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Research Article

INTERACTION OF '*ICHNOCARPUS FRUTESCENS*' WITH BIOGENIC AMINES FOR ITS ANTI-DEPRESSANT ACTIVITY

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ABSTRACT

The research interest has focused on various herbs that possess antidepressant properties and may be useful adjuncts in helping the management of depression in humans. The present study was therefore designed to investigate the antidepressant-like effects of methanol leaf extract of *Ichnocarpus frutescens* in animal models of depression. The mice were divided in to different groups and treated with two different doses of methanol extracts (200 & 400 mg/kg), same doses of extracts with different antagonist of 5HT, NA & DA (Ondansetran, Terazosin & Chlorpromazine) and standard groups treated with imipramine 10mg/kg . All groups of animals were separately submitted to forced swim test (FST), and Tail suspension test (TST) tests for the bio-screening of leaf extract with antidepressant profile. Results revealed that the immobility time in the FST & TST was significantly reduced with extracts alone and extracts with Chlorpromazine treated group compared to negative control. There is no significant reduction in immobility time was observed particularly in rats orally administered extracts with Ondansetran & Terazosin when compared with their respective controls. Thus, it is suggested that methanol leaf extract of *Ichnocarpus frutescens* exhibited significant antidepressant-like effects in animal models of depression and may be served as a potential resource for natural psychotherapeutic agent, against depression and it's may act mostly through modifying serotonergic and noradrenergic neurons.

KEYWORDS: *Ichnocarpus frutescens,* Ondansetran, Terazosin, forced swim test & Tail suspension test.

INTRODUCTION

Mental depression is one of the common chronic illnesses that affect the mood, thought, physical health and behaviour of an individual. In the recent years it was recognized as a major health problem. According to the World Health report[1], approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020[2]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models[3]. In modern medicine several types of synthetic antidepressant drugs are available to treat depression. However, these drugs have the disadvantages of relatively low response rates and high side effects[4]. Thus, the search for a quick acting new antidepressant drug without or with minimum side effects is need of the hour and it is continuing. In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs[5]. Based on the above information the present study designed to evaluate the anti-depressant activity in plant.

Neurotransmitters, especially noradrenaline and serotonin (also called 5-HT) are believed to be key in the control of mood and emotional behavior. The neurotransmitters we are concerned with monoamines all share a similar chemical structure and are also known as biogenic amines. These neurotransmitters include adrenaline, dopamine, serotonin and nor adrenaline.

Ichnocarpus frutescens L. is a plant from the family Apocynaceae, is extensively cultivated in most regions of the world and common avenue tree, commonly called as black creeper in English. The literature survey reveal that *Ichnocarpus frutescens* L. roots has been used as tonic, diuretic, demulscent, diaphorectic, dyspepsia, stones in gall bladder, skin troubles and diabetic. Young stems and leaves are also used for depressant, delirium, epilepsy and diabetes .Young stem and leaves contain triterpenoids, α-amyrin and its acetalupeol and its acetate, friedelin, epifriedelinol and β-sitosterol. Flowers and fruit contain flavonoids-quercetin, kaempferol-3-glucoside,

sorbopyranoside and flavonoids, and is used as an anti-depressant agents and the recent studies on anti-depressant activity claim that flavonoids promote significant anti-depressant property[6,7]. However, the literature reveals no scientific data on anti-depressant activity on *Ichnocarpus frutescens* Leaves. In view of this, the present study is taken up to investigate the possible anti-depressant property of *Ichnocarpus frutescens* L. leaves in mice. So this study is essential and justifiable.

MATERIALS AND METHODS

Plant collection, authentication, preparation of the extract and phytochemical screening

The leaf part of *Ichnocarpus frutescens* was collected from local distributor in Tirupathi in the month of February. The leaf *Ichnocarpus frutescens* was identified and authenticated by K. Madhava Chetty, Assistant professor from Sri Venkateswara University, Tirupathi, Chittoor district, Andhra Pradesh.

The dried leaves of *Ichnocarpus frutescens* were crushed into fine particles (powder) using a mixer. The Powdered leaves were packed in a Sohxlet apparatus and subjected to continuous hot Percolation at temperature 50ºc using methanol as solvent till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40ºc. Concentrated extract was dried and packed in an air tight container. The methanol extracts of leaves of *Ichnocarpus frutescens* was subjected to qualitative analysis for various phytoconstituents. [8]

Experimental Animals

All the experiments were carried out using male, Swiss Albino mice (25-30 g) procured from the Sri Venkateswara Enterprises, Bangalore, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 23 ± 2ºC and relative humidity of 30–70%. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial rat chaw pellets.

Acute oral toxicity studies[9]

Organization for Economic co-operation and Development (OECD) regulates guidelines for oral acute toxicity study. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. The acute toxicity study was carried out as per OECD 423 Guidelines. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The methanol extract of *Ichnocarpus frutescens* had good margin of safety and did not shown any lethal effects on the animals up to the doses of 2000mg/kg. Hence the LD50 of *Ichnocarpus frutescens* was considered as 2000mg/kg. Studies were carried out with 1/10 of the LD50 as therapeutic dose 200mg/kg and double the dose of therapeutic dose 400mg/kg.

ANTI-DEPRESSANT ACTIVITY

Experimental study design

Wistar albino mice were divided into ten groups of six rats each.

Group I	:	Negative control, administer water 2 ml/kg orally.
Group II	:	Positive control and receive standard drug imipramine 10 mg/kg orally.
Group III	:	Receive MEIF 200 mg/kg Orally.
Group IV	:	Receive MEIF 400 mg/kg orally.
Group V	:	MEIF 200 mg/kg with Ondansetran (OND) 2mg/kg receive orally.
Group VI	:	MEIF 400 mg/kg with Ondansetran 2mg/kg receive orally.
Group VII	:	MEIF 200 mg/kg with Terazosin (TZ) 1mg/kg receive orally.
Group VIII	:	MEIF 400 mg/kg with Terazosin 1mg/kg receive orally.
Group IX	:	MEIF 200 mg/kg with Chlorpromazine (CP) 5mg/kg receive orally.
Group X	:	MEIF 400 mg/kg with Chlorpromazine 5mg/kg receive orally.

Forced swim test (FST)

For the determination of antidepressant activity, FST protocol was employed[10]. During the test, animals were individually placed in a glass cylinder (30 cm in height, 15 cm in diameter) filled 15 cm high with water at 25 ± 2°C. Treatment was given 60min prior to study as described by study design. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test after one hour of treatment. Immobility period was regarded as the time spent by the mouse to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.

Tail suspension test (TST)

The test was carried out according to the method described by Steru *et al.* [11] Tail suspension test is behaviour despair model of depression, employed in rodents to predict antidepressant potential by decreasing immobility period produced by several different classes of antidepressant drugs. It has been reported that tail suspension test is less stressful and has higher pharmacological sensitivity than forced swim test, the other commonly employed model to study antidepressant activity. Treatment was given 60min prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed

approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 4 min period after one hour of treatment. The animal was considered immobile when it did not show any movement of the body except for those required for respiration and hanged passively.

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 tests. P<0.05, P<0.01 & P< 0.001was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening

The methanol leaf extract of *Ichnocarpus frutescens* was subjected to Preliminary Phytochemical results showed the presence of alkaloids, carbohydrates, flavonoids or polyphenols etc.

Table 1: Preliminary phytochemical screening of methanol extract of *Ichnocarpus frutescens* leaf

S. No.	Constituents	Tests	Methanol
1	Alkaloids	Mayer's test Dragendroff's test Hager's test Wagner's test	+ + + +
2	Sterols	Burchard test Salkowski	+ -
3	Carbohydrates	Molisch's test Fehling's test Benedict's test Barfoed's test	+ + + +
4	Glycosides	Legal test Keller kiallani test Borntrager's test	+ + +
5	Fixed oils & Fats	Spot test Saponification test	- -
6	Phenolic Compounds	Ferric chloride	+
7	Proteins & amino acids	Biuret test Ninhydrin test Millon's test	+ + +
8	Terpenoids & Saponins	Foam test Hemolysis test	- -
9	Tannins	Gelatin test $Fecl_3$ test	+ +
10	Gums & mucilage	Precipitation to 90%alcohol	-
11	Flavonoids	Shinoda test Lead acetate test Ferric chloride test Zinc HCL test	+ + + +

+ve: Present**, -ve:** Absent

Forced swim test

The standard (imipramine 10mg/kg) and methanol extract alone (200 & 400mg/kg) treated groups, the peak values of immobility time significantly decreased to 64.4 ± 5.91, 96.6 ± 4.7 and 78.2 ± 5.8 when compare to control 198 ± 6.66. Thus, the both doses of methanol extract along with Ondansetran and terazosin significantly increases the immobility time 170 ± 8.66, 148.2 ± 7.94, 164.6 ± 6.51 and 130 ± 3.53 compare to extract alone treated groups but 400 mg/kg shows no significant and 200 mg/kg shows less significant to negative control. There is no significant variation in extract with Chlorpromazine treated group immobility time compare to group III & IV. But it is reveals significant variation compare to negative control (Table 3).

Table 3: Effect of MEIF on Immobility time in Forced Swim test model in mice

Group	Treatment	Immobility time in seconds	% Inhibition
I	Negative control	198 ± 6.66^{b}	0
II	Positive control	$64.4 \pm 5.91^{***a}$	67.47
III	MEIF 200mg/kg	$96.6 \pm 4.7^{**a}$	51.21
IV	MEIF 400mg/kg	$78.2 \pm 5.8^{***a}$	60.50
V	MEIF 200mg/kg+ OND	170 ± 8.66^{nsb}	14.14
VI	MEIF 400mg/kg+ OND	$148.2 \pm 7.94^{*b}$	25.15

VII	MEIF 200mg/kg+ TZ	164.6 ± 6.51^{nsb}	16.86
VIII	MEIF 400mg/kg+ TZ	130 ± 3.53*^{b}	34.34
IX	MEIF 200mg/kg+ CP	114.6 ± 6.51**^{a}	42.12
X	MEIF 400mg/kg+ CP	96.2 ± 3.53**^{a}	51.41

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; N=6 in each group; Significance at****p<0.001,* ***P* < 0.01, **P*<0.05, Non Significance (ns) at *P* > 0.05 Vs negative control. Mean bearing same superscripts do not differ significantly. Mean bearing different superscript differ significantly.

Tail suspension test

The standard (imipramine 10mg/kg) and methanol extract alone (200 & 400mg/kg) treated groups, the peak values of immobility time significantly decreased to 35.2 ± 5.91, 62.9 ± 9.7 and 42.3 ± 7.8 when compare to control 145 ± 8.25. Thus, the both doses of methanol extract along with Ondansetran and terazosin significantly increases the immobility time 146.8± 5.66, 124.5 ± 11.94, 110.7 ± 5.51 and 110.7 ± 5.51 compare to extract alone treated groups but it shows no significant to negative control. There is no significant variation in extract with Chlorpromazine treated group immobility time compare to group III & IV. But it is reveals significant variation compare to negative control (Table 4).

Table 4: Effect of MEIF on Immobility time in Forced Swim test model in mice

Group	Treatment	Immobility time in seconds	% Inhibition
I	Negative control	145 ± 8.25^{b}	0
II	Positive control	35.2 ± 5.91***^{a}	75.72
III	MEIF 200mg/kg	62.9 ± 9.7**^{a}	56.62
IV	MEIF 400mg/kg	42.3 ± 7.8***^{a}	70.82
V	MEIF 200mg/kg+ OND	146.8± 5.66^{nsb}	-1.2
VI	MEIF 400mg/kg+ OND	124.5 ± 11.94^{nsb}	14.48
VII	MEIF 200mg/kg+ TZ	110.7 ± 5.51^{nsb}	24.13
VIII	MEIF 400mg/kg+ TZ	95.1 ± 6.53*^{b}	34.41
IX	MEIF 200mg/kg+ CP	76.4 ± 6.51*^{b}	47.31
X	MEIF 400mg/kg+ CP	61.5 ± 9.53**	57.58

Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; N=6 in each group; Significance at***p<0.001, **p < 0.01, *p<0.05, Non Significance (ns) at p > 0.05 Vs negative control. Mean bearing same superscript does not differ significantly. Mean bearing different superscript differ significantly.

DISCUSSION

The incidence of depression in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Despite the widely popular use of *Ichnocarpus frutescens* for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of different doses of the methanol extract of *Ichnocarpus frutescens* in mice was able to induce antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants [11]. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [12]. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [13].

Results showed that the administration of the MEIF produced a diminution of immobility time of mice exposed to the both forced swimming and tail suspension tests. In the present study, methanol extract of *Ichnocarpus frutescens* (200 and 400 mg/kg, po) administered to mice, produced significant antidepressant like effect in both TST and FST and both MEIF 200, 400mg/kg immobility time increasing with ondansetran and terazosin ($5HT_3$ antagonist and alpha adrenergic antagonist).

Data in the literature demonstrated that ondansetran and terazosin possibly act $5HT_3$ and α adrenergic receptors on the nerve terminals and inhibit the release/ block post neuron binding site/ inhibit the reuptake of 5HT and NA, which indicates that the plant extract exerts antidepressant effects by significantly modifying release of 5HT & NA in the terminals. It has been established that the shortening of immobility time in the forced swimming and the tail suspension tests depends mainly on the enhancement of central 5-HT and NA. There is a little effect was found due to the increase of dopamine also. Exact mechanisms underlying the antidepressant action might be due to

the extract act through the serotonergic and adrenergic neuron and increase the same. The action of the extract may due to presence of large number of phytochemicals in the MEIF. However, the antidepressant activity may be attributed to the presence of flavonoids, tannin, phenolic compounds, alkaloids and glycoside in the extract.

CONCLUSION

As per our results obtained from experiments performed on the mice were succeeded and showing very positive results. We believe that *Ichnocarpus frutescens* has the marked efficacy in the treatment of depression and other mood disorders.

From all the above findings, the present investigation suggests that the methanol extract of *Ichnocarpus frutescens* may possess antidepressant activity by inhibiting reuptake of Serotonin and noradrenaline or increase the release of the neurotransmitter nonselectively. Therefore lend pharmacological credence to the unclaimed 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 methanol extract of *Ichnocarpus frutescens.* Further investigation should be carried out to isolate and identify the chemical constituent which is responsible for its antidepressant activity.

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