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Article

Anxiolytic effects of *Dolichandrone falcata* Seem., Bignoniaceae, stem-bark in elevated plus maze and marble burying test on mice

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RESUMO: "Efeitos ansiolíticos das cascas de *Dolichandrone falcata* Seem., Bignoniaceae, em teste do labirinto em cruz elevada e teste de esconder esferas, em camundongos" *Dolichandrone falcata* Seem., Bignoniaceae, é uma árvore do tipo decidua, comumente conhecida como "Medshingi" na da região Toranmal de Maharashtra, na Índia. Uma pasta da casca é aplicada em fraturas ou luxação dos ossos e usada como veneno de peixe; o suco da casca é usada em casos de menorragia e leucorréia. Das folhas da planta foi isolado crisina-7-rutinoside. O presente estudo foi realizado para investigar os efeitos ansiolíticos do extrato metanólico (DFBM), acetato de etila (DFBEA) e compostos isolados DFB (V + VI) de *D. falcata* utilizando a casca do tronco em modelos animais. Os efeitos ansiolíticos foram estudados por labirinto em cruz elevada (EPM) e o ensaio de esconder esferas de mármore (MBT). Os extratos bruto e seco DFBM DFBEA foram preparados em doses de 100, 200 e 400 mg/kg, enquanto que o composto DFB (V + VI) foi preparado em doses de 50, 100 e 200 mg/kg e foram administrados em camundongos para avaliação da atividade ansiolítica. DFBEA 400 and DFB (V+VI) 200 mg/kg produziram efeitos ansiolíticos significantes (p<0.01), de maneira dose-dependente, por aumentar o tempo despendido e o número de entradas nos braços abertos do EPM e por diminuir o número de esferas escondidas pelos camundongos no teste do MBT. Este estudo mostrou que os extratos DFBM, DFBEA e o composto isolado DFB (V + VI), contendo flavonóides com potencial farmacológico (como a crisina) podem ser o responsável pela atividade ansiolítica.

Unitermos: *Dolichandrone falcata,* Bignoniaceae, efeito ansiolítico, benzodiazepínicos, Labirinto em cruz elevada, Marble burying, Diazepan, Fluoxetina.

ABSTRACT: *Dolichandrone falcata* Seem., Bignoniaceae, is a deciduous tree commonly known as Medshingi in local areas of Toranmal region of Maharashtra, India. Its bark paste is applied on fractured or dislocated bones, used as a fish poison; bark juice is used in cases of menorragia and leucorrhoea. The leaves of the plant have afforded chrysin-7-rutinoside. The present study was carried out to investigate the anxiolytic effects of methanol extract (DFBM), ethyl acetate extract (DFBEA) and isolated compound DFB (V+VI) of *D. falcata* stem-bark using animal models. Anxiolytic effects were studied by elevated plus maze (EPM) and marble burying test (MBT) assay. The crude dried DFBM and DFBEA extract was prepared in doses of 100, 200 and 400 mg/kg whereas DFB (V+VI) compound was prepared in doses of 50, 100 and 200 mg/kg and were administered orally to mice for evaluation of anxiolytic activity. DFBEA 400 and DFB (V+VI) 200 mg/kg produced highly significant (p<0.01) anxiolytic effects in dose dependent manner by increasing the time spent on and the number of entries into the open arms of the EPM and by decreasing the number of marbles buried by mice in MBT test. This study showed that the DFBM, DFBEA extracts and DFB (V+VI) isolated compound possesses potential pharmacological active constituents flavonoids (like chrysin) which may be responsible for the anxiolytic activity.

Keywords: *Dolichandrone falcata,* Bignoniaceae, anxiolytic effect, benzodiazepine, Elevated plus maze, Marble burying, Diazepam, Fluoxetine.

INTRODUCTION

Anxiety disorders are the most common mental illness in the world and became a very important area of research interest in psychopharmacology. Benzodiazepines are among the first line of drugs that have been extensively used for the last 45 years to treat several forms of anxiety (Jordan et al., 1996; Lader &

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Morton, 1991).

Although benzodiazepines have well-known benefits, their side effects are prominent, including sedation, muscle relaxation, anterograde amnesia and physical dependence (Kaplan & Sadock, 2005). However, the fact that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects (Griffiths et al., 1987). Thus, there is a need of robust anxiolytic compounds that have lesser side effects than benzodiazepines and an immediate onset of action than currently available 5-HT1A receptor acting drugs (Trabera & Glaser, 1987).

Dolichandrone falcata Seem., Bignoniaceae, is a deciduous tree commonly known as Medshingi in local areas of Toranmal region of Maharashtra, India. It occurs as a tree and shrub small to medium sized, 6 to 15 m in height, frequently in hill forest, occasionally seen in dry scrub forests. Sometimes growing on hedges of cultivated fields. Its bark paste is applied on fractured or dislocated bones (Patil, 2003). The bark is used as a fish poison. A decoction of the fruits is being used to procure abortion (Asia, 2009). Bark juice is used in cases of menorrhagia, leucorrhoea (Vidyasagar & Prashantkumar, 2007). The leaves of the plant *D. falcata* have afforded chrysin-7-rutinoside (Subramanian et al., 1972). Chrysin is a flavone widely distributed in plants, which was reported to have many different biological activities including anti-oxidant, anti-allergic, anti-inflammatory, anti-cancer, antiestrogenic and anxiolytic activities (Shin et al., 1999). Despite those traditional claims and successful isolation of the bioactive compounds, no in-depth scientific study has been performed on *D. falcata* stem-bark pharmacological properties. Thus, the aim of the present study was to determine anxiolytic properties of the DFBM, DFBEA extract and DFB (V+VI) isolated compound of *D. falcata* stem-bark using two animal models of anxiety.

MATERIALS AND METHODS

Plant material

Dolichandrone falcata Seem., Bignoniaceae (Figure 1), stem-bark was collected during the month of May from Toranmal Hills of Satpuda region in Maharashtra, India. The plant was identified and authenticated by Dr. PG Diwakar, Joint Director, Botanical Survey of India, Pune, and a voucher specimen of the sample (vishal 1) was deposited in the Herbarium collection at department. The stem-bark was cleaned, dried in the shade, and then powdered in a pulverizer. The sample was stored in an airtight container.

Figure 1. *Dolichandrone falcata* Seem., Bignoniaceae. A. Whole tree; B. flower, fruit and stem-bark.

Preparation of extract

Methanol extract (DFBM)

The dried stem-bark powder (1 kg) was extracted with (4x 3 L) of methanol by continuous hot extraction using Soxhlet extractor. The methanol containing extract was filtered and distilled on a water bath. The resulting methanol extract solution was concentrated in vacuum using a Rotavapor to obtain a greenish brown powder corresponding to 13% of the original powder (DFBM; yield 130 g).

Ethyl acetate extract (DFBEA)

Twenty grams of the methanol extract was suspended in water (500 mL) and fractionated with *n*-hexane (3x 800 mL), chloroform (4x 600 mL), ethyl acetate (4x 500 mL) and *n*-butanol (4x 500 mL), successively. Evaporation of each solvent yielded *n*-hexane (1 g), chloroform (7 g), ethyl acetate (4 g), and *n*-butanol (2.4 g) extracts (Sinaphet et al., 2006). The ethyl acetate (DFBEA) extract was used for the pharmacological screening.

Isolation and TLC of DFB (V+VI)

The method described by Sinaphet et al. (2006) with slight modifications was used for isolation of DFB (V+VI) compound. Total methanol extract (DFBM) was defatted with *n*-hexane, aqueous part *i.e*. extract remained after defatting was taken and subjected to column chromatography. By using column chromatography, 115 fractions of 30 mL were collected using ethyl acetate:methanol as a mobile phase. Fractions were grouped according to their TLC profile and grouped from DFB-I to DFB-XI. From this, DFB-V and DFB-VI were grouped due to similarity between their thin layer chromatography (TLC) profiles. Both grouped fractions were subjected to preparative thin layer chromatography (PTLC) that gave three prominent bands that were scrapped and separated further for TLC and purification. A middle band (crystalline powder, brownish in color) DFB (V+VI) was then purified and analyzed by TLC. This powder was evaluated by R_f value by TLC method using various mobile phases that are frequently used for detection and identification of chrysin (flavonoid) (Lleana et al., 2004).

Standard drugs/chemicals solutions

Diazepam and Fluoxetine were used as standard drugs for EPM and MBT methods respectively.

Phytochemical Screening

Phytochemical nature of extracts was analyzed by using following methods. The presence of alkaloid was detected by using Dragendroff, Mayer's, Hager's and Wagner's reagents. Since glycosides, presence was detected by using different chemical test like general chemical test, test for flavonoids (Shinoda and lead acetate), Keller-Kiliani test, Borntraeger test, and frothing test for saponins. Whereas for steroids detection Liberman-Burchard and Salkowski tests were pereformed. Phenols presence was also evaluated by using different tests such as $FeCl_3$, lead acetate and gelatin tests. Finally Molisch and Fehlings tests were conducted for presence of carbohydrate content (Gokhale et al., 2008).

Pharmacological studies

Test animals

Adult Swiss albino mice weighing 20-25 g of either sex, obtained from Institute of Animal Health and Veterinary Biological Products, Mhow, India, were used for behavioral experiments. Animals were housed in acrylic cages (45×60×25 cm) with water and food available *ad libitum* under an artificial 12 h light/dark cycle (light on at 7:00) and at a constant temperature (22±2 °C). Mice were housed in the departmental room for one week before testing to ensure adaptation to the new environment. All of the behavioral experiments were performed between 10:00 am to 17:00 pm. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: RCPIPER/IAEC/2008-09/13) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Acute toxicity test

Acute toxicity tests were performed according to OECD-2006 guideline no. 425. Animals were weighed and marked, a single high dose of DFBM and DFBEA as recommended by the OECD guidelines was administered to the first animal. After single administration, animals were observed for the sign of toxicity up to 24 h. If mortality was observed, one-step lesser dose of the previous one was given to the next animals. If the animal survived, the same dose was given to the next five animals. Further, all the animals were observed for the presence of signs of toxicity and mortality for fourteen days. The body weights of the animal were also recorded. Additional observations like changes in skin, eyes and mucous membranes, and respiratory circulatory, autonomic and central nervous system and behavior pattern were performed. Attention was also given to observed precipitation of tremors and convulsions. The LD50 of the test drug was calculated using a computer assisted statistical programme-AOT425statPgm (OECD 2006).

Elevated plus maze test (EPM)

The EPM for mice (Lister, 1987) was used with few modifications and designed as two perpendicular open arms (16×5 cm) and two closed arms (16×5×12 cm) in a perpendicular position. The open and closed arms were connected by a central platform (5×5 cm). The platform and the lateral walls of the closed arms were made of transparent acrylic. The floor was made of black acrylic. The maze was 25 cm above the floor. One hour after oral treatment, the animal was placed at the centre of the plus maze with its nose in the direction of one of the open arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The plus maze was carefully cleaned with a wet towel after each animal test. The time of permanence measures the time spent by the animal in the open and closed arms. Anxiolytic compounds reduce the natural animal's aversion to the open arms and promote the exploration thereof. On the other hand, the forced or voluntary passages of the animal into the open arms of the elevated plus maze are associated with hormonal and behavioral changes indicative of increased anxiety. The mice were

divided into eleven groups (six animals/group). Standard drug Diazepam (1 mg/kg, *p.o*.) used as the positive control and carboxy methyl cellulose (CMC) (0.5%) was used as vehicle control. The CMC was given to vehicle control group and the dosage volume of which was equivalent to volume of dose administered to treatment groups. *Dolichandrone falcata* stem-bark extracts DFBM, DFBEA at doses of 100, 200, and 400 mg/kg, *p.o*., and DFB (V+VI) isolated compound at dose of 50, 100, 200 mg/kg was administered in remaining nine groups. After each trial, the EPM apparatus was wiped clean with alcohol (70%) solution.

Marble burying test (MBT)

The marble burying test was conducted as described by Yamada et al. (2002) with slight modifications. Individual mice were placed for 30 min in a plastic cage with the same dimensions as their home cage and that contained sawdust as a bedding material; they were then returned to their home cages (habituation trial). Twenty four clear glass marbles (diameter, 15 mm) were spaced 3 cm apart in four rows of six, on approx. 5-cm layer of sawdust bedding which was lightly pressed down to make a flat even surface, in a plastic habituation cages used for each individual test. Mice were reintroduced to these cages (each test mouse was returned to the same cage in which they had been habituated). After 30 min, the test was terminated, and the number of buried marbles (marbles more than 2/3 covered with bedding materials) was counted. After each trial, the sawdust was replaced, and the test apparatus and glass marbles were washed by water and cleaned with 70% alcohol (Broekkamp et al., 1986; Njung'e & Handley, 1991; Friede & Freudenstein, 2002). All the doses of test extracts were same as used in EPM test except Fluoxetine (10 mg/kg, *p.o.*) which was used as standard drug for positive control. Since group treated with vehicle, CMC (0.5%) was taken as negative control.

Data statistical analysis

The statistical analysis of all the results were carried out using one-way ANOVA followed by Dunnett's multiple comparison test. p values <0.05 were considered statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical analysis of *Dolichandrone falcata* stem-bark extract indicates that methanol extract (DFBM) contains flavonoids, phenols, glycosides and carbohydrates. Whereas ethyl acetate extract (DFBEA) shows presence of flavonoids.

Isolation and TLC of DFB (V+VI)

The crystalline brown powder, which obtained as a isolated compound DFB (V+VI) was found similar to chrysin. In addition, the R_f value of this compound showed similarity between previously reported mobile phases for chrysin by using TLC (Table 1) (Lleana et al., 2004).

Acute toxicity test

Dolichandrone falcata stem bark extracts did not produce any mortality even at the dose of 5000 mg/kg, *p.o*., thus it was found to be non-toxic. On the basis of above results, three doses (100, 200, 400 mg/kg, *p.o*.) of *D. falcata* were selected for further pharmacological studies. The results showed no clinical signs and mortality of the animal therefore an LD50>5000 mg/kg body weight might be assumed.

Effect of *D. falcata* on the Elevated Plus-Maze (EPM)

In the elevated plus maze test, our results show that similarly to diazepam which increased the time spent in the open arms, all the extracts of *D. falcata* stem bark in the doses of 100, 200 and 400 mg/kg also increased this parameter (Table 2). The effect was dose-dependent and highly significant at the dose of 400 mg/kg for DFBM and DFBEA extract, while for DFB (V+VI) isolated compound all the doses *i.e.* 50, 100 and 200 mg/kg showed highly significant (p<0.01) increase in time spent in open arms in a dose dependent manner when compared to the control group. Furthermore, significant increases in the number of entrances in the open arms were also observed with DFBEA (400 mg/kg) and DFB (V+VI) (100 and 200 mg/kg) treatment groups as compared to control (Table 3). The time spent in closed arm was also observed and DFBM, DFBEA and DFB (V+VI) showed significant (p<0.01) decrease in this parameter (Table 4).

Marble-Burying Test (MBT)

In MBT, the effects of the representative of *D. falcata* stem-bark extracts and isolated compound were studied on marble burying behavior of test animals. DFBM extract reduced number of marbles buried by mice in dose dependent manner. Whereas DFBEA extract (200, 400 mg/kg) and DFB (V+VI) isolated compound (50, 100 and 200 mg/kg) showed highly significant (p<0.01) effects by reducing number of marbles buried by mice as compare to vehicle control group. As expected, positive control Fluoxetine (10 mg/kg, *p.o.*) exhibited significant decrease in the marble burying behavior of test animals (Table 5).

DISCUSSION

There has been a considerable popular interest

Table 1. Co-TLC of compound DFBV+VI with chrysin R_f values, using various mobile phases.

Sr. No.	Mobile phase	Ratio (volume/volume)	No. of separated compounds	R_f value	
				Chrysin (standard)	DFB (V+VI) compound
1.	Acetonitrile	-	1	0.90	0.90
2.	Ethyl acetate	-		-	-
3.	Ethyl acetate:methanol	5:5	3	0.50	0.21, 0.50, 0.75
4.	Hexane:ethyl acetate	7:3	5	0.87	0.12, 0.30, 0.50, 0.72, 0.87

Table 2. Effect of oral administration of *Dolichandrone falcat*a stem-bark extracts on time spent (Seconds) in open arm in elevated plus maze.

Treatment	Control	DFBM	DFBM	DFBM	DFBEA	DFBEA	DFBEA	DFB (V+VI)	DFB (V+VI)	DFB (V+VI)	STD. (Diazepam)
Dose (mg/kg)	-	100	200	400	100	200	400	50	100	200	1
1	2	2	6	10	3	4	8	12	12	14	15
2	1	2	2	7	4	2	9	11	14	15	14
3	3	1	1	6	2	5	5	13	15	17	18
4	2	1	1	8	4	7	7	10	14	16	16
5	1	2	4	9	1	2	8	9	13	15	19
6	4	1	3	11	5	5	11	12	16	18	20
Average	2.2±0.48	1.3±0.33	2.8±0.79	8.5±0.76**	3.2±0.60	4.2±0.79	8.0±0.82**	11±0.60**	14±0.58**	16±0.60**	17±0.97**

Values represent mean±SEM, n=6. One way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01 compare with control group.

Table 3. Effect of oral administration of *Dolichandrone falcata* stem-bark extracts on number of entries in open arm in elevated plus maze.

Treatment	Control	DFBM	DFBM	DFBM	DFBEA	DFBEA	DFBEA	DFB (V+VI)	DFB (V+VI)	DFB (V+VI)	STD. (Diazepam)
Dose (mg/kg)	-	100	200	400	100	200	400	50	100	200	1
1	0	1	0	1	1	2	2	1	2	1	4
2	0	0	1	1	1	1	2	0	1	1	1
3	1	0	1	1	2	2	1	1	2	2	3
4	0	1	0	0	0	0	2	0	2	4	5
5	0	0	2	2	1	4	3	1	3	2	4
6	0	1	0	0	2	0	1	1	2	3	5
Average	0.17±0.17	0.50±0.22	0.67±0.33	0.83±0.31	1.2±0.31	1.5±0.62	1.8±0.31*	0.67±0.21	2.0±0.26**	2.2±0.48**	3.7±0.61**

Values represent mean±SEM, n=6. One way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01 compare with control group.

Table 4. Effect of oral administration of *Dolichandrone falcata* stem-bark extracts of on time spent (Seconds) in closed arm in elevated plus maze.

Treatment	Control	DFBM	DFBM	DFBM	DFBEA	DFBEA	DFBEA	DFB (V+VI)	DFB (V+VI)	DFB (V+VI)	STD. (Diazepam)
Dose (mg/kg)	-	100	200	400	100	200	400	50	100	200	1
1	298	273	249	237	284	291	220	271	240	217	175
2	295	275	250	215	273	264	246	210	208	198	187
3	275	270	247	230	250	270	264	215	219	189	200
4	284	260	239	240	270	267	242	199	223	210	208
5	295	264	253	226	266	231	214	228	205	206	197
6	270	271	258	234	274	230	220	240	224	220	192
Average	290±4.8	270±2.3	250±2.6**	230±3.7**	270±4.6	260±9.8*	230±8.0**	230±11**	220±5.1**	210±4.8**	190±4.7**

Values represent mean±SEM, n=6. One way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01 compare with control group.

Table 5. Effect of oral administration of *Dolichandrone falcata* stem-bark extracts of on number of marble burying in mice.

Treatment	Control	DFBM	DFBM	DFBM	DFBEA	DFBEA	DFBEA	DFB (V+VI)	DFB (V+VI)	DFB (V+VI)	STD. (Fluoxetine)
Dose (mg/kg)	-	100	200	400	100	200	400	50	100	200	10
1	21	18	21	18	22	3	11	12	1	1	1
2	22	19	22	14	18	7	8	6	6	5	2
3	20	21	20	17	12	17	7	15	4	3	2
4	23	20	18	19	20	8	13	3	9	4	1
5	22	24	16	14	18	16	9	5	4	3	0
6	20	16	12	15	12	14	8	5	6	0	1
Average	21±0.49	20±1.1	18±1.5	16±0.87	17±1.7	11±2.3**	9.3±0.92**	7.7±1.9**	5.0±1.1**	2.7±0.76**	1.2±0.31**

Values represent mean±SEM, n=6. One way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01 compare with control group.

in the use of the so-called natural remedies, or herbal products, to treat anxiety and depression. Recently, several plants have been reported to possess anxiolytic effects in different animal models of anxiety. Various traditional herbal medicines have also been suggested to possess anxiolytic activity. Some herbs such as St. John's wort and ginseng have been introduced in the clinic for the treatment of anxiety (Friede & Freudenstein, 2002; Bhattacharya & Mitra, 1991; Park et al., 2005; Cha et al., 2005). The elevated plus-maze is one of the many tests for the identification of anxiolytic or anxiogenic effect of a drug in rodents (Pellow et al., 1985). Several plants increase the exploration of open arms in the elevated plus-maze test and are used to diminish anxiety in folk medicine.

Effects of diazepam on mice anxiety behavior in EPM

The elevated plus-maze is currently one of the most widely used animal model of anxiety, having been employed by many research laboratories in the last decade and has been extensively validated for use in both rats and mice (Lister, 1987, Espejo, 1997). Its validity in our study was supported by the observation that diazepam, a classic anxiolytic, significantly increased the time spent in the open arms. The classic anxiolytic benzodiazepine, diazepam 10 mg/kg was used as positive control. These results confirm the suitability of the method used in the present study.

Effects of DFBM, DFBEA extracts and DFB (V+VI) isolated compound on the EPM

In the present study, DFBM extract does not produce open arm exploration significantly at all administered doses, since we have demonstrated that DFBEA extract and DFB (V+VI) isolated compound, following acute oral administration, produced a dose-dependent anxiolytic-like effect in mice as measured by an increased open arm exploration in the EPM.

Searching for safer BDZ-receptor (BDZ-R) ligands it has been demonstrated the existence of a new family of ligands that have a flavonoids structure, first isolated from plants used as tranquilizers in folkloric medicine. Some of those compounds, such as 6,3-dinitroflavone were found to have a very potent anxiolytic effect (Wolfman et al., 1996). These compounds exhibit a high affinity for the benzodiazepine receptors. Due to their selective pharmacological profile and low intrinsic efficacy at the benzodiazepine receptors, flavonoids derivatives, such as those described, could represent an improved therapeutic tool in the treatment of anxiety.

Since DFBEA dose up to 400 mg/kg was taken to clarify anxiety effects of flavonoids in EPM model. Many flavonoids were found to be ligands for the γ-aminobutyric acid type A (GABAA) receptors in the central nervous system (CNS); which led to the hypothesis that they act as benzodiazepine-like molecules. Their behavioral effects in animal models of anxiety, sedation and convulsion (Marder & Paladini, 2002; Paladini et al., 1999) support this. Some members of the family of flavonoids have been demonstrated to have moderate binding affinities for the benzodiazepine-site. *In vivo* studies revealed that these compounds were mostly partial agonists of $GABA_A$ receptors, and only a few flavonoids were shown to possess antagonistic activities (Wang et al., 2005). Considering some plant species, Wolfman et al. (1994) have postulated that chrysin, a natural monoflavonoid, is a ligand for central benzodiazepine receptors. A major problem of anxiolytic compounds is that their anxiolytic activity cannot be easily separated from sedation (Costa & Guidotti, 1996; Atack, 2003). At high doses, for example, diazepam starts to reduce the activity of the mice, as hinted at by the significantly reduced unpunished licks in the Vogel conflict test, a parameter related to locomotor activity (Nazar et al., 1997; Kennett et al., 1998). The anxiolytic-like effect of DFBEA extract observed in the present study seems not to be associated with any motor effects, since no significant behavior change of mice was observed in the open field. This leads to the assumption that the anxiolytic-like effect of *Dolichandrone falcata* stem bark extracts is selective without producing benzodiazepine-like side effects such as sedation, muscle relaxation or ataxia.

Using this test the DFBEA extract and DFB

(V+VI) isolated compound increased the time spent and number of entries in the open arms similarly to diazepam. These results are suggestive that DFBEA extract and DFB (V+VI) isolated compound have an anxiolytic-like effect in the plus-maze test.. Our data represents that DFBEA (ethyl acetate) extract of plant has a highly significant anxiolytic effect and isolated compound DFB (V+VI), which may be flavonoid chrysin that showed a very highly potent anxiolytic effects similar to diazepam.

Effects of DFBM, DFBEA and DFB (V+VI) on the marble burying test (MBT)

DFBM extract does not decreased number of marbles buried by mice significantly, while DFBEA extract and DFB (V+VI) isolated compound decreased the number of marbles buried. This activity was similar to marble burying which was reduced by acute administration of different classes of antidepressants with slow-onset anxiolytic properties in the clinic such as selective serotonin reuptake inhibitors (SSRI) (citalopram, paroxetine, fluoxetine), serotonin and noradrenaline reuptake inhibitors (SNRI) (duloxetine), tricyclic antidepressants (TCA) (clomipramine) and MAOI (phenelzine) (Borsini et al., 2002). Fluoxetine which is SSRI was used as anxiolytic agent based on the discussion that preclinical and clinical studies suggest that there exists a neurobiological link between emotional and cognitive processes. Accordingly, anxiety disorders may result from an over expression of aversive memories. Clinically effective anxiolytics, then, may reduce anxiety through a disruption of the association between emotion and cognition. Evidence in support of this theory comes from both animal and human research. For example, rats injected with benzodiazepines or SSRI, such as fluoxetine, show diminished aversive memory formation that may contribute to their well-documented anxiolytic effects in animal models and in the clinic (Degroot & Nomikos, 2005).

Our data represents that DFBEA extract and DFB (V+VI) isolated compound of plant has a highly significant and potent anxiolytic effect.

CONCLUSION

Different extracts and isolated compound (chrysin) of *Dolichandrone falcata* stem-bark produced significant anxiolytic effects when subjected to EPM and MBT models of anxiety. The DFBM extract showed satisfactory effects whereas DFBEA extract showed highly significant activities. DFB (V+VI) isolated compound was very potent in effect similar to standard such as diazepam and fluoxetine in experimental animals. Since isolated compound DFB (V+VI) TLC data suggest that it may be chrysin, further investigation could be done by characterizing isolated compound DFB (V+VI) for its spectral studies and physical data.

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REFERENCES

Asia Pacific Medicinal Plant Database Broader topics Medicinal Plant Database. Available at: http://219.93.41.233/wapi/mctweb.dll/getObject?MID=MEDICINALPLANT&ObjID=1841. Accessed on 14 October 2009.

Atack JR 2003. Anxioselective compounds acting at the GABA (A) receptor benzodiazepine binding site. *CNS Neurol Disord Drug Targets 2*: 213-232.

Bhattacharya SK, Mitra SK 1991. Anxiolytic activity of *Panax ginseng* roots: an experimental study. *J Ethnopharmacol 34*: 87-92.

Borsini F, Podhorna J, Marazziti D 2002. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology (Berl) 163*: 121-141.

Broekkamp CL, Rijk HW, Joly-Gelouin D, Lloyd KL 1986. Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice. *Eur J Pharmacol 126*: 223-229.

Cha HY, Park JH, Hong JT, Yoo HS, Song S, Hwang BY 2005. Anxiolytic-like effects of ginsenosides on the elevated plus-maze model in mice. *Biol Pharm Bull 28*: 1621-1625.

Costa E, Guidotti A 1996. Benzodiazepines on trial: a research strategy for their rehabilitation. *Trends Pharmacol Sci 17*: 192-200.

Degroot A, Nomikos G 2005. Fluoxetine disrupts the integration of anxiety and aversive memories. *Neuropsychopharmacology 30*: 391-400.

Espejo E 1997. Structure of the mouse behavior on the elevated plus-maze test of anxiety. *Behav Brain Res 86*: 105-112.

Friede M, Freudenstein J 2002. Antidepressant and anxiolytic effect of St. John's wort extract ZE-117. *Eur Psychiatr 17(Suppl. 1)*: 96-96.

Gokhale SB, Purohit AP, Kokate CK 2008. *A Textbook of Pharmacognosy.* Nirali Prakashan, Pune India, p. 201.

Griffiths RR, Ator NA, Roache JD, Lamb RJ 1987. Abuse liability of triazolam: experimental measurements in animals and humans. *Psychopharmacol Ser 3*: 83-87.

Jordan AD, Kordik CP, Reitz AB, Sanfillipo PJ 1996. Novel anxiolytic agents- 1994 to present. *Expert Opin Ther Patents 6*: 1047-1060.

Kaplan HI, Sadock BJ 2005. Comprehensive Textbook of Psychiatry, 8th ed. New York: Lippincot Williams and Wilkins.

Kennett GA, Trail B, Bright F 1998. Anxiolytic-like actions of BW723C86 in the rat Vogel conflict test are 5-HT2B receptor mediated. *Neuropharmacology 37*: 1603-1610.

Lader M, Morton S 1991. Benzodiazepine problems. *Br J Addict 86*: 823-828.

Lister RG 1987. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl) 92*: 180-185.

Lleana S, Oana RP, Loana S 2004. The study of separation of active components from the propolis extract. *Annals West Uni Timi Series Chem 15*: 9-14.

Marder M, Paladini AC 2002. GABAA-receptor ligands of flavonoid structure. *Curr Top Med Chem 2*: 853-867.

Nazar M, Jessa M, Plaznik A 1997. Benzodiazepine-GABAA receptor complex ligands in two models of anxiety. *J Neural Transm 104*: 733-746.

Njung'e K, Handley SL 1991. Evaluation of marble-burying behavior as a model of anxiety. *Pharmacol Biochem Behav 38*: 63-67.

OECD 2006. Guidelines for Testing of Chemicals. Revised draft Guidelines 425: Acute oral toxicity-up and down method, Revised document.

Paladini C, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH 1999. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. *J Pharmacy Pharmacol 51*: 519-526.

Park JH, Cha HY, Seo JJ, Hong JT, Han K, Oh KW 2005. Anxiolytic-like effects of ginseng in the elevated plus-maze model: comparison of red ginseng and sun ginseng. *Prog Neuropsychopharmacol Biol Psychiatry 29*: 895-900.

Patil DA 2003. Flora of Dhule and Nandurbar districts (Maharashtra). Bishen Singh Mahendra Pal Singh Publishers and Distributors of Scientific Books. Dehradun. p. 440.

Pellow S, Chopin P, File SE, Briley M 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods 14*: 149-167.

Shin JS, Kim KS, Kim MB, Jeong IJH, Kim BK 1999. Synthesis and hypoglycemic effects of chrysin derivatives. *Bioorg Med Chem 9*: 869-874.

Sinaphet B, Noiarsa P, Kanchanapoom T 2006. Dolichandroside, a new phenolic triglycoside from *D. serrulata. J Nat Med 60*: 251-254.

Subramanian SS, Nagarajan S, Sulochana N 1972. Chrysin-7-rutinoside from the leaves of *Dolichandrone falcata. Phytochemistry 11*: 438-439.

Trabera J, Glaser T 1987. 5-HT1A receptor-related anxiolytics. *Trends Pharmacol Sci 8*: 432-437.

Vidyasagar GM, Prashantkumar P 2007. Traditional herbal remedies for gynecological disorders in women of Bidar district, Karnataka, India. *Fitoterapia 78*: 48-51.

Wang F, Shing M, Huen Y, Tsang SY, Xue H 2005. Neuroactive flavonoids interacting with GABA-A receptor complex. *CNS Neurol Disord Drug Targets 4*: 575-585.

Wolfman C, Viola H, Marder M, Wasowski C, Ardenghi P, Izquierdo I, Paladini AC, Medina JH 1996. Anxioselective properties of 6,3'-dinitroflavone, a high-affinity benzodiazepine receptor ligand. *Eur J Pharmacol 318*: 23-30.

Wolfman C, Viola H, Paladini A, Dajas F, Medina JH 1994. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea. Pharmacol Biochem Behav 47*: 1-4.

Yamada K, Wada E, Yamano M, Sun YJ, Ohara-Imaizumi M, Nagamatsu S 2002. Decreased marble burying behavior in female mice lacking neuromedin-B (NMB-R) implies the involvement of NMB/NMB-R in 5-HT neuron function. *Brain Res 942*: 71-78.