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ANTIDEPRESSANT ACTIVITY OF CHLOROFORM EXTRACT OF *INDIGOFERA BARBERI* IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Depressive disorder is a prevalent psychiatric disorder, which affects 21% of the world population. The presently using drugs can impose a variety of side effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain and sleep disorder. During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders. Antidepressant activity of fractions of chloroform extract of *Indigofera barberi* was investigated by using Forced swimming test (FST) and Tail suspension test (TST) models. Imipramine was used as reference standard. It has been observed from our study that fraction D of chloroform extract of *Indigofera barberi* showed significant reduction in immobility in Forced swimming test (FST) and Tail suspension test (TST) models of depression comparable to Imipramine. Further, it is concluded that fraction D is responsible for antidepressant activity.

Key words: *Indigofera barberi*, Depression, Forced swimming test, Tail suspension test.

INTRODUCTION

Depression is considered as affective mood disorder, which is characterized by change in mood, lack of confidence, lack of interest in surroundings, has been estimated to affect 21% of world's population and it may range from mild to severe depression, which is called as psychotic depression. According to the World Health Organization report, approximately 450 million people suffer from a mental or behavioral disorder. Today depression is the leading cause of suicides. It is to be estimated that 3000 people lost their lives in each day and 1 million people lost their lives yearly due to suicide. Depression is the

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most prevalent disorder and the symptoms associated with depression changes the neurotransmitter levels in brain such as norepinephrine, serotonin and dopamine[1]. There are various synthetic drugs are available for the treatment of depression are selective serotonin reuptake inhibitors (SSRI) - Flouxetine, Fluvoxamine, Sertraline, Paroxetine, Tricyclic antidepressants (TCA) - Imipramine, Amitriptyline, Clomipramine, Desipramine, Doxepin, Monoamine oxidase inhibitors (MAOIs) - Selegiline, Atypical antidepressants - Bupropione, Duloxetine, Venlafaxine, Mirtazapine, Trazodone and many more drugs[2]. But with their effective treatment these drugs also associated with side effects such as sexual dysfunction[3], nausea[4], insomnia, mania[5], tremor[6], dystonia[7], dry mouth and hyprtention[8].

Indigofera barberi of Fabaceae family is high valued endemic herb of Tirumala Hills. Vernacularly known as Adavineelimanadu mokka. It is an under shrub grows up to 1 m tall. Its branchlets faintly angled. Leaves 3 foliolate, leaflets ovate-oblong, pubescent, obtuse, mucronate. Flowers pink in color arranged in axillary congested racemes. Pods sub-terete, deflexed, appressed, white-tomentose, sharply pointed. Seeds 2 to 4 in number. Flowering and fruiting season is September to December[9].

Leaf powder (5 g) is taken orally along with butter milk for controlling diabetes. Leaves (50 g), garlic (1 g) and pepper (1 g) made into paste and prepared pills of peanut size, 5 pills are taken once a day for 5 days to cure jaundice as prescribed by Nakkala and other tribal physicians. It is used as a dye and coloring agent. Whole plant powder (5 g) is taken along with rice washed water once a day for 10 days to expel intestinal worms and to cure several types of skin diseases and peptic ulcers[10]. Leaf juice is used as an antiseptic to cure wounds, cuts, burns and boils. Keeping these facts in view, the present study was undertaken to create a scientific base for the use of the fractions of chloroform extract of *Indigofera barberi* as an analgesic agent.

EXPERIMENTAL

Materials and methods

Plant material

The whole plant of *Indigofera barberi* was collected from the deciduous forest of Tirumala Hills in Andhra Pradesh, India. Samples were authenticated by Dr. K. Madhava Chetty, Plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, India. The whole plant of *Indigofera barberi* were sorted, cleaned and air-dried at room temperature for one week. By using the laboratory hammer mill, these were ground to powder. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of extracts

The powdered materials of *Indigofera barberi* (whole plant) were extracted successively each for 18 hrs with petroleum ether, ethyl acetate, chloroform, ethanol and distilled water in soxhlet apparatus. The extracts were concentrated to dryness in Rota evaporator till free from the solvents.

Acute toxicity studies

Acute toxicity studies were conducted using albino mice (20-25 g) for chloroform extract according to OECD guidelines. Healthy adult mice, starved overnight were divided into groups (n=5) and chloroform extract was orally administered at the doses of 100, 300, 500, 1000, 1500, 2000, 3000 mg/Kg body weight. Animals were observed for over 14 days for mortality and physical/behavioral changes.

Isolation of fractions

Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5 mm (TLC plates, Merck). The spots were visualized in UV light and 10% of H_2SO_4 in methanol. The chloroform extract was subjected to column chromatography (silica gel 60-100) for further purification. The column was equilibrated for one hour with petroleum ether at flow rate 5 mL/min. The sample (1 g dissolve in methanol) was loaded on to the column, 11 fractions were collected using Petroleum ether (100%), Petroleum ether: Ethyl acetate (4:1), Petroleum ether: Ethyl acetate (2:3), Petroleum ether: Ethyl acetate (3:2), Ethyl acetate (100%), Chloroform: Methanol (2:3), Chloroform: Methanol (3:2). Above yielded product were pooled into four fractions based on TLC. The yield and appearance of four fractions was fraction A 150 mg/g & yellow, fraction B 200 mg/Kg & dark brown greenish, fraction C 150 mg/g & light green and fraction D 300 mg/Kg & saffron.

Phytochemical analysis

Phytochemical analysis[11] of fractions was carried out for the presence of alkaloids, tannins, saponins, glycosides, terpenoids, carbohydrates, flavonoids, proteins, amino acids, fixed oils, steroids & sterols by different methods.

Animals

Albino rats of wistar strain weighing 150-200 g were taken. The rats were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25 ± 2°C and relative humidity of 55–65%. A 12 hrs light and dark schedule was maintained in the air conditioned animal house. All the rats were fed with common diets for 1 week after arrival and then

divided into groups with free access to food and water. All experimental animals were handled according to institutional and international guidelines guiding the use of experimental animals.

Study design

The rats were randomly allocated into ten groups of five rats for different experimental animal models.

Group I: Control (Received normal saline)

Group II: Imipramine (20 mg/Kg)

Group III: Fraction A (70 mg/Kg)

Group IV: Fraction A (150 mg/Kg)

Group V: Fraction B (70 mg/Kg)

Group VI: Fraction B (150 mg/Kg)

Group VII: Fraction C (70 mg/Kg)

Group VIII: Fraction C (150 mg/Kg)

Group IX: Fraction D (70 mg/Kg)

Group X: Fraction D (150 mg/Kg)

Test for antidepressant activity

Forced swim test (FST)

FST or behavior despair was proposed as a model to test for antidepressant activity by Porsolt and Bertin[12] Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15 cm height and maintained at 25^{o}C. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering standard and test to the groups as mentioned above.

Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru and Chemat[13] Depression was produced by suspending the

animal from the edge of a table 50 cm above the floor by an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

Statistical analysis

Results are presented as mean ± SEM. Data comparisons between treatments groups were done by use of one-way ANOVA followed by Dunnetts multiple comparison test. Values were considered statistically significant at $P < 0.05$, < 0.01 and < 0.001.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Phytochemical screening revealed the presence of alkaloids and carbohydrates in fraction A, saponins in fraction B, glycosides in fraction C and flavonoids in fraction D. The results are shown in Table 1.

Table 1: Preliminary phytochemical screening of chloroform extract of *Indigofera barberi*

Phytochemicals	Fraction A	Fraction B	Fraction C	Fraction D
Alkaloids	+	---	---	---
Tanins	---	---	---	---
Saponins	---	+	---	---
Glycosides	---	---	+	---
Terpinoids	---	---	---	---
Carbohydrates	+	---	---	---
Flavonoids	---	---	---	+
Proteins	---	---	---	---
Aminoacids	---	---	---	---
Fixed oils	---	---	---	---
Steroids & Sterols	---	---	---	---

Acute toxicity studies

Acute toxicity studies were carried by up-down regulation method. It was found that the extract at a limit dose from 1500 to 3000 mg/Kg is safe and does not show any mortality.

Forced swim test (FST)

The effect of fractions of chloroform extract of *Indigofera barberi* is fractions A, B and C did not produce significant reduction in immobility count of the rats. Oral administration of fraction D at a dose of 50 mg/Kg and 100 mg/Kg produced dose dependent decrease in the immobility duration. This reduction in immobility time was significant when compared with vehicle control. Imipramine was used as reference positive standard, significantly reduced the immobility time in FST. Percent decrease in immobility time at 90 mins with fraction D at 50 mg/Kg and 100 mg/Kg are 30.05% and 60.58% and Imipramine at 60 mins at 20 mg/Kg was 80.41%, respectively. The results are furnished in Table 2.

Table 2: Antidepressant effect of fractions of chloroform extract of *Indigofera barberi* by forced swim method

Treatment design	Reaction time					
	0 min	30 min	60 min	90 min	120 min	180 min
Normal saline	3.97 ± 0.11	3.86 ± 0.09	3.84 ± 0.08	3.91 ± 0.06	3.93 ± 0.05	3.95 ± 0.04
Imipramine (20 mg/kg)	3.83 ± 0.01	1.56 ± 0.02 (59.26)	0.75 ± 0.06 (80.41)	0.99 ± 0.09 (74.15)	1.32 ± 0.07 (65.53)	2.14 ± 0.10 (44.12)
Fraction A (50 mg/kg)	3.79 ± 0.02	3.56 ± 0.05 (6.06)	3.34 ± 0.02 (11.87)	3.09 ± 0.12 (18.46)	2.99 ± 0.09 (21.10)	3.23 ± 0.06 (14.77)
Fraction A (100 mg/kg)	3.79 ± 0.06	3.32 ± 0.11 (12.40)	2.76 ± 0.09 (27.17)	2.34 ± 0.08 (38.25)	2.11 ± 0.07 (44.32)	2.41 ± 0.06 (36.41)
Fraction B (50 mg/kg)	3.89 ± 0.03	3.62 ± 0.05 (6.94)	3.51 ± 0.06 (9.76)	3.16 ± 0.09 (18.76)	3.33 ± 0.06 (14.39)	3.56 ± 0.05 (8.48)
Fraction B (100 mg/kg)	3.81 ± 0.07	3.32 ± 0.07 (12.86)	3.23 ± 0.11 (15.22)	2.85 ± 0.13 (25.19)	2.98 ± 0.06 (21.78)	3.11 ± 0.09 (18.37)
Fraction C (50 mg/kg)	3.86 ± 0.04	3.56 ± 0.06 (7.77)	3.45 ± 0.07 (10.62)	3.38 ± 0.09 (12.43)	3.48 ± 0.08 (9.84)	3.51 ± 0.09 (9.06)

Cont…

Treatment design	Reaction time					
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Fraction C (100 mg/kg)	4.01 ± 0.11	3.46 ± 0.05 (13.71)	3.21 ± 0.07 (19.95)	3.03 ± 0.11 (24.43)	3.29 ± 0.11 (17.95)	3.36 ± 0.09 (16.20)
Fraction D (50 mg/kg)	3.96 ± 0.02	3.22 ± 0.06 (18.68)	3.02 ± 0.09 (23.73)	2.77 ± 0.12 (30.05)	2.98 ± 0.02 (24.74)	3.13 ± 0.04 (20.95)
Fraction D (100 mg/kg)	3.78 ± 0.05	2.56 ± 0.08 (32.27)	2.04 ± 0.06 (46.03)	1.49 ± 0.01 (60.58)	1.68 ± 0.11 (55.55)	2.43 ± 0.09 (35.71)

Results were analyzed by one way ANOVA using Dunnett's multiple comparison test:
Values were considered statistically significant at $P < 0.05$, < 0.01 and < 0.001

Tail suspension test (TST)

The effect of fractions of chloroform extract of *Indigofera barberi* is fractions A, B and C did not produce significant reduction in immobility count of the rats. Oral administration of fraction D at a dose of 50 mg/Kg and 100 mg/Kg produced dose dependent decrease in the immobility duration. This reduction in immobility time was significant when compared with vehicle control. Imipramine was used as reference positive standard, significantly reduced the immobility time in TST. Percent decrease in immobility time at 90 mins with fraction D at 50 mg/kg and 100 mg/Kg are 31.62% and 61.37% and Imipramine at 60 mins at 20 mg/Kg was 78.88%, respectively. The results are given in Table 3.

Table 3: Antidepressant effect of fractions of chloroform extract of *Indigofera barberi* by tail suspension method

Treatment design	Reaction time					
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Normal saline	4.87 ± 0.01	4.85 ± 0.02	4.89 ± 0.06	4.76 ± 0.05	4.79 ± 0.11	4.84 ± 0.11
Imipramine (20 mg/kg)	4.83 ± 0.02	2.21 ± 0.01 (54.24)	1.02 ± 0.03 (78.88)	1.68 ± 0.02 (65.21)	2.11 ± 0.09 (56.31)	2.65 ± 0.11 (45.13)
Fraction A (50 mg/kg)	4.76 ± 0.04	4.32 ± 0.05 (9.24)	3.99 ± 0.03 (16.17)	3.82 ± 0.05 (19.74)	4.28 ± 0.07 (10.08)	4.45 ± 0.09 (6.51)
Fraction A (100 mg/kg)	4.85 ± 0.07	4.09 ± 0.09 (15.67)	3.76 ± 0.02 (22.47)	3.64 ± 0.07 (24.94)	3.85 ± 0.12 (20.61)	3.99 ± 0.07 (17.73)

Cont…

Treatment design	Reaction time					
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Fraction B (50 mg/kg)	4.88 ± 0.05	4.39 ± 0.04 (10.04)	4.21 ± 0.04 (13.72)	3.97 ± 0.08 (18.64)	4.25 ± 0.06 (12.90)	4.56 ± 0.08 (6.55)
Fraction B (100 mg/kg)	4.91 ± 0.01	4.01 ± 0.05 (18.32)	3.84 ± 0.05 (21.79)	3.66 ± 0.02 (25.45)	3.75 ± 0.08 (23.62)	4.11 ± 0.09 (16.29)
Fraction C (50 mg/kg)	4.81 ± 0.06	4.28 ± 0.06 (11.01)	4.09 ± 0.05 (14.96)	3.85 ± 0.03 (19.95)	3.96 ± 0.09 (17.67)	4.11 ± 0.07 (14.55)
Fraction C (100 mg/kg)	4.75 ± 0.04	3.73 ± 0.09 (21.47)	3.41 ± 0.04 (28.21)	3.22 ± 0.07 (32.21)	3.47 ± 0.07 (26.94)	3.84 ± 0.05 (19.15)
Fraction D (50 mg/kg)	4.87 ± 0.06	3.95 ± 0.07 (18.89)	3.68 ± 0.06 (24.43)	3.33 ± 0.09 (31.62)	3.65 ± 0.08 (25.05)	3.91 ± 0.06 (19.71)
Fraction D (100 mg/kg)	4.79 ± 0.03	3.32 ± 0.07 (30.68)	2.42 ± 0.07 (49.47)	1.85 ± 0.11 (61.37)	2.03 ± 0.10 (57.62)	2.95 ± 0.08 (38.41)

Results were analyzed by one way ANOVA using Dunnett's multiple comparison test:
Values were considered statistically significant at $P < 0.05$, < 0.01 and < 0.001

The present study demonstrated the antidepressant activity of fraction D of chloroform extract of *Indigofera barberi* in FST and TST, a valid animal model for screening antidepressant drugs[14-20]. The test model of depression (forced swim test and tail suspension test) are based on the observation that rats or mice when forced to swim or suspended in a restricted space from which there is no possibility of an escape, eventually cease to struggle, surrendering themselves (despair or helplessness) to the experimental conditions. This suggested that, helplessness or despair behavior reflected a state of lowered mood in laboratory animals and could serve as a valuable test for screening antidepressant drugs[21]. The forced swimming induced state of immobility in animals claimed to represent a condition similar to human depression[22]. These models are widely accepted to screen antidepressants including tricyclics, selective serotonin re-uptake inhibitors, monoamine oxidase inhibitors[23]. In present study, fraction D was administered by oral route the mice at different dose levels. It significantly reduced the immobility time of mice in FST and TST compared to other fractions. This reduction in immobility time was found to be dose dependent in both the models. Plant sources can also be the effective alternative remedy to treat the depression is once again proved by our findings.

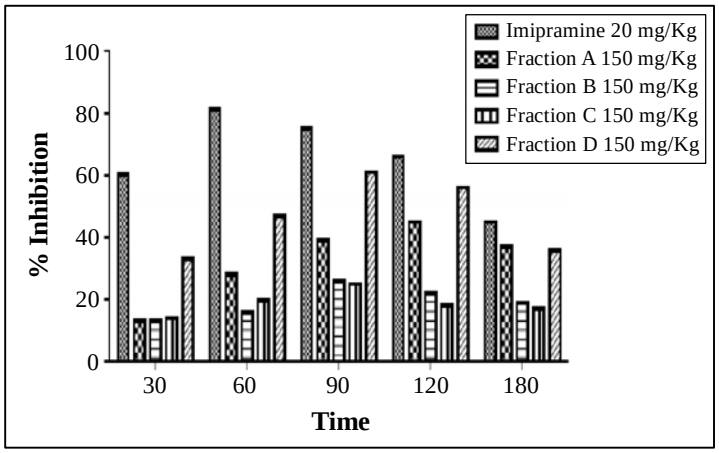

Fig. 1: Antidepressant effect of fractions of chloroform extract of *Indigofera barberi* by forced swim method

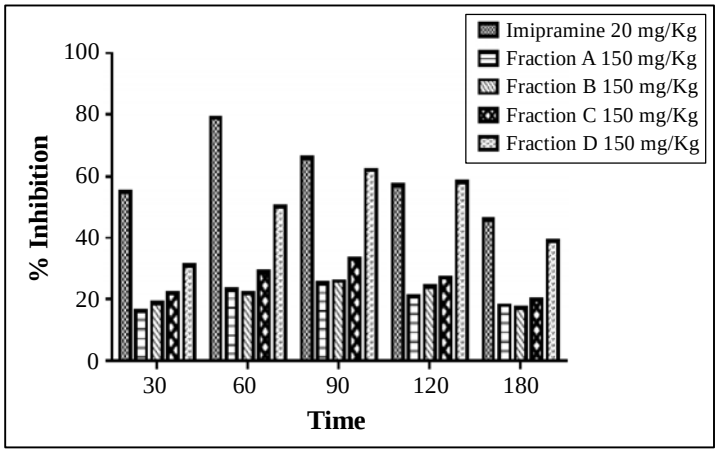

Fig. 2: Antidepressant effect of fractions of chloroform extract of *Indigofera barberi* by tail suspension method

CONCLUSION

Antidepressant drugs used for the depression treatment may cause side effects such as vomiting, nausea, irritation etc. To overcome this, natural medicines are used for treatment, which will have very less side effects. Secondary metabolites such as the flavonoids have been reported to be present in fraction D. Many flavones derivatives were found to be ligands for $GABA_A$ receptors in the CNS and thus they bind to the benzodiazepine binding site with resulting depressant actions in mice[24]. In conclusion, the

fraction D of chloroform extract of whole plant of *Indigofera barberi* demonstrated promising antidepressant property in the various animal models used in this study, confirming its efficacy. *Indigofera barberi* is one of the plant used in traditional a medicine, which was proved to possess antidepressant activity in rats in our present study. The result is similar to that of standard drug. However, further studies are needed to characterize the mechanism of the antidepressant effect of *Indigofera barberi* and extend these results before the safe application in humans.

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