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Evaluation of Antidepressant Activity of Ethanolic Extract of *Dacus Carota* in Mice

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Abstract

The present study was undertaken to evaluate the antidepressant potential of *Dacus carota* (DC) in different animal models like forced swim test (FST), tail suspension test (TST), Apomorphine Induced Hypothermia (AIH), Reserpine Induced Hypothermia (RIH), 5-HTP Potentiation of Head twitches in mice (HTPPH). Inbred adult male Swiss Albino mice weighing 25-30g were used in the study. Fluoxetine (25 mg/kg) was used as a standard drug in FST, TST and HTPPH models and Desipramine (20 mg/kg) was used as a standard drug in AIH and RIH models. Duration of immobility was noted in FST and TST models. Rectal temperature was noted in AIH model. In RIH model rectal temperature along with Ptosis was noted. Head twitches were noted in HTPPH model. In this study, both fluoxetine and DC significantly reduced the duration of immobility in both tail suspension and forced swim tests as compared to the animals in the control group. The antidepressant activity of DC (400 mg/kg) was comparable to that of standard drugs. The results of the present study indicate the potential for use of DC as an adjuvant in the treatment of depression.

Keywords: Antidepressant activity, *Dacus carota*, Forced swim test, Tail suspension test, Apomorphine and Reserpine Induced Hypothermia.

INTRODUCTION

Depression is defined as a "state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being". It is characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. According to the World Health report [1], approximately 450 million people suffer from a mental or behavioral disorder. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 [2]. Psychiatric illness is also often associated with suicide and there are between 10 and 20 million suicide attempts every year. Depression is the most prevalent mental disorder. The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation. In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem [3]. Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. These considerations implicate the search for new antidepressant agents that have a fast onset of action, with less side effects and a wider safety margin. Various plants are being used in complementary and alternative medicines for management of mood disorders.

On the basis of the above information, the roots of DC were selected for evaluating its antidepressant activity due to its traditional use in the management of diarrhoea, acidity, heartburn and ulcers [4]. The plant was reported to possess medicinal values such as antifungal, antibacterial, enzyme protective, hepatoprotective activities [5]. It is a remedy for fever, gonorrhoea, anorexia, dysentery, sores, and skin diseases. Chemical constituents in DC include polyacetylenes like Falcarinol and falcarindiol, pyrrolidine, β-carotene, and lesser amounts of α-carotene and γ-carotene [6]. So far there has been no scientific report in literature about the antidepressant activity (in experimental animal models) of this species. Therefore, the present study has been undertaken to investigate the effect of Ethanolic Extract of *Dacus carota* (EEDC) on depression in mice.

MATERIALS AND METHODS

Plant material

The root part of *Dacus carota* (Carrot) was collected from local distributor in Tirupathi, Andhra Pradesh, in the month of February. The plant material was identified and authenticated by Dr. T. Vijaya, Associate professor, Department of Botany, Sri Venkateswara University, Tirupathi, Chittoor district, Andhra Pradesh.

Preparation of extract

The dried Carrots were crushed into fine particles (powder) using a mixer. The powdered Carrots were packed in a soxhlet apparatus and subjected to continuous hot percolation at temperature 50ºc using ethanol as solvent till clear solvent was observed in siphon tube. Extract was concentrated in rotary vaccum evaporator. Concentrated extract was dried and packed in an air tight container.

Preliminary phytochemical screening

The preliminary phytochemical investigations were carried out with the Ethanolic root extract of *Dacus carota* for qualitative identification of phytochemical constituents like alkaloids, carbohydrates, gums, proteins and amino acids, glycosides, steroids, flavanoids and triterpinoids by using standard procedures.

Animals

Albino mice of either sex weighing between 25-30 gm were used in this study. The animals were procured from animal house, Shri Vishnu College of Pharmacy, Bhimavaram and

are acclimatized for 7 days and were housed under standard laboratory conditions of temperature (25±2 ^{0}C) and relative humidity (55±5%) with a 12:12 light-dark cycle in polypropylene cages.

All the animals were fed with synthetic standard diet (National Institute for Nutrition, Hubsiguda, Hyderabad) and water was supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of Shri Vishnu College of Pharmacy. All the animal studies were performed as per the rules and regulations in accordance to the guidelines of CPCSEA with registered number as 439/0/a/CPCSEA.

All the animals were fasted 3hrs prior to oral administration of vehicle/standard/test compounds during the experiment. All the experiments were carried out during the light period (9:00 to 17:00 hrs) to avoid circadian rhythms.

Drugs and Chemicals

Fluoxetine (Sigma Life Sciences, Bangalore), Desipramine (Sigma LifeSciences, Bangalore), Apomorphine (Sigma Life Sciences, Bangalore), Reserpine (Sigma Life Sciences, Bangalore), 5-HTP (Sigma Life Sciences, Bangalore), Glacial acetic acid, 1N Sodium hydroxide.

Acute oral toxicity studies

Toxicity studies of extract were carried out in Swiss albino mice weighing between 25-30g. They were performed according to OECD guideline No. 423 [7]. Four groups of mice comprising three animals each were treated with 5, 50, 300 and 2000mg/kg of the extract orally, via gastric catheter. The animals were then observed continuously for the first 4hrs for any behavioural changes and for mortality if any at the end of 72hrs. All four doses were found to be safe since no animal died even at the dose of 2000 mg/kg when administered orally and the animals did not showed any gross behavioural changes.

EXPERIMENTAL DESIGN

Animals were divided into six groups of six animals each.

Group I – Control (Vehicle treated group, p.o)

Group II – Standard (Fluoxetine 25 mg/kg, p.o in FST, TST, HTPPH models and Desipramine 20 mg/kg, p.o in AIH, RIH models).

Group III – Low dose of EEDC (200 mg/kg, p.o)

Group IV – High dose of EECD (400 mg/kg, p.o)

Forced Swim Test (FST)

The experiment was carried out in a narrow glass cylinder (13 cm in diameter *24 cm high) containing water (25ºC) to a depth of 10cm, from which they cannot escape. All the animals were fasted for 3 hrs prior to the administration of vehicle/standard/test compounds. Thirty minutes later, the animals were subjected to swim. Test was carried out for 6 minutes; the first two minutes the animal was allowed to adjust to the new conditions; for the next four minutes the immobility time that alternated with conditions of enhanced motor activity was measured with a stopwatch. Immobility time was the time during which the animal floated on the surface with front paws together and made only those movements which were necessary to keep afloat [8].

Tail Suspension Test (TST)

The test and standard compounds were administered p.o., 60 minutes prior to testing. The mice were suspended on the edge of a shelf 58 cm above the table top by adhesive tape placed approx. 1 cm from the tip of tail. The duration of immobility was recorded for the period of 6 minutes by using stopwatch. After the initial period of vigorous motor activity, the mice would become still. Mice were considered immobile when they hang passively and completely motionless [9].

Apomorphine Induced Hypothermia (AIH)

All the animals were fasted for 3 hrs prior to oral administration of vehicle/standard/test compounds. One hour after oral administration of the test compounds or the vehicle, 16 mg/kg apomorphine was injected s.c to the animals. The rectal temperature of each mouse was measured by an electronic thermometer prior to apomorphine administration and 10, 20, 30 & 60 minutes after apomorphine administration. During the entire experiment, animals were housed in groups in glass jars at room temperature [10].

Reserpine Induced Hypothermia (RIH)

All the animals were fasted for 3 hrs prior to oral administration of vehicle/standard/test compound. The basal rectal temperature was measured by inserting an electronic thermometer to a constant depth of 3 cm. On the day before testing, animals were injected with 2 mg/kg body weight of animal subcutaneously with reserpine. The animals were housed in a climate controlled animal colony and have free access to food and water for 18 hrs. After 18 hours of the administration of Reserpine, once again rectal temperature was measured. Rectal temperature was measured every 1/2 hr, 1 hr, 2 hr & 4 hrs after oral administration of the compounds [11].

Scoring of ptosis in mice is as follows:

Normal – 0; 1/4 th closed eyes – 1; 1/2 closed eyes – 2; 3/4 th closed eyes – 3; Fully closed eyes – 4.

5 - HTP Potentiation Of Head Twitches In Mice (HTPPH)

The animals were fasted for 3 hrs prior to oral administration. Animals were administered with vehicle/standard/test compounds orally to the respective groups. After 60 minutes, animals were injected with 75 mg/kg pargyline hydrochloride, subcutaneously. 90 minutes after pargyline, the animals were injected with 5-HTP, 10 mg/kg i.p. The number of head twitches and behavioural parameters like escape tendency, hind limb abduction, tremors, fore limb clonus and lardosis were calculated for half an hour.

The scoring of various behavioural parameters is as follows:Escape tendency - 0 or 1; Hind limb abduction - 0-2; Tremors - 0-4; Fore limb clonus - 0-2; Lardosis - 0-2.

Statistical Analysis

Results were presented as Mean ± SEM. The data was subjected for statistical analysis by One way analysis of variance (ANOVA) followed by Dunnett's t test and $P<0.05$*, 0.01** and 0.001*** were considered as significant, $P>0.05$ was considered as non-significant (ns) Vs Control group.

RESULTS

TABLE 1: Phytochemical constituents of Ethanolic Extract of *Dacus carota* (EEDC)

S. No.	Phytochemical constituents	Ethanolic Extract of *Dacus carota*
1	Alkaloids	+
2	Flavonoids	+
3	Gylcosides	+
4	Phenols	-
5	Saponins	-
6	Steroids	-
7	Carbohydrates	+
8	Terpinoids	+
9	Proteins and Amino acids	+

TABLE 2: Effect of EEDC on Immobility time in Forced Swim test model in mice

GROUP	IMMOBILITY TIME (MEAN ± SEM) ; N=6			
	30min	60min	120min	240min
Control	140 ± 11.66	139.2 ± 7.94	114.6 ± 6.51	110 ± 3.53
Flouxetine (25 mg/kg, p.o)	82.4± 25.38*	42.2 ± 14.22**	60.4 ± 17.91*	55.8 ± 14.50**
EEDC (200 mg/kg , p.o)	99.6 ± 4.7	88.6 ± 4.16*	77.2 ± 8.8	75.2 ± 10.15
EEDC (400 mg/kg, p.o)	63 ± 13.60**	56.6 ± 19.09**	60.2 ± 20.42*	54.4 ± 10.11**

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at $^{**}P < 0.01$, $^{*}P<0.05$ Non Significance (ns) at $P > 0.05$ Vs control.

TABLE 3: Percentage inhibition of EEDC in Forced Swim test model in mice

GROUP	PERCENTAGE INHIBITION (%)			
	30min	60min	120min	240min
Control	------	------	------	------
Fluoxetine (25 mg/kg, p.o)	41.4	69.6	47.2	49.2
EEC (200 mg/kg , p.o)	28.85	36.3	32.6	34.72
EEC (400 mg/kg, p.o)	55	59.6	47.4	50.5

TABLE 4: Effect of EEDC on Immobility time in Tail Suspension model in mice

GROUP	IMMOBILITY TIME (MEAN ± SEM); N=6
Control	128.2 ± 11.57
Flouxetine (25 mg/kg, p.o)	53.6 ± 7.17**
EEDC (200 mg/kg , p.o)	69.6 ± 21.10*
EEDC (400 mg/kg, p.o)	54.6 ± 8.45**

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at $^{**}P < 0.01$, $^{*}P<0.05$ Non Significance (ns) at $P > 0.05$ Vs control.

TABLE 5: Percentage inhibition of EEDC in Tail Suspension test in mice

GROUP	PERCENTAGE INHIBITION (%)
Control	------
Fluoxetine (25 mg/kg, p.o)	58.19
EEC (200 mg/kg , p.o)	45.70
EEC (400 mg/kg, p.o)	57.41

TABLE 6: Effect of EEDC on temperature in Apomorphine induced hypothermia model in mice

GROUP	INITIAL	RECTAL TEMPERATURE (MEAN ± SEM) ; N=6			
	0min	10min	20min	30min	60min
Control	37.6 ± 0.25	36.8 ± 0.37	36.4 ± 0.25	37 ± 0.45	36.8 ± 0.37
Desipramine (20 mg/kg)	37.9 ± 0.33	40.8 ± 0.37**	41.4 ± 0.40**	39.6 ± 0.40**	39 ± 0.55**
EEDC (200 mg/kg)	38.2 ± 0.20	37.8 ± 0.20	36.6± 0.24	36.6 ± 0.24	37 ± 0.36
EEDC (400mg/kg)	38.7 ± 0.20	38.2 ± 0.20*	37.4 ± 0.24*	36 ± 0.24	36.6 ± 0.24

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at $^{**}P < 0.01$, $^{*}P<0.05$ Non Significance (ns) at $P > 0.05$ Vs control.

Degree of Hypothermia:

TABLE 7: Effect of EEDC on degree of hypothermia in Apomorphine induced hypothermia model in mice

GROUP	DEGREE OF HYPOTHERMIA			
	10min	20min	30min	60min
Control	-0.8	-1.2	-0.6	-0.8
Desipramine (20 mg/kg, p.o)	2.9	3.5	1.7	1.1
EEC (200 mg/kg , p.o)	-0.4	-1.6	-1.4	-1.2
EEC (400 mg/kg, p.o)	-0.5	-1.3	-2.3	-2.1

TABLE 8: Effect of EEDC on temperature in Reserpine induced hypothermia model in mice

GROUP	INITIAL	RECTAL TEMPERATURE (MEAN ± SEM) ; N=6			
	0min	30min	60min	120min	240min
Control	40.58 ± 0.20	32.3 ± 0.56	33.6 ± 0.67	34 ± 0.68	34.5 ± 0.67
Desipramine (20 mg/kg)	40.08 ± 0.08*	32.5 ± 0.34	36 ± 0.26*	36.6 ± 0.21**	36 ± 0.37
EEDC (200 mg/kg)	39.25 ± 0.11**	32.16 ± 0.60*	36 ± 0.61	33.33 ± 0.41	33.16 ± 0.4
EEDC (400mg/kg)	39.25 ± 0.11**	30.83 ± 0.40	30.83 ± 0.40**	31 ± 0.26**	30.5 ± 0.22**

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at $^{**}P < 0.01$, $^{*}P<0.05$ Non Significance (ns) at $P > 0.05$ Vs control.

Degree of Hypothermia:

TABLE 9: Effect of EEDC on Degree of hypothermia in Reserpine induced hypothermia model in mice

GROUP	DEGREE OF HYPOTHERMIA			
	30min	60min	120min	240min
Control	-8.2	-6.9	-6.5	-6
Desipramine (20mg/kg, p.o)	-7.55	-4.05	-3.45	-4.05
EEC (200mg/kg , p.o)	-7.58	-6.58	-6.41	-6.58
EEC (400mg/kg, p.o)	-8.7	-8.7	-8.5	-9

TABLE 10: Effect of EEDC on Ptosis in Reserpine induced hypothermia model in mice

GROUP	INITIAL	PTOSIS SCORE; N=6			
	0 min	30 min	60 min	120 min	240 min
Control	4	4	4	4	4
Desipramine (20mg/kg, p.o)	4	3	3	3	3
EEDC (200 mg/kg , p.o)	4	4	4	4	4
EEDC (400 mg/kg, p.o)	4	4	4	4	3

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at $^{**}P < 0.01$, $^{*}P<0.05$ Non Significance (ns) at $P > 0.05$ Vs control.

TABLE 11: Effect of EEDC on Number of Head twitches in 5-HTP potentiation of Head twitches model in mice

GROUP	NUMBER OF HEAD TWITCHES (MEAN ± SEM) ; N=6					
	5min	10min	15min	20min	25min	30min
Control	6.2 ± 0.37	7.6 ± 0.51	11.4 ± 0.58	13.8 ± 0.58	12.4 ± 0.68	10.8 ± 0.5
Fluoxetine (25 mg/kg)	10 ± 0.71**	13 ± 1.30**	15.4 ± 0.51**	19.6 ± 0.68 **	17.6 ± 0.68**	16.8 ± 0.7**
EEDC (200 mg/kg)	7.2 ± 0.37	10 ± 0.71	13 ± 0.71*	16.2 ± 0.66*	13.8 ± 0.58	12.6 ± 0.6
EEDC(400 mg/kg)	8.4 ± 0.51*	12.2 ± 1.07**	15.2 ±1.07**	19 ± 1.14**	16.8 ±1.39**	15.2 ± 1.1**

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at $^{**}P < 0.01$, $^{*}P<0.05$ Non Significance (ns) at $P > 0.05$ Vs control.

DISCUSSION

The present work was subjected to investigation for the evaluation of the antidepressant activity of Ethanolic root extract of *Dacus carota* in mice. The extract was primarily subjected to phytochemical investigation and acute oral toxicity study. In Acute Oral Toxicity study, EEDC did not show any lethal effect even up to the doses of 2000 mg/kg, p.o and complete absorption of drug through GIT was observed. The effect of EEDC was investigated for its putative antidepressant activity by using various experimental models in mice viz. Tail Suspension test, Forced Swim test, Apomorphine induced Hypothermia, Reserpine induced Hypothermia and 5HTP potentiation of Head twitches in mice.

Forced Swim test & Tail Suspension test are the most commonly used preliminary screening tests for characterizing potential antidepressant drugs. In these models EEDC at doses of 200 mg/kg, p.o and 400 mg/kg, p.o showed significant increase in the motor activity of mice which elevate depressed mood by decreasing immobility time of mice [12]. The parameters observed in this model are immobility time of mice. Drugs which decrease immobility time leads to increase in the motor activity of mice which inhibit depression developed due to swimming and tail suspension of mice in these tests and offer protection against depression induced by these methods. In the present study, EEDC (200 & 400 mg/kg, p.o) has shown a significant dose dependent activity i.e. increase in the dose of the drug proportional to decrease in the immobility time threshold and offers good percentage protection as compared to control group. Similarly, the standard drug Fluoxetine (25 mg/kg, p.o) had significant percentage protection. Fluoxetine was selective serotonin reuptake inhibitor work on the serotonin balance by inhibiting a transporter that selectively pumps serotonin back into the neurons [14].

In Apomorphine induced hypothermia model, Apomorphine induces hypothermia which cannot be antagonized by the EEDC (200 & 400 mg/kg, p.o) but Desipramine (Standard-20 mg/kg-p.o) antagonized hypothermia revealed that the test component might be acting through Serotonin reuptake inhibition. The parameters observed in this model are rectal temperature readings of the mice. In this model, Desipramine and EEDC (200 & 400 mg/kg, p.o) had significantly showed opposite actions against hypothermia induced by Apomorphine.

In Reserpine induced hypothermia model, Reserpine induces hypothermia which cannot antagonized by the EEDC (200 & 400 mg/kg, p.o) and Fluoxetine (Standard-25 mg/kg-p.o) which revealed that EEDC might be acting through Serotonin reuptake inhibition like Fluoxetine. The parameters observed in this model are rectal temperature readings and Ptosis Score.

In 5-HTP model, EEDC at doses of 200 mg/kg and 400 mg/kg, Standard drug, Fluoxetine (25 mg/kg, p.o) had significant effect in potentiation of head twitches in mice compared to control group.

The action of EEDC in Apomorphine, Reserpine induced hypothermia and 5-HTP potentiation of head twitches models showed that the extract might have affect on the Serotonin reuptake inhibition like Serotonin reuptake inhibitors to exert its antidepressant activity.

CONCLUSION

In the present study, EEDC was evaluated by using various experimental models. EEDC at doses of 200 mg/kg, p.o and 400 mg/kg, p.o showed significant increase in the motor activity of mice which elevate depressed mood by decreasing immobility time of mice in Forced Swim test and Tail Suspension test. In Apomorphine & Reserpine induced hypothermia model, EEDC didn't show any antagonism against hypothermia produced by the Apomorphine and Reserpine. EEDC showed significant potentiation of head twitches in mice in 5-HTP model. From all the above findings, the present investigation suggests that the Ethanolic extract of *Dacus carota* may possess antidepressant activity by inhibiting reuptake of Serotonin which acts through Serotonergic receptors (G-protein coupled receptors) as mood elevator [15]. Therefore lend pharmacological credence to the traditional use of this plant in the treatment of depression.

However, an extensive Pharmacological study of this plant is required for complete understanding of the antidepressant activity of Ethanolic extract of *Dacus carota*.

Further investigation should be carried out to isolate and identify the chemical constituent which is responsible for its antidepressant activity.

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