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RESEARCH ARTICLE

Anxiolytic, Antidepressant and Anti-Inflammatory Activities of Methanol Extract of *Momordica Charantia* Linn Leaves (Cucurbitaceae)

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ABSTRACT

Methanol extract of dried leaves of *Momordica charantia* Linn (Cucurbitaceae) was investigated for anxiolytic, antidepressant and anti-inflammatory activities in animal models. Anxiolytic activity of methanol extract of dried leaves of *Momordica charantia* Linn was tested by elevated plus maze test. The results showed a significant anxiolytic effect comparable, with diazepam in all the tested doses. Behavior despair test was used to assess antidepressant activity of methanol extract of *M. charantia* Linn leaves. The extract treatment showed antidepressant effect by decreasing mobility time of subjected rats to forced swimming dose of 300 mg/kg extract, the swimming behaviour of the animals was comparable to the standard drug imipramine. The anti-inflammatory activity was studied by Carrageenin-induced edema in rats and 60 % oedema inhibitions was observed with 300 mg/kg methanol extract of dried leaves of *Momordica charantia* Linn, which was nearly equivalent to that of 10 mg/kg of indomethacin**.**

Keywords: *Antidepressants, Anxiolytic, Anti-inflammatory, Momordica charantia*

The plant *Momordica charantia* Linn (Family: Cucurbitaceae) is a well known plant and widely distributed and cultivated in many parts of India. It is known as bitter gourds in English, pavakay in Tamil, karela in Hindi and Bengali, Karke in Marathi, and Kaippa or kaippa-valli in Malayalam [1]. The fruit of the plant is widely used as vegetable as well as it is used in ayurvedic and unani system of medicines for the treatment of many diseases. The fruits and leaves of *M. charantia* Linn are useful in piles, leprosy, jaundice, diabetes, snake-bite and it is found to have vermifuge and antioxidant properties [2]. The earlier reports showed that the plant also has anti- malarial, anti-plasmodial properties [3, 4] and insecticidal activity against mustered saw fly [5]. The present study was carried out to investigate the anxiolytic, antidepressant and anti-inflammatory potential of methanol extract of dried leaves of *Momordica charantia* Linn.

MATERIALS AND METHODS

Plant material

The leaves of *Momordica charantia* Linn were collected from Tirunelveli District, Tamilnadu, India, during July 2005. The voucher specimens were identified and authenticated. The leaves of *Momordica charantia* Linn were dried in shade, pulverized by a mechanical grinder and passed through 40-mesh sieve to get the fine powder.

Preparation of extract

Coarsely powdered dry leaves of *Momordica charantia* Linn were successively extracted with 95% methanol in cold for 72 hour at room temperature. The whole extract was collected in a 5 liter conical flask, filtered, and the solvent was evaporated to dryness under reduced pressure. The yield of the prepared extract was 19.65%w/w.

Preliminary phytochemical group test

The preliminary phytochemical group test of the methanol extract of dried leaves of *Momordica charantia* Linn was performed by the standard methods [6-9].

Test for Alkaloids

Small quantity of the methanol extract of dried leaves of *Momordica charantia* Linn was treated with few drops of diluted hydrochloric acid, filtered and di-

Table 1. Preliminary phytochemical group tests for the methanol extract of leaves *Momordica charantia* Linn.

	Phytoconstituents	Methanol extract of leaves of *Momordica charantia* Linn
1	Alkaloids	+
2	Steroids	+
3	Triterpenoids	+
4	Amino Acids	+
5	Flavonoids	+
6	Gums	-
7	Reducing Sugar	+
8	Tannins	+
9	Saponins	+

-, Absence, +, Presence.

vided into four portions. The first portion was treated with Mayer's reagent and formation of yellowish buff colored precipitate indicated positive test for alkaloids. The second portion treated with Dragendroff's reagent and the development of orange brown precipitate shows the presence of alkaloids. The third portion was treated with Wagner's reagent and the formation of reddish brown precipitate suggested the presence of alkaloids. Final portion was treated with Hager's reagent and the development of yellowish precipitate demonstrated the positive presence of alkaloids.

Test for amino acids

Small amount of the methanol extract of dried leaves of *Momordica charantia* Linn was dissolved in a few milliliters of distilled water and treated with Ninhydrin at the pH range of 4 to 8. The formation of purple color suggested the presence of amino acids.

Test for flavonoids

Small quantity of the methanol extract of dried leaves of *Momordica charantia* Linn was dissolved in ethanol and was hydrolyzed with 10% sulphuric acid and cooled. Next, it was extracted with diethyl ether and divided into three portions in three separate test tubes. One ml of diluted sodium carbonate solution, 1ml of 0.1M sodium hydroxide solution and 1ml of diluted ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

Test for steroids and triterpenoids

The presence of *steroids and triterpenoids* in methanol extract of dried leaves of *Momordica charantia* Linn was confirmed through Libermann-Burchard reaction, by dissolving 10 mg of methanol extract of dried leaves of *Momordica charantia* Linn in 1 ml of chloroform and 1 ml of acetic anhydride and add 1-2 ml of concentrated sulphuric acid slowly. A reddish violet ring at the junction of the two layers confirmed the presence of triterpenoids and steroids.

Salkowski test was utilized to confirm the presence of steroids. Concentrated sulphuric acid was added to the chloroform solution of methanol extract of dried leaves of *Momordica charantia* Linn, appearance of reddish-blue color in the chloroform layer and green fluorescence in acid layer, suggested the presence of steroids.

Test for reducing sugar:

Aqueous solution of methanol extract of dried leaves of *Momordica charantia* Linn was prepared by dissolving sufficient quantity of methanol extract of dried leaves of *Momordica charantia* Linn in minimum amount of distilled water. The aqueous solution of extract was filtered and divided into several portions. Equal volume of Benedict's reagent was mixed with same portion of the aqueous extract in a test tube and heated for few minutes. Formation of brick red precipitate confirmed the presence of reducing sugars.

Equal volume Fehling's solution was added to the aqueous solution of extract in a test tube and heated for few minutes. Development of brick red color demonstrated the presence of reducing sugars.

Test for Gums:

The equal of volume of aqueous solution of extract and concentrated sulphuric acid were mixed and treated with Molish's reagent. Formation of red-violet ring at the junction of sulphuric acid layer and aqueous solution of extract indicated the presence of gums (Molish's test).

Test for Tannins:

The aqueous solution of extract was treated separately with 10% aqueous potassium dichromate solu-

Table 2. Anxiolytic effects of methanol extract of leaves of *Momordica charantia* Linn (MEMC) using elevated plus maze test.

Treatment	Dose (mg/kg)	Number of entries in open arm for 5 min	Time spent in open arm for 5 min (Sec)
Propylene glycol	5 ml kg^{-1}	1.6 ± 0.24	29.6 ± 3.18
Diazepam	4	6.2 ± 0.31	211 ± 13.8[a]
MEMC	100	3.2 ± 0.24[d]	24 ± 3.67[b]
MEMC	200	4.5 ± 0.60[a, c]	55 ± 11.5[b]
MEMC	300	5.5 ± 0.60[a, c]	98 ± 11.5[b]

n = 6; [a]P<0.001, [d]P<0.05 when compared to control, [b]P<0.001, [c]P<0.05 when compared to diazepam; Values are expressed as mean ± SEM. Data were analyzed using One way ANOVA followed by Tukey multiple comparison test.

Table 3. Antidepressant effects of methanol extract of leaves of *Momordica charantia* Linn (MEMC) on immobility using behavior despair test.

Treatment	Dose (mg kg^{-1})	Immobility time for 5 min (Sec)
Propylene glycol	5 ml kg^{-1}	111 ± 4.58
Imipramine	5	86 ± 6.41[a]
MEMC	100	41 ± 7.48[b,c]
MEMC	200	53 ± 4.16[b,c]
MEMC	300	65 ± 3.54[b,c]

n = 6; [a]P<0.05; [b]P<0.001 when compared to control; [c]P<0.001 when compared to imipramine; Values are expressed as mean ± SEM. Data were analyzed using One way ANOVA followed by Tukey multiple comparison test

tion, 5% ferric chloride solution and 10% aqueous lead acetate solution. Development of yellowish brown precipitate, greenish black color and yellow color precipitate, respectively, demonstrated the presence of tannins.

Tests for Saponins:

Small quantity of methanol extract of dried leaves of *Momordica charantia* Linn was dissolved in minimum amount of distilled water and shaken in a graduated cylinder for 15 minutes. Formation of stable foam suggested the presence of saponins.

Animals

Swiss albino male mice weighing 20-25g and albino Wister rats of either sex weighing 160-180g each were housed in standard metal cages at room temperature. They were provided with food and water *ad libitum.* The rats were allowed a one-week acclimatization period before the experimental sessions.

Anxiolytic activity

Elevated plus maze test

Elevated plus maze test was performed in five groups of six male Wistar rats, after 30 minutes of oral administration of 5 ml kg^{-1} of propylene glycol (vehicle control); 100, 200 and 300 mg kg^{-1} of methanol extract of *M. charantia* Linn leaves to the group I-IV respectively and intraperitoneal injection of 4 mg kg^{-1} of diazepam (drug control) to the last group of animals. Elevated plus maze test consists of a plus shaped maze, elevated 45 cm above ground level. It has two open (10 X 50 X 40 cm) arms. The test rat was placed in the central square area (10 X 10 cm) of the plus maze and time spends by the animals in open arm during a 5 min observation period was noted. Data for vehicle control, diazepam and different doses of the methanol extract (100, 200 and 300 mg kg^{-1}) of *M. charantia* leaves treated groups were compared [10].

Antidepressant activity

Behaviour despair test

Behaviour despair test was performed in five groups of 6 Swiss albino male mice. Propylene glycol as vehicle control (5 ml kg^{-1}); 100, 200 and 300 mg kg^{-1} of methanol extract of *M. charantia* Linn leaves were administered orally to the groups I to IV respectively and 5 mg kg^{-1} of imipramine (drug control) was administered intraperitonealy. Mice were forced to swim individually in a plexiglas cylinder (height 45 cm, diameter 20 cm) containing fresh water up to 15 cm for 15 min and the animals were observed for five minutes. In this test, after a brief spell of vigorous activity, animals show a posture of immobility which is characterized by floating motionless in the water making only those movements necessary to keep the head above the water. This immobility reflects the state of depression. Each mouse was subjected to this test 24 h prior and 1 h after administration of vehicle, extract and drug for 5 minutes in test session, and the duration of immobility during the last 3 minutes was recorded [11].

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

The rats were divided into five groups, each group consisting of six Wistar rats weighing 160-180g each. Oedema was induced by subplantar injection of 0.1 ml of freshly prepared 1% carrageenan suspension into the right hind paw of each rat. The paw volume was measured at 0 h and 3 h after injection of carrageenan by using a plethysmometer [12, 13]. The methanol extract of *M. charantia* Linn leaves at 100, 200 and 300 mg kg^{-1} were administered orally to first three groups of rats. While the fourth and fifth group of animals received 5 ml kg^{-1} propylene glycol as vehicle control or 10 mg kg^{-1} indomethacin as drug control respectively, for comparative pharmacological assessment [14]. Drug pretreatment was given 1 h before the injection of carrageenan. The percentage inhibition of oedema was cal-

Table 4. Anti-inflammatory effect of methanol extract of leaves of *Momordica charantia* Linn (MEMC) on Carrageenin induced rat paw oedema.

Treatment	Dose (mg kg^{-1})	Increase in paw volume (ml)	% inhibition of paw volume
Propylene glycol	5 ml kg^{-1}	0.72 ± 0.03	-
Indomethacin	10	0.24 ± 0.02	66.66
MEMC	100	0.42 ± 0.04*	41.66
MEMC	200	0.36 ± 0.04*	50.00
MEMC	300	0.28 ± 0.04*	61.11

* p<0.001 when compared to control; Values are expressed as mean ± SEM; Data were analyzed by using Student *t*- test.

culated by using the following formula:

$V_c - V_t / V_c X 100$

Where, Vc = The average increase in paw volume of control.

V_t = The average increase in paw volume after the administration of test and standard drug.

Statistical analysis

The results were expressed as mean ± S.E.M. The significance of results was evaluated by one way ANOVA followed by Tukey multiple comparison test and by Student's t-test [15].

RESULTS

The preliminary phytochemical group tests were performed by the standard protocol [6-9] and the results are presented in Table 1. The results showed the presence of steroids, triterpenoids, alkaloids, flavanoids, tannins, amino acids, reducing sugar and saponins in methanol extract of *M. charantia* Linn leaves.

Elevated plus maze test

Dose dependent anxiolytic activity was observed with methanol extract of *M. charantia* Linn and the results are presented in Table 2. The methanol extract and diazepam treated animals showed more number of entries and time spent in open arm compared to control group of animals. The effect of the extract treated group of animals was significant at higher dose level and it can be compared well with diazepam.

Behaviour despair test

The results of the antidepressant effect of methanol extract of *M. charantia* leaves, presented in Table 3, revealed that the mobility time was significantly decreased and the extract treated rats are subjected to force swimming. The effect of the leaf extract at 300 mg kg^{-1} is comparable well with imipramine at the doses used.

Carrageenan-induced rat paw oedema

The effect of methanol extract of leaves of *Momordica charantia* Linn on carrageenin induced oedema in rats as shown in Table 4 revealed that the oedema suppressant was 61 percent at 300 mg kg^{-1}, which was nearly equivalent to that of 10 mg kg^{-1} of the standard drug indomethacin. The anti-inflammatory effect was significant (p<0.001) in the dose of 100, 200 and 300 mg kg^{-1} of methanol extract, when compared to the control group (Table 4).

DISCUSSION AND CONCLUSION

Modern day life style leads to numerous stress conditions, among which anxiety and depression are general and widely prevalent senile neurological disorders. The methanol extract of leaves of *Momordica charantia* Linn under study, at the tested doses, exhibited anxiolytic and anti depressant actions. The anxiolytic activity of novel substances including herbal remedies is generally assessed by using elevated plus-maze test. Oral administration of different doses (100, 200 and 300 mg kg^{-1}) of a methanol extract of *M. charantia* Linn was able to increase significantly the number of entries to open arms and time spent in open arm compared to control group of animals [16]. The data was highly significant compared with that produced by the diazepam, the standard anxiolytic drug.

The widely used animal models for assessing antidepressant like activity in small animals are forced swimming test and tail suspension test. It is expected that immobility occurs in these two tests will reflect a state of behavioral despair or unable to adapt the stress as seen in human [17, 18]. The basic concept of forced swimming test is animal will get immobile posture when subjected to the short-term or inescapable stress. The methanol extract of *M. charantia* Linn at the dose of 300 mg kg^{-1} produced significant antidepressant effect in forced swimming test, as is evident from the reduction in the immobility time and the effect was comparable to the standard drug, Imipramine. Numerous neural pathways are involved in the pathophysiology of depression and anxiety states. Therefore, a great number of neurotransmitters are thought to involve in underlying mechanisms of these diseases, as evident by the anxiolytic and antidepressant drugs [19].

The effective anti-inflammatory activity was observed with extract treated animals for three hour measurement. The methanol extract of *M. charantia* Linn at 300 mg kg^{-1} showed 61% reduction in oedema which was nearly equivalent to that of 10 mg kg^{-1} of the standard drug indomethacin. The most widely used primary test to screen new anti-inflammatory agent's measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent [12]. Carrageenin-induced paw oedema was taken as a prototype of exudative phase of inflammation. This oedema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins [20]. The development of oedema in the rat paw after the injection of carrageenan has been described as a biphasic event [21]. The initial phase starts immediately after injection and reduces within 1 h, is attributed to the release of histamine and serotonin [22]. The second phase of swelling which begins at 1 h and remains through 3 h, is due to the release of prostaglandin-like substances [18]. It has been reported that the second phase of oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. Generally NSAIDs strongly inhibit the second phase of carrageenan-induced edema while some others are inhibit the both phases. Indomethacin seems to block both phases [21, 23].

The significant anti-inflammatory effect shown by the methanol extract of *Momordica charantia* Linn leaves may be due to inhibition of prostaglandin-like substances. The anoxilytic, antidepressant and anti-inflammatory activities of methanol extract of *M. charantia* Linn may be due to a combination of different biologically constituents rather than any single compound, being the most interesting the alkaloids, the ster-

oids, the flavonoids and the triterpenoids. The presence of steroids, triterpenoids, alkaloids, and flavanoids was confirmed by the preliminary pytochemical analysis. Recent reports have also indicated that many flavonoids possess anti-inflammatory activity [24, 25]. In conclusion, the present study demonstrates that the leaf extract of *Momordica charantia* Linn can produce significant anoxilytic, antidepressant and anti-inflammatory activities. However, to know the exact mechanism by which *M. charantia* leaf extract produce these effects is a subject of further study.

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