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ADAPTOGENIC ACTIVITY OF SIDDHA MEDICINAL PLANT *INULA RACEMOSA*

[Roots]

**GNANASEKARAN D^{1*}, REDDY CU², JAIPRAKASH B³, NARAYANAN N⁴, KIRAN
YR⁵ AND ELIZABETH H⁶**

1: Professor, Dept. of Pharmacology, Bharathi college of Pharmacy, Bharathinagar, India

2: Professor, Dept. of Pharmacology, Sri Ramachandra college of Pharmacy, Porur, Chennai,
India

3: Director, Gayathri college of Pharmacy, Orissa, India

4: Director Jaya college of Pharmacy, Chennai, India

5: PG scholar, Dept. of Pharmacology, Bharathi college of Pharmacy, Bharathinagara,
Karnataka, India

6: Scholar Dept. of Zoology, Thiruvallur University, Vellore, Tamilnadu, India

***Corresponding Author: Email: gnanasekaran_99@yahoo.co.uk; Mob.: +919986493815**

ABSTRACT

The present study carried out on the extract *Inula racemosa* (roots) to show the adaptogenic activity. Forced swimming test (FST) is a screening model for antidepressants / adaptogens. Two swimming sessions were conducted: a 15 min pre-test followed 24 hr later by a 6 min test. The total duration of immobility behaviour was recorded during the second 6 min test. Mouse was judged immobile, when it remained floating in water, in an upright position making only small movements to keep the head above water. The experimental animals were euthanized and their

brains were removed immediately, and the prefrontal cortexes (PFC) were dissected out on ice for biochemical analysis. LD₅₀ of the test drug was found to be greater than 2000mg/kg body weight. The animals treated with formulation of extract (100mg/kg) and (200mg/kg) showed significant decrease in the immobility period with simultaneous increase in anti oxidant markers as well as adrenaline and serotonin levels. The above study indicates positive adaptogenic activity of the extract *Inula racemosa* (roots), by forced swim test and resultant biochemical studies.

Keywords: *Inula racemosa*, Forced Swim Test, Prefrontal Cortexes, Antidepressants.

INTRODUCTION

Siddha system of medicine also known as Siddha vaidya in India, considered as the crown of all the traditional arts of the ancient world owing to its richness and simplicity, practiced by siddhars. Siddha medicine is in usage of herbs, metals, minerals as well as animals in preparing highly effective medicines, is the oldest medical system in existence. The hallmark of traditional Siddha system is KAYAKARPAM i.e., imparting immunity to diseases [1]. The work presented in this paper is on plant mentioned as Kayakarpam plants in published as well as unpublished palm leaf literatures. The Pharmacological experiments have been evaluated with special reference to their rejuvenation activity. The parameters chosen are also indicative of their potential use in Kayakarpam. Kaya Regeneration Therapy, one of the techniques used for longevity in

Siddha Vaidya is practiced in Tamilnadu, "the emerald state," in South India. This special technique of body treatment is traditionally used for physical, emotional and sexual health. It enhances vitality, physical beauty, functionality and productivity. As part of the broad spectrum of medical practices which constitute the Siddha Vaidya medical system, it acts as an immune-modulator, cyto-protector and physical regenerator. This translates into such things as remedies for stroke management following the critical period, and improvement in cases of scleroderma, rheumatoid arthritis and systemic lupus erythematosus. It also helps past traumas that continue to cause pain but register no physical evidence. *Inula racemosa* is a member of the Asteraceae family. It grows in the temperate and alpine western Himalayas, and it is common in

Kashmir. The roots are widely used locally in indigenous medicine as an expectorant and in veterinary medicine as a tonic. The rhizome is sweet, bitter and acrid in taste with a neutral potency and act as antiseptic, anti-bacterial, anti-fungal, anti-inflammatory, analgesic and mild diuretic. It is used in the treatment of contagious fevers, angina pectoris, heart disease and ischemic heart disease. It is also used in cough, hiccup, bronchial asthma, indigestion, flatulence, inanorexia and in fever. Externally, the paste of its roots is used effectively, in dressing the wounds and ulcers as the herb possesses antiseptic property. Also used to boost the appetite [2]. It is stated in traditional siddha literature under the author Bhava Mishra, 'Bhava Prakash Nigandu' [2]. Roots of this plant (nagapala) used in liver diseases, rejuvenation and anti ageing. But it has not been explored properly and remains a silent drug in herbal medicine. The present study carried out on the extract *Inula racemosa*(roots) to show the adaptogenic activity.

MATERIALS AND METHOD

Plant Source

The roots *Inula racemosa* was collected in the surrounding areas of Bidadi, Ramnagar Dist, Karnataka.

Extraction

One kg of powdered roots of *Inula racemosa* was taken in a soxhlet and 2500 ml of 90% ethanol was added. It was refluxed for 72 hours and filtered through muslin cloth while hot. The alcohol extract was dried under vacuum and suspended in carboxy methyl cellulose before use [3].

Total Extract

Formulation is done by dissolving total extract in 'Amuri.'

Preparation of Amuri

Amuri is a primordial liquid elixir obtained from plantain tree named *Musa paradisiaca* through a special process.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (18-22g) were used for the study. Animals were housed in polycarbonate colony cages (6/cage) in a well ventilated room (air cycle: 15/min; 70:30) under an ambient temperature of $23\pm 2^{\circ}\text{C}$ and 40–65% relative humidity, with artificial photoperiod 12-h light/12-h dark cycle. They were provided with standard rodent pellet diet (Provimi, India) and purified water ad libitum (RIOS, USA). Experimental animals were

acclimatized for 7 days to the laboratory conditions prior to experimentation. Guidelines of “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were strictly followed throughout the study. Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai, India, approved the study protocol. IAEC/XIV/SRU/100/2008.

Acute Oral Toxicity Study

Acute oral toxicity study was conducted according to the OECD test guideline 423- Acute toxic class method. Female Sprague Dawley rats (160-180g) were divided into two groups of 3 animals / group. Animals were housed individually in a well ventilated polypropylene cage. A 12-h light / 12-h dark artificial photoperiod was maintained. Room temperature 22°C ($\pm 3^\circ$) and relative humidity 50–70% were maintained in the room. Animals had free access to pelleted feed. (Nutrilab rodent, Tetragon Chemie Pvt Ltd., India) and Reverse osmosis (Rios, USA) purified water *ad libitum*. The test drug was administered once orally via gastric intubation at a dose level of 2000 mg/kg b.wt. Lethality, abnormal clinical signs and body weight

changes were observed on the day of dosing and thereafter for 13 days. Gross pathological changes were also observed on the termination of the experiment [4, 5].

Forced Swim Test

Forced swimming test (FST) is a screening model for antidepressants / adaptogens. Mice were placed individually in a Plexiglas cylinder (height 40 cm, diameter 10 cm) filled with tap water ($27 \pm 2^\circ\text{C}$) to a height of 18 cm. Two swimming sessions were conducted: a 15 min pre-test followed 24 h later by a 6 min test. After the pre-test, the animals were removed from the water, dried before a room heater and returned back to their home cages. The total duration of immobility behaviour was recorded during the second 6 min test. Mouse was judged immobile, when it remained floating in water, in an upright position making only small movements to keep the head above water [6, 7].

Treatment and Groups

To test the adaptogenic effect of drugs in FST, animals were pretreated with drugs at 100 mg/kg, p.o daily for a period 14 days. Test drugs were prepared in 0.5% CMC. There were nine groups: Group I: vehicle – received no CMC or FST; Group II: vehicle

received 0.5% CMC (at dose volume of 10ml/kg, p.o) + FST; Group III: Fluoxetine (15 mg/kg, p.o) + FST; Group IV: TE + FST (100mg/kg, p.o); Group V: TE + FST (200mg/kg, p.o); Group VI: FE + FST (100mg/kg, p.o); Group VII: FE + FST (200mg/kg, p.o); Group VIII: Formulation + FST (100mg/kg, p.o) and Group IX: Formulation + FST (200mg/kg, p.o). Mice from groups I and II, injected with saline solution, served as controls. Fluoxetine was used as a classical antidepressant. All groups were treated daily for 14 days. FST was performed 1h after the last dose. The experimental animals were euthanized and their brains were removed immediately, and the prefrontal cortexes (PFC) were dissected out on ice for biochemical analysis [7].

Biochemical Estimation

Superoxide Dismutase

Superoxide dismutase (SOD) was assayed by taking 0.05 ml of brain homogenate followed by the addition of 0.3 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.025 ml of PMS (186 μ M) and 0.075 ml of NBT (300 μ M in buffer, pH 8.3). Reaction was started by addition of 0.075 ml of NADH (780 μ M in buffer of pH 8.3). After incubation of the reaction mixture at 30 °C for 90 s, the

reaction was stopped by addition of glacial acetic acid (0.25 ml). Then, the reaction mixture was stirred vigorously and shaken with 2.0 ml of n-butanol. The mixture was allowed to stand for 10 min and centrifuged. 1.5 ml of n-butanol alone served as blank. The colour intensity was read at 560nm using spectrophotometer (Perkin Elmer, λ 25, USA). Enzyme activity was expressed as 1 Unit = 50% inhibition/minute/mg of protein [8].

Reduced Glutathione

Glutathione (GSH) content was estimated by following Jollow et al. (1974) method. 0.25 ml of tissue homogenate was added to an equal volume of ice cold 5% TCA. The precipitate was removed by centrifugation at 4000rpm for 10 min. To 1ml aliquot of supernatant, 0.25 ml of 0.2M phosphate buffer (pH 8.0) and 0.5 ml of DTNB (0.6mM in 0.2M phosphate buffer, pH 8.0) were added and mixed well. The absorbance was read at 412nm using spectrophotometer (Perkin Elmer, λ 25, USA). The values were expressed in nanomoles/g tissue [9].

Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was evaluated by measuring the TBARS content according to

the TBA test described by Ohkawa et al. (1979) with slight modifications. 0.2 ml of the brain tissue homogenate was taken and to this 0.8 ml saline, 0.5 ml of BHT and 3.5 ml TBA reagent (0.8%) were added and incubated at 60 °C in a boiling water bath. After cooling, the solution was centrifuged at 2000rpm for 10 min. The absorbance of the supernatant was determined at 532nm using spectrophotometer (Perkin Elmer, λ25, USA) against the blank. TBARS content were expressed in nanomoles/mg tissue. Standard calibration was plotted using 1,1,3,3-tetraethoxy propane in the concentration range of 0.50–4.00 µg [10].

Protein Content

Protein content in the brain homogenate was estimated by Lowry et al., method (1951) [11].

Determination of Norepinephrine (NE) and 5-Hydroxytryptamine (5-HT) Levels

The levels of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) were determined by reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection. Briefly, a C₁₈ reverse-phase column (Waters 150 mm 3 4.6 mm, 5µm), an electrochemical detector (ESA, 5600A, Bedford, MA, USA), and a liquid

chromatography work station were employed. Following decapitation, the brains were rapidly removed and dissected on ice; prefrontal cortex was isolated. The tissues were weighed, sonicated for a few seconds in 0.15 M HClO₄ (1 ml/100 mg tissue), and centrifuged. The supernatant was filtered using 0.2 µm and stored at -80°C until HPLC-ED analysis. The mobile phase composed of phosphate buffer (pH of 3.9, 90 mmol/L NaH₂PO₄, 50 mmol/L citric acid, 1.7 mmol/L octanesulfonic acid sodium salt, and 0.05 mmol/L Na₂EDTA); with 0.6 ml/min flow rate. The sample (20 µl) was injected and the oxidation potential was fixed at 450 mv. The peak areas of the external standards were used to quantify the sample peaks.

Statistical Analysis

Data are expressed as mean ± SEM. All data were analyzed using one-way analyses of variance (ANOVA), followed Dunnett's Multiple Comparison Test as post hoc. P value < 0.05 was considered to be statistically significant. GraphPad Prism version 4.0 software was used for the statistical analysis

RESULTS

Acute Oral Toxicity Study

- There were no treatment related deaths, abnormal clinical signs, remarkable body

weight changes or gross pathological changes were observed in the experimental animals.

- From the above results, LD₅₀ of the test drug was found to be greater than

2000mg/kg b.wt. Hence, the test drug falls in the “category-5” or “unclassified” in accordance to the Globally Harmonized System.

Body Weight of the Experimental Animals is Shown in (Table1)

Table1: Body Weight of the Experimental Animals

Treatment	Body weight (g)					
	-1 day	-1 hr before test substance administration Day 1	6h on Day 1	Day 2	Day 7	Day 14
Control	154.33±2.39	143.00±5.52	167.67±5.68	168.33±5.86	175.53±9.67	193.78±10.88
<i>Inula racemosa</i>	152.00±4.99	149.00±6.65	164.78±7.93	164.64±5.59	185.63±10.56	196.53±14.84

Values expressed in mean±SEM; n=3

Swim Endurance

Table 2: Effect of Formulation of Extract on Swim Endurance

S No	Group		Immobility period
1	Vehicle-treated (saline, p.o) + Non-FST		-
2	Vehicle-treated (saline, p.o) + FST		230.17±12.76
3	Fluoxetine (15 mg/kg, p.o) + FST		128.33±9.29**
4	<i>Inula racemosa</i>	(100 mg/kg, p.o) + FST	226.77±1.2**
5	Formulation of extract	(200 mg/kg, p.o) + FST	203.14±3.18**

Values expressed in mean±SEM; n=6 per group

Effect of Formulation of extract on swim endurance shown in (Figure 1)

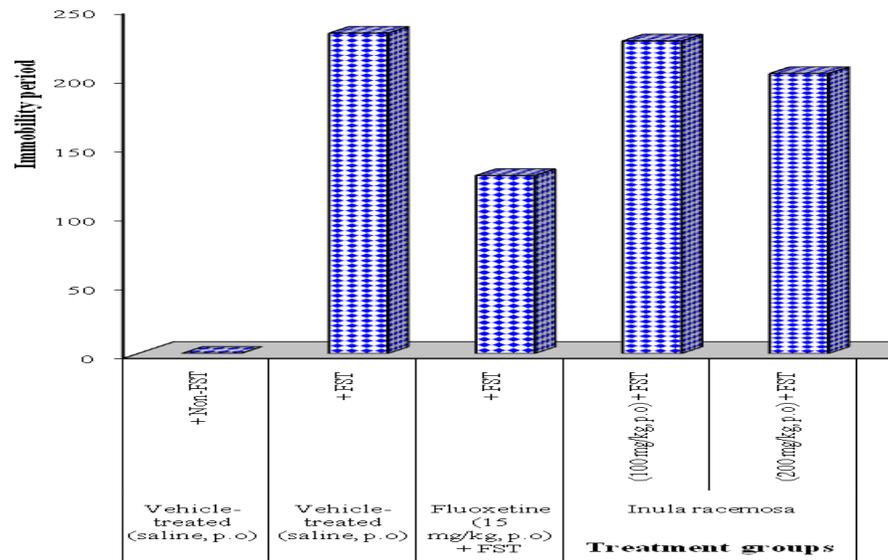


Table 3: Effect of Formulation of Extract on Antioxidant Markers In Mouse Brain

S. No.	Group	SOD (Units/min/mg protein)	GSH (nano moles/g tissue)	LPO (nano moles/mg protein)
1	Vehicle-treated (saline, p.o) + Non-FST	23.16±1.82	3.20±0.15	0.19±0.01
2	Vehicle-treated (saline, p.o) + FST	7.89±0.69 ^{##}	1.12±0.34 ^{##}	2.46±0.34 ^{##}
3	Fluoxetine (15 mg/kg, p.o) + FST	14.78±2.89	1.75±0.22	1.78±0.13 [*]
4	<i>Inula racemosa</i> (100 mg/kg, p.o) + FST	11.27±1.24 ^{**}	2.63±0.056	1.95±0.014 [*]
5	Formulation of extract (200 mg/kg, p.o) + FST	17.32±0.24 ^{**}	2.91±0.013 ^{***}	1.99±0.018 ^{**}

Where, ^{##} represents significant at p<0.001 vs control group, ^{*} represents significant at p<0.05, ^{**} represents highly significant at p< 0.01, and ^{***} represents very significant at p<0.001 vs control + FST

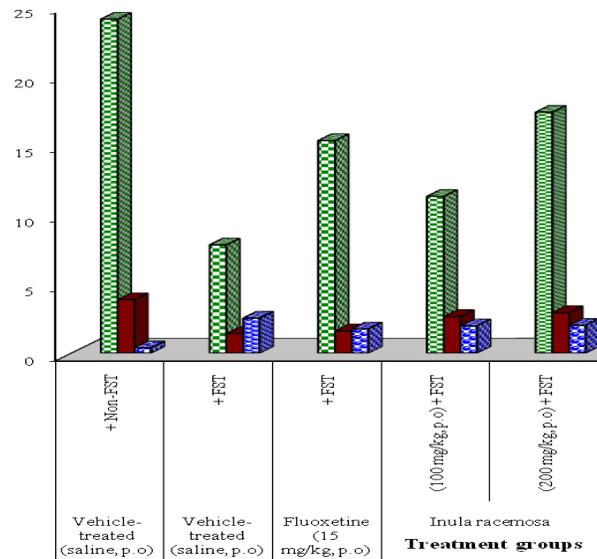


Figure 2: Effect of Formulation of Extract on Antioxidant Markers In Mouse Brain

Table 4: Effect of Formulation of Extract on NE and 5-HT Levels

S. No.	Group	Norepinephrine (NE)	5-Hydroxytryptamine
1	Vehicle-treated (saline, p.o) + Non-FST	88.46±9.16	45.47±3.28
2	Vehicle-treated (saline, p.o) + FST	85.75±15.37	40.73±7.36
3	Fluoxetine (15 mg/kg, p.o) + FST	83.62±11.82	39.88±5.73
4	<i>Inula racemosa</i> Formulation of extract	(100 mg/kg, p.o) + FST	49.84±8.38
5		(200 mg/kg, p.o) + FST	35.36±4.57

DISCUSSION AND CONCLUSION

Adaptogens are the plant derived biologically active substances that improves the immunity and physical endurance. Many herbals preparations have been evaluated for their adaptogenic activity during exposure to stressful conditions. In response to stressor, a series of behavioural, neurochemical and immunological changes occur that ought to serve in an adaptive capacity. If stress increases the organism may become diseased [12]. Stress is one of the basic factors which cause a number of diseases such as atherosclerosis, coronary heart disease, aging and liver disease [13]. In the present the adaptogenicity potential of formulation of extract was investigated in the forced swim test model. In the present study, vehicle treated rats showed increased immobility period, decreased brain noradrenaline and serotonin levels. The animals treated with formulation of extract (100mg/kg) and (200mg/kg) showed significant decrease in the immobility period with simultaneous increase in adrenaline and serotonin levels. These effects can be speculated to their influence on the modulation of neurotransmitters in experimental animal brains.

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