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## EVALUATION OF ANTIDEPRESSANT ACTIVITY OF *SPINACIA OLERACEAE* BY USING ALBINO RATS

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**ABSTRACT:** Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. Spinach is a leafy green vegetable that came originally from south-western Asia and is now grown in most parts of the world. Scientifically it is known as *Spinacia oleracea* Linn. (Family-Chenopodiaceae). It is a good source of the bioflavonoid quercetin with many other flavonoids which exhibits anti-oxidant, anti-proliferative, anti-inflammatory, antihistaminic, in addition to its many other benefits. Treatment with *Spinacia oleraceae* (200mg/kg body wt) decreases the immobility time against forced swim test and tail suspension test. Fluoxetine (30mg/kg, i.p) was selected as reference standard and it showed significant antidepressant activity in rodents. Results suggest that *Spinacia oleraceae* exerts anti-depressant activity as shown by its effects on different experimentally induced different models.

**INTRODUCTION:** Depression is a serious disorder in today's society with estimates of lifetime prevalence as high as 21% of the general population in some developed countries. As a therapeutic alternative, effective herbal drugs may offer advantages in terms of safety and tolerability, possibly also improving patient compliance<sup>1</sup>.

Even though many medications are available the risk of relapse and reoccurrence remains high. Thus, there is a need for more effective and less toxic agents.

Plants extracts are some of the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of depression<sup>2</sup>. Herbal drug used in depression are *spinacia oleraceae*, *Centella asiatica*, *Hypericum perforatum*, *Rhodiola rosea*, *Pfaffia paniculata*, *Rauwolfia serpentine*, *Rhododendron molle*. *Spinacia oleracea* Linn. (Family-Chenopodiaceae) possess anti-inflammatory, anti-diabetic, anti-oxidant, anti-proliferative, antihistaminic and anthelmintic activity<sup>3-7</sup>.

Preliminary phytochemical studies showed the presence of Saponins, Tannins, Alkaloids, Phenols, flavonoids, glycosides and carbohydrates. Since the spinach has not been examined for their antidepressant activity hence the present study has been undertaken to evaluate the anti-depressant

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activity of *Spinacia oleraceae* against FST and TST.

**MATERIALS AND METHODS:** The plant was collected from local market of Bhongir. It was identified and authenticated by Prof. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant herbarium was prepared and deposited in the Department of Pharmacognosy for further reference. The plant was identified as *Spinacia oleracea* Linn. (Family-Chenopodiaceae) under the voucher no: SSCP/2012-2013-001.

**Instruments and Chemicals used:** The solvents used for extraction were, Benzene, Chloroform, Ethyl acetate, Ethanol and Distilled Water. Other reagents used were of laboratory grade and obtained from various other commercial sources. All the reagents used were of laboratory and analytical grade. Solvents are obtained from SD Fine-Chem Ltd. (Mumbai), Virat Lab company (Hyderabad), Accord labs (Secunderabad), Rolex laboratory reagent (Mumbai), Nova Biotech (Kolkata), Diazepam tablets (Barr Laboratories).

#### **Pharmacognostic studies:**

**Extraction:** Leaves were washed, air dried under shade and powdered with the help of grinder. Powder was weighed and packed in Soxhlet. Solvent used for Soxhletion was in the order of increasing polarity. Soxhletion was continued at the temperature of 50°C till clear solvent was observed in siphon tube. Extract was collected in water bath at 40°C. Concentrated extract was dried at 40c in hot air oven. Dried extract was packed in air tight container.

#### **Preliminary Phytochemical Screening<sup>8-14</sup>:**

The extract so obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books to determine the nature of chemical constituents present in the plant.

**Animals:** Wistar albino rats (150-200 g) of either sex and of approximate same age used in the present studies were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet and water ad libitum. All the animals were housed in

polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light.

The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) after scrutinization. The animals received the drug treatments by oral route as well as intra peritoneal.

**Selection of dose and standard drug preparation:** Different doses of 5, 50, 300, 2000mg/kg of ethanolic and aqueous extracts of leaves of *Spinacia oleracea* use administrated orally. The alcoholic and aqueous extracts of leaves of *Spinacia oleracea* were screened for acute toxicity study by OECD guidelines (423) for determining the LD<sub>50</sub>. The results showed that LD<sub>50</sub> was found to be 2000mg/kg.

For the assessment of antidepressant activity dose level was chosen in such a way that dose was approximately one tenth of the maximum dose during acute toxicity studies. Therefore dose was fixed on 200mg/kg. Fluoxetine was used as the reference drug for evaluating the antidepressant activity. Fluoxetine suspension was prepared using saline.

**Experimental design:** On the day of the experiment, the animals were divided randomly into control and experimental groups (n=6). Group 1 received the vehicle, 5% gum acacia (10ml/kg) and served as the control group, groups 2 and 3 received the test drugs in doses of 200mg/kg, and group 5 received the standard drug fluoxetine (10mg/kg) i.p. Drugs/vehicle was administered to the animals 60 minutes prior to the behavioral evaluation in acute study.

The antidepressant activity of the test drug was evaluated using the following experimental models of depression TST and FST:

**Tail Suspension Test (TST)<sup>15</sup>:** The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be

immobile only when they hung passively and were completely motionless.

**Forced Swim Test (FST)** <sup>16</sup>: Each animal was placed individually in 5 liter glass beakers, filled with water upto a height of 15 cm and were observed for a duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test.

**TABLE 1: PHYTOCHEMICAL SCREENING OF *SPINACIA OLERACEAE***

S. No.	Phytoconstituents	Chemical test	Benzene	Chloroform	Ethyl acetate	Ethanol	Aqueous
1.	Steroids and Triterpenoids	Liebermann-Burchard test	+	-	-	-	-
2.	Phenolic and tannins	5% Alcohol FeCl <sub>3</sub> solution	-	-	++	+	++
3.	Flavonoids	Shinoda test	-	+	++	+	++
4.	Saponins	Foam test	-	+	-	+	++
5.	Alkaloids	Dragendorff and Mayer reagent	-	-	-	+	-
6.	Antraquinones glycosides	Borntrager test	-	-	-	-	+
7.	Carbohydrates	Molisch's reagent and Fehling solution	-	-	+	+	++

**Acute toxicity study:** The extract was studied for acute toxicity at doses of 2000mg/kg. The extract was found devoid of mortality of all animals. So, the dose selected for the antidepressant activity was 200mg/kg.

#### Evaluation of Antidepressant activity:

**Forced swimming test:** It was observed that ethanol and aqueous extracts at a dose of 200mg/kg exhibited significant reduction in immobility time when compared to control in dose dependent manner (fig. 1). Similarly the animals treated with fluoxetine (10mg/kg) as expected showed significant decrease in immobility time (Table 2).

**TABLE 2: EFFECT OF *SPINACIA OLERACEAE* ON DURATION OF IMMOBILITY TIME IN FORCED SWIM TEST**

S. No.	Group	Treatment	Immobility time
1	CONTROL	5% Gum Acacia	39 ± 7.14
2	STANDARD	Fluoxetine (10mg/kg i.p)	11 ± 2.01**
3	TEST 1	Ethanol Extract (200mg/kg p.o)	15 ± 2.74*
4	TEST2	Aqueous Extract (200 mg/kg p.o)	03 ± 0.54***

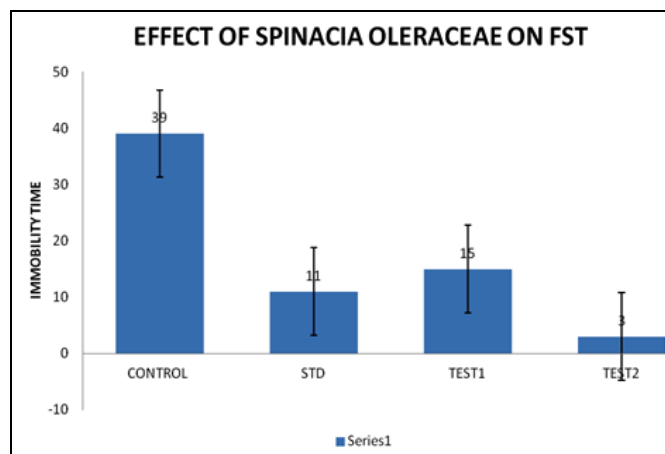
Values are expressed as mean ± S.E.M (n = 6), in each group \*p < 0.02, \*\*p < 0.01, \*\*\*p < 0.001 when compared to control.

**Tail suspension test:** In tail suspension test, the ethanolic and aqueous extracts of leaves of

**Statistical Analysis:** The mean±S.E.M. values were calculated for each group. The data were analyzed using one-way ANOVA followed by Dunnet's multiple comparison test. P < 0.001 was considered to be statistically significant.

#### RESULTS:

**Preliminary Phytochemical Screening:** The extracts showed the presence of saponins, tannins, alkaloids, phenols, flavonoids, glycosides and carbohydrates.



**FIGURE 1: EFFECT OF *SPINACIA OLERACEAE* ON DURATION OF IMMOBILITY TIME IN FORCED SWIM TEST**

*Spinacia oleraceae* at a dose of 200 mg/kg p.o. significantly decreased the immobility time (fig. 2).

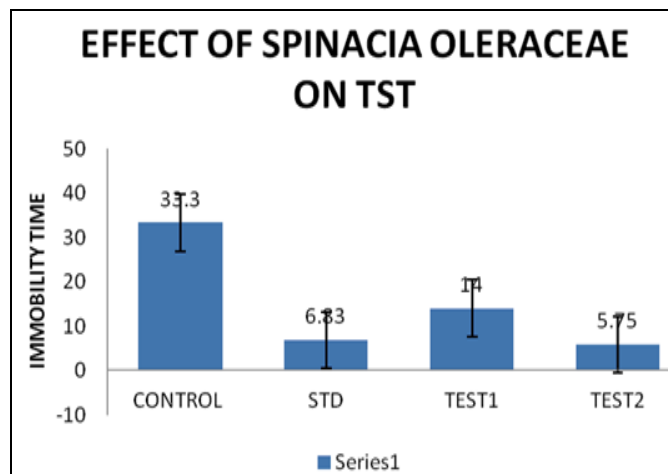
The magnitude of the antidepressant effects of 200 mg/kg p.o. of ethanolic and aqueous leaves of

*Spinacia oleraceae* was comparable to that of fluoxetine 10 mg/kg i.p. (Table 3).

**TABLE 3: EFFECT OF SPINACIA OLERACEAE ON DURATION OF IMMOBILITY TIME IN TAIL SUSPENSION TEST**

S. No.	Group	Treatment	Immobility time
1	Control	5% Gum Acacia	33.3 ± 6.20
2	Standard	Fluoxetine (10mg/kg i.p)	6.83 ± 1.272**
3	TEST 1	Ethanol extract (200mg/kg p.o)	14 ± 2.608*
4	Test 2	Aqueous extract (200 mg/kg p.o)	5.75 ± 1.071***

Values are expressed as mean ± S.E.M (n = 6), in each group \*p < 0.02, \*\*p < 0.01, \*\*\*p < 0.001 when compared to control.



**FIGURE 2: EFFECT OF SPINACIA OLERACEAE ON DURATION OF IMMOBILITY TIME IN TAIL SUSPENSION TEST**

**DISCUSSION:** The widespread use of FST is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rodents following initial escape-oriented movements develop an immobile posture when placed inside an inescapable cylinder filled with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal's ability to cope with stressful stimuli. Markedly showed a significant decrease in the time spent immobile by rodents. By performing tail suspension test, the reduced immobility time directed the antidepressant effect. Antidepressant effects may be due to the flavonoid<sup>17</sup>.

Flavonoids glycosides are mostly hydrolyzed into their aglycons by mucosal and bacterial enzymes in the intestines, and then converted to conjugated metabolites during the absorption process<sup>18, 19</sup>. Transportation of these metabolites into the brain tissues via the blood brain barrier and their effect

on the central nervous system (CNS) has been recently argued<sup>20, 21</sup>.

Therefore, one of the antidepressant mechanism of *Spinacia oleraceae* is thought to involve flavanoids and glycosides which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance and consequently, exerting an antidepressant effect. Thus, extracts of *Spinacia oleraceae* may have potential therapeutic value for the management of depressive disorders.

**CONCLUSION:** The present results suggest that aqueous and ethanolic extract of *spinacea oleraceae* produces the antidepressant like effect as it decreases the immobility time during depression in animal model (FST & TST). It was found to be similar to that of fluoxetine. The aqueous extract of *spinacea oleraceae* was found to be more potent when compare to ethanol extract. The antidepressant like effect of the aqueous extracts seems mostly likely to be mediated through an interaction with adrenergic, dopaminergic and serotonergic system. Thus aqueous extract of *spinacea oleraceae* may have potential therapeutic value for the management of depressive disorders.

However, further studies are required to identify the phytoconstituents responsible for the observed anti-depressant effect.

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