±5.89**
EERC 500 mg/kg	154.3±17.96**	112.6±1.85**
EERC 1000 mg/kg	127.5±13.61**	116.3±2.09**
Fluoxetine (20 mg/kg)	130.1±26.41**	87.8±2.3**

n= 10 in each group, values are mean ± S.E.M. Data was analyzed by one- way ANOVA followed by post hoc, *p<0.05, **p<0.001 vs. control mice group, #p<0.05, ##p<0.001 vs. standard mice group

Discussion

In the present study, ethanolic extract of *Raphanus caudatus* in the doses of 250, 500, and 1000 mg/kg, p.o. administered to mice for 60 successive days. The extract resulted in notable antidepressant-like activity in both FST

and TST that are comparable to standard antidepressant fluoxetine. Both of these tests are successfully and widely used to evaluate antidepressant-like activity in rodents in term of reduction in immobility time (Rodrigues et al., 2OO2; Suzuki et al., 2001). Actually, this immobility is a condition of hopelessness indicative of depression (Steru et al., 1985).

Raphanus caudatus has been reported to contain significant amount of sulforaphane (Pocasap, et al., 2013, Sangthong and Weerapreeyakul, 2016) and in a recent study, sulforaphane has been demonstrated to possess potent antidepressant like activity in mice using FST and TST. Furthermore, sulforaphane also blocked HPA-axis activity and resulted in decreased serum corticosterone along with inhibition of adrenocorticotropic hormone (ACTH) and interleukin-6 in mice. Thus, the presence of sulphoraphane might have been responsible for antidepressant like activity of *Raphanus caudatus* in the present study

Raphanus sativus is a rich source of flavonoids, alkaloids, anthocynins and isothiocyanate compounds. Hence, it may be safely suggested that antidepressant-like activity of *Raphanus caudatus* in the current investigation could be due to presence of these bioactive constituents. Nevertheless, this must be evaluated and confirmed by characterization of active constituents.

Regarding depression, the amine hypothesis is still widely accepted according to which reduced levels of brain biogenic amines such as serotonin, noradrenaline and dopamine are associated with depression whereas antidepressant drugs work by increasing the levels of such amine neurotransmitters (Belmaker and Agam, 2008, Krishnan and Nestler, 2008). In this regard, there could be possibility that extract might be involved in modulation of such neurotransmitters. However, it must be confirmed by mechanism based studies in future.

Present investigation clearly indicates that *Raphanus caudatus* have significant antidepressant capability in rodent models of depression

Conclusion

The findings of present study suggest that ethanolic extract from *Raphanus caudatus* possess significant antidepressant-like activity in FST and TST behavioral paradigms in mice. Thus, the plant extract could be used to ameliorate depression-like behavior alone, or in combination with antidepressant agents. Chemical characterizations of active antidepressant constituents of plant along with molecular targets are necessary to establish newer antidepressant agent/s from the plant.

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Conflict of interest

Authors declare that they have no conflict of interest.

Compliance with Ethical standards and Ethical Approval

The present research work and ethical clearance was approved by the Board of Advanced Studies and Research of University of Karachi vide BASR number 02419/Pharm. All the selected animals were handled using ethical principles of research. Specifications described in Helsinki Resolution 1964 were followed for animal handling in this research.

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