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An anxiolytic effect of *Dolichandrone Falcata* Leaves extract In experimental animals.

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ABSTRACT

The aim of the present study is to investigate the effects of aqueous extract of *Dolichandrone falcata* leaves. This study evaluated the effects of aqueous extracts of *Dolichandrone falcata* (DF) leaves in mice and rats submitted to the elevated plus maze, social interaction, light/dark and hole board tests. Diazepam was used as the standard drug. In the elevated plus maze, aqueous extract of DF (300mg/kg; p.o) and diazepam (2 mg/kg, i.p) increasing the percentage of time spent in open arms and the percentage of open arm entries. In social interaction test, the aqueous extract of DF (300mg/kg; p.o) and diazepam treated animals significantly increased social interaction time of low light, unfamiliar test condition. In the light/dark test, DF aqueous extract (300mg/kg; p.o) and diazepam (2 mg/kg, i.p) increased the time spent in light area, latency to enter dark chamber and number of times tunnel crossing. In hole board test, DF aqueous extract (300mg/kg; p.o) and diazepam (2 mg/kg, i.p) significantly increased the head dipping counts and decreased the head dip latency. These results suggested that the aqueous extract of DF, in contrast to diazepam, had no effect on locomotion in these tests, its side effect profile might be considered superior to the benzodiazepines. Thus, present findings indicate that DF exhibited anxiolytic-like effect.

Keywords: *Dolichandrone falcata;* Anxiolytic property; Elevated plus maze; Social interaction; light/dark; Hole board.

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INTRODUCTION

Anxiety disorders are marked by excessive fear (and avoidance), often in response to specific objects or situations and in the absence of true danger, and they are extremely common in the general population. According to a recent epidemiological study, the lifetime prevalence of any anxiety disorder is 28.8% [1]. Anxiety disorders are associated with impaired workplace performance and hefty economic costs [2]. Anxiety is also an important component of many other psychiatric or medical conditions. Effective treatments such as anxiolytic drug therapy or cognitive behavioural therapy exist but, many patients remain untreated, experience adverse effects of benzodiazepines, or do not benefit from full symptom control. It has been estimated that 43% of anxiety sufferers use some form of complementary therapy. The most popular treatments include herbal medicines [3].

The increasing awareness of herbal medicine is acknowledged by WHO. WHO estimate about three-quarters of the world's population currently use herbs and other forms of traditional medicines to treat their diseases. WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, almost for several hundred years. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. herbal dugs constitute only those traditional medicines, which are primarily use medicinal plants preparations for therapy [4].

The *Dolichandrone falcata seem* Family-Bignoniaceae, is traditional medicinal plant has been used in ayurvedic medicine for fish poison and to procure abortion [5]. It is reported, that possess active constituents flavonoids like chrysin, chrysin 7-rutinoside and chrysin 7- glucoside [6]. It is reported that plant possess chemical constituents having antioxidant activity 'Dolichandroside A, a new α-glucosidase inhibitor and DPPH free –radical scavenger from *Dolichandrone falcata* seem' [7].

The aims of present study were to investigate the anxiolytic effect of aqueous extract of *Dolichandrone falcata* leaves in animal models of anxiety. This is being carried out with the intention of giving a scientific validity and justification of such herb indicated for the treatment of anxiety and related disorders.

MATERIALS AND METHODS

Animals

Albino male Wistar rats and Albino mice weighing between 180 to 200g and 18 to 22 gm respectively were procured form registered breeders (146/999/CPCSEA, Mahavir Enterprise, Hyderabad). Male Swiss mice were housed in cages of 5 at 22 ± 1^{0}C in a 12-h light/dark cycle. Food and water were freely available with the exception of the brief test periods. Male Wistar rats were individually housed in polycarbonate cages under a 12-h light/dark cycle in temperature control 22 ± 1^{0}C animal facility. Food and water were freely available with the

exception of the brief test periods. Animals were handled gently every day for at 7 days. All experiments were carried out in a quit room under dim light between 9.00 a.m. and 2.00 p.m. The experiments were performed following the approval of the CPCSEA on the Institutional Animal Ethics Committee (IAEC) of Luqman College of Pharmacy, Gulbarga.

Plant material

Leaves of *Dolichandrone falcata* were collected from Solapur province of India and authenticated by P.G Diwakar, Joint Director, Botanical Survey of India, Pune where the specimen voucher (DVKDF1) has been deposited for future reference.

Preparation of extracts

The plants collected were carefully and leaves were separated. The leaves were washed with tap water and left to dry for 15 days in the shade at room temperature. Then they were stored in well sealed cellophane bags, so as to prevent from the environmental effects. The shade dried powdered leaves were used for the extraction with distilled water. The powder weighing approximately 225-250 gm was extracted by adding 1000 ml of distilled water. The extract was filtered and the filtrate evaporated to dryness under reduced pressure using a rotary evaporator [8].

Drugs

Diazepam hydrochloride I.P - Chackosons Chemicals Private Limited, Kerala, India, was used as a reference standard and suspended in 2% gum acacia. Aqueous extract of *Dolichandrone falcata* leaves were administered in 100, 300 mg/kg; p.o. doses in distilled water. All drugs were freshly prepared before each experiment.

Procedure

Elevated plus maze test

Anxiolytic activity was measured using the Elevated plus maze [9]. Elevated plus maze (EPM) one of the commonly used animal model for testing anxiolytic drugs. It is based on the apparent natural aversion of rodents to open and high spaces animals have tendency to spend more time in enclosed arms than in the open arms [10]. The maze consisted of two open (28 cm x 5 cm) and two closed (28 cm x 5 cm x 14 cm) arms, extending from the central platform (5 cm x 5 cm) and elevated up to the height of 40 cm above the floor. The entire maze was made of clear Plexiglass. All testing occurred in a dimly illuminated laboratory during dark phase of light cycle. Mice were randomly assigned to four experimentally groups and then adjusted slightly to mach the average body weight of the groups (n = 6). The control group received 2 % gum acacia per oral. The standard drug diazepam was administered i.p. 30 min prior to testing and aqueous extract dose 100 mg/kg and 300 mg/kg were administered p.o 45 min prior to testing. Mice

were individually placed on the centre of the maze facing an open arm, and the number of entries and the time spend in closed and open arm during a 5 min observation period. Arm entries were defined as entry of all four paws in to the arm. The percentage of open arm entries (100 x open/total entries) was calculated for each animal.

Social interaction test

The general design was essentially as reported by File and Hyde [11]. The test was validated for male, adult rats, and there are some important sex differences in that female do not increase social interaction as markedly in response to increasing familiarity with the test arena [12]. The test was conducted in a Perspex box with opaque walls 65 x 65 cm with walls 47 cm high. The test condition was manipulated by altering both the light level and familiarity of rats to the test arena. In this study low light unfamiliar test condition was chosen. In low light unfamiliar condition, the apparatus was illuminated by red bulb on the ceiling.

Each rat was tested for social interaction with unknown test partner that did not differ by more than 10 g in weight. In low light unfamiliar condition, 24 male rats were assigned to four experimental groups. Both members of pair had the same familiarisation experience and the same drug treatment. The control group received 2 % gum acacia per oral. The standard drug diazepam was administered i.p. 30 min prior to testing and aqueous extract dose 100 mg/kg and 300 mg/kg were administered p.o 45 min prior to testing. The experiments perform between 09.00 and 14.00. Pairs of rats were placed in opposite corners of the arena and then left for 10 min. their behaviours were recorded with a video camera and observed on a monitor in an adjacent room. The total time of active social interaction including sniffing, nipping, grooming, following, mounting, kicking, boxing, wrestling, jumping on, crawling under or over the partner and locomotion activity (the number of square crossed) were recorded for each pair by two blind observers and the average scores used for subsequent analysis. Passive body contact was not regarded as a social interaction.

Light and dark transition test

The light/dark transition test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodent in response to mild stressors, that is, novel environment and light [13].

The apparatus for light/dark transition test consist of two compartments: one light area (27 L x 27 W x 27 H cm), illuminated by 100 W desk lamp was painted white, and the other dark area (18 L x 27 W x 27 H cm) was painted black. The two compartments were separated by a partition with a tunnel (7.5 x 7.5 cm) to allow passage from one compartment to the other [14]. Mice were randomly assigned to four experimental groups and then adjusted slightly to mach the average body weight of the groups (n= 6). The control group received 2 % gum acacia per oral. The standard drug diazepam (2 mg/kg) was administered, i.p. 30 min prior to testing and aqueous extract dose (100 mg/kg) and (300 mg/kg) were administered p.o 45 min prior to

testing. The experiments were performed between 09:00 and 14:00. Animal was placed in the centre of the light area with its back to the opening. The following parameter were recorded during 5 min: Latency time for the first crossing to the dark compartment, the number of transition between the light and the dark compartment (tunnel crossing), the total time spent in the light compartment. The apparatus was cleaned thoroughly between trials.

Statistical analysis

The data obtained were analysed using the GraphPad Prism 5 software programme Version 5.0 and expressed as mean ± S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by Dunnet's test. *P*-values less than 0.05 ($P<0.05$) were considered as significant.

RESULTS

Elevated plus maze test

Administration of diazepam (2 mg/kg) significantly increased the percentage of time spent and of arm entries in open arms ($P<0.001$) as compare to control group. The results are sown in Fig.1 and Table 1. The DF at dose (300 mg/kg) resulted in a significant increase in the percentage of time and entries into open arm ($P<0.001$), compared to control groups. The DF at dose (100 mg/kg) resulted in a significant increase in the open arm time ($P<0.1$) but not entry. (Table 1, Figure 1- a and b).

Social interaction test

The results of the social interaction test were shown in Fig. 2 and able. 2. DF at 300 mg/kg could increase the time spent in social interaction ($P<0.01$) under low light unfamiliar condition. (Diazepam 2 mg/kg) could increase social interaction time ($P<0.01$) in under low light unfamiliar condition and had significantly decreased squared entered ($P<0.01$).
(Table 2, Figure 2- a and b).

Light/dark transition test

Results of light/dark test were shown in Table.3 and Fig.3. Diazepam treatment had an effects on time spent in the light area ($P<0.001$), latency to dark chamber and no. of tunnel crossings ($P< 0.01$). DF at dose of 300 mg/kg significantly increase of time spent in light area ($P<0.01$), latency to dark chamber and no. of tunnel crossings ($P<0.05$). (Table 3, Figure 3-a, b and c)

DISCUSSION

The present study investigated the putative behavioural effects in mice of the aqueous extracts from the leaves of *Dolichandrone falcata*. The results demonstrate that aqueous

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extracts have anxiolytic-like effects at some selected tested doses in these three murine models of anxiety.

Table 1
Effects of DF in the 5 min elevated plus-maze test in mice.

Drugs	Dose mg/kg	% open arm time	% close arm time	% open arm entry	% closed arm entry
CTL	-	34.92 ± 3.94	65.07 ± 3.94	39.63 ± 3.02	60.37 ± 3.02
DIZ	2	61.54 ± 0.52 ***	38.45 ± 0.52 ***	68.02 ± 3.04 ***	31.97 ± 3.04 ***
DF1	100	43.33 ± 2.27*	55.83 ± 2.99 *	49.84 ± 3.76	50.15 ± 3.76
DF2	300	58.44 ± 0.89 ***	41.55 ± 0.89 ***	67.15 ± 3.16 ***	32.12 ± 2.48 ***

Values represent means ± S.E.M (n=6). *P<0.05, ***P<0.001 compared with vehicle.

Table 2
Effects of DF in 10 min Social interaction test in rats.

Drugs	Dose (mg/kg)	Social interaction time (sec)	Locomotion
CTL	-	58.78 ± 22.05	145 ± 10.74
DIZ	2	190.1 ± 23.21 *	111.0 ± 8.73 *
DF 1	100	78.51 ± 30.18	133.7 ± 4.41
DF 2	300	169.0 ± 24.80 *	134 ± 8.32

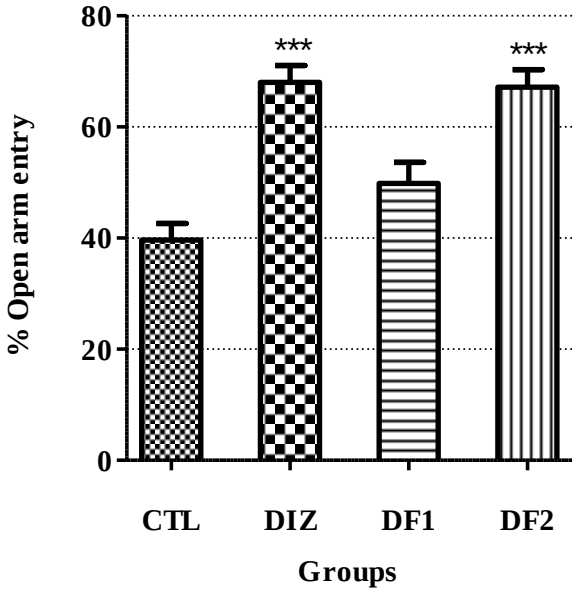
Values represent means ± S.E.M (n=3 pairs pr group). Testing condition were low light, unfamiliar (LU) *P<0.05, compared with vehicle.

Table 3
Effects of DF in 5 min light/dark transition test in mice.

Drugs	Dose (mg/kg)	Time in light area (sec)	Latency to enter dark (sec)	Tunnel crossing
CTL	-	100.0 ± 7.55	23.67 ± 3.28	11.17 ± 0.87
DIZ	2	155.0 ± 9.89 ***	44.33 ± 3.19**	16.67 ± 0.88 **
DF 1	100	126.3 ± 9.97	26.00 ± 2.33	10.67 ± 0.88
DF 2	300	142.0 ± 7.13 **	40.00 ± 5.00 *	15.00 ± 1.29 *

Values represent means ± S.E.M (n=6). *P<0.05, compared with vehicle.

(a)



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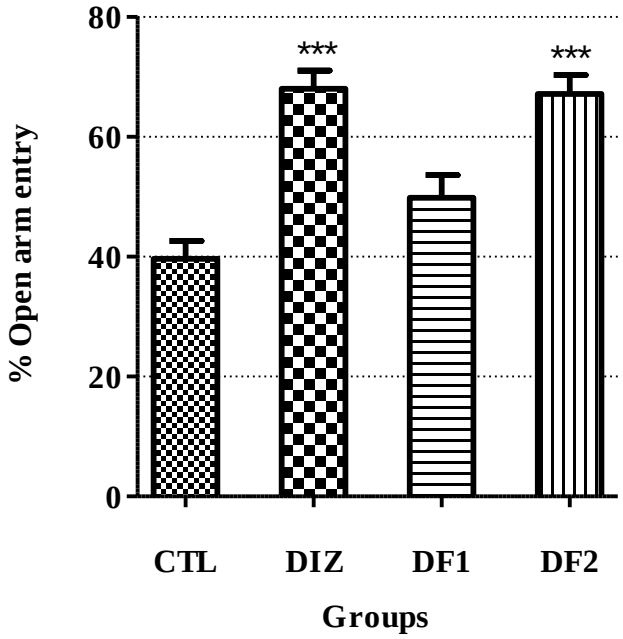
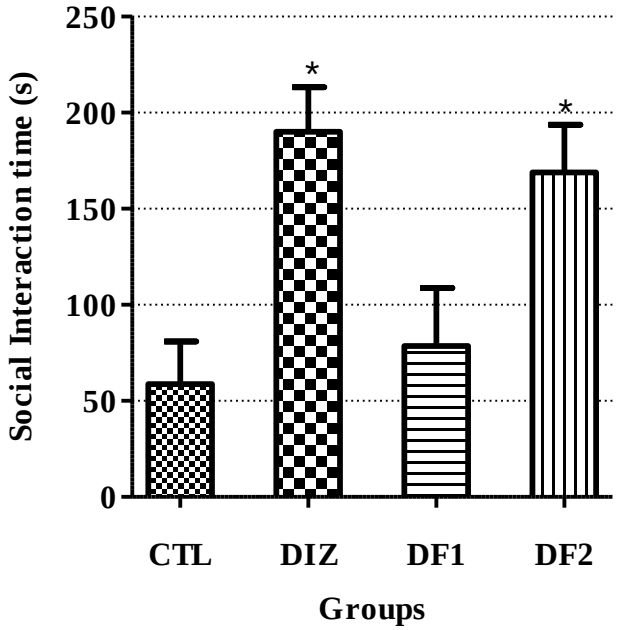


Figure 1 (a and b): Effects of diazepam and extracts of *Dolichandrone falcata* in the elevated plus maze test in mice. Results are expressed as mean ± S.E.M. (n=6). The following parameters are shown: (a) The percentage of time spent in open arms. (b) The percentage of open arm entries. **P*< 0.05, ****P*<0.001 compared with vehicle-treated animals.

(a)



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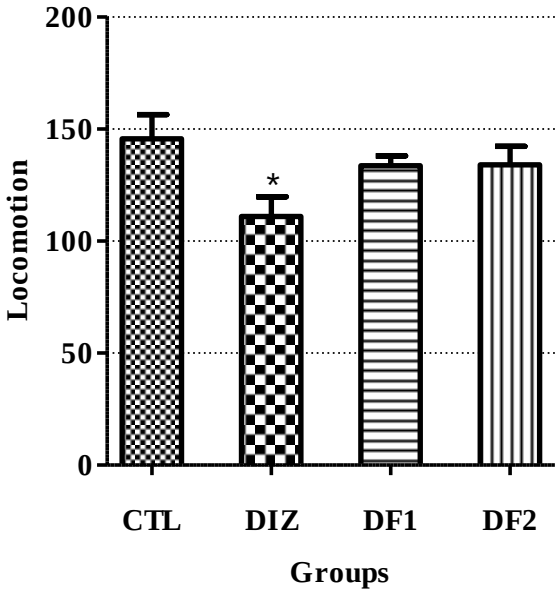
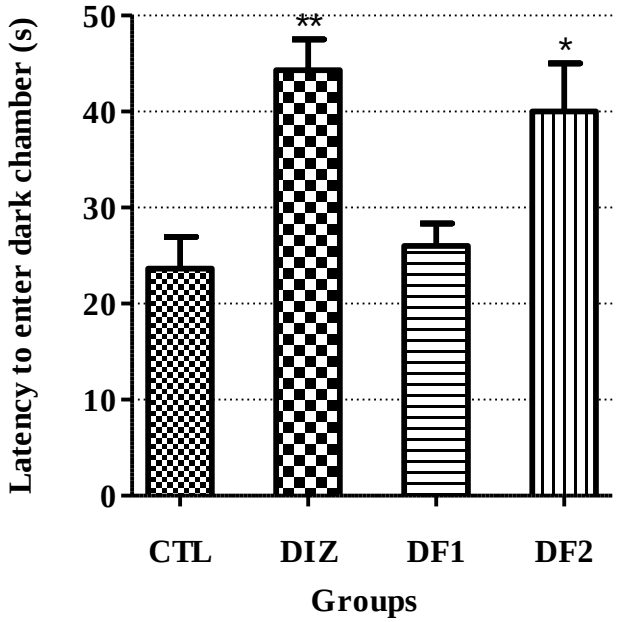


Figure 2 (a and b):Effects of diazepam and extracts of *Dolichandrone falcata* in the social interaction test in mice. Results are expressed as mean ± S.E.M. (n=3 pairs per group). Test condition were low light, unfamiliar (LU). The following parameters are shown: (a) social interaction time-sec (b) locomotion: **P*< 0.05, ****P*<0.001 compared with vehicle-treated animals.

(a)



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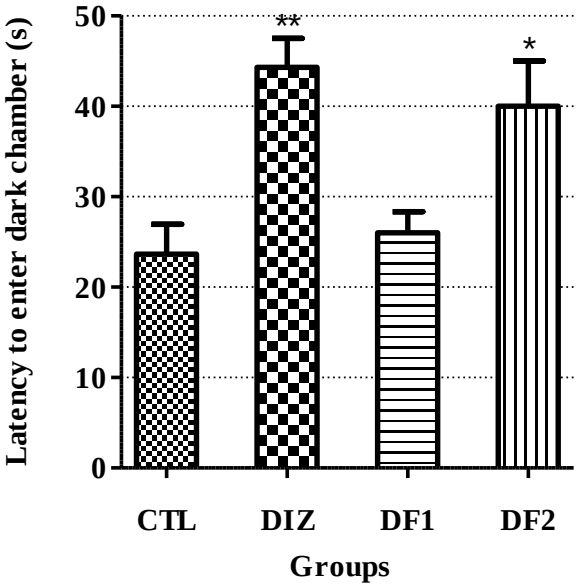
(b)



(c)

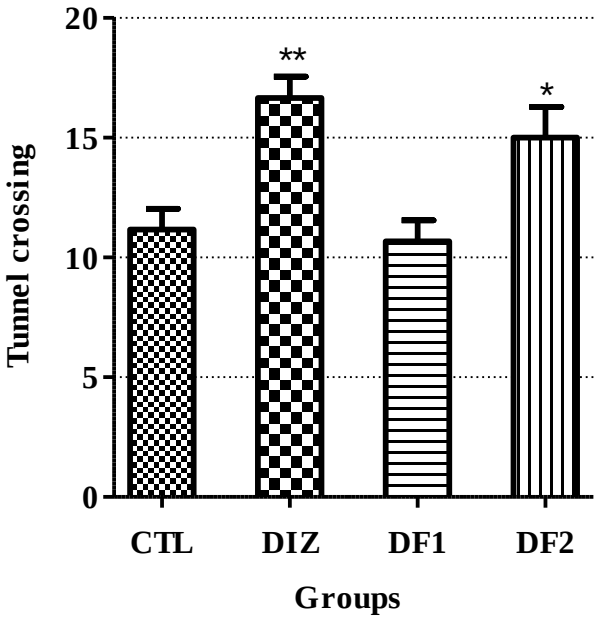


Figure 3 (a, b and c): Effects of diazepam and extracts of *Dolichandrone falcata* in the light/dark transition test in mice. Results are expressed as mean ± S.E.M. (n=6). The following parameters are shown: (a) the time spent in light chamber-sec (b) the latency to enter dark chamber-sec and (c) tunnel crossings. *P< 0.05, ***P<0.001 compared with vehicle-treated animals.

The EPM stands as of the most popular in vivo animal test currently in use. The test was further validated as an animal model of anxiety on pharmacological, physiological and behavioural grounds [15]. Diazepam increased the percentage of open arm entries and the time spent in the open arms [16] confirming the anxiolytic effect The dose DF1 shows increase

percentage of time spent in open arm but not entry. The DF2 (300 mg/kg) had increased the percentage in time spent and entry in to open arm with decreased in closed arm. It can be suggested that DF2 (300 mg/kg) shows clearly anxiolytic effects similar to the standard drug as result animal spent more time in open arm and less time in closed arm. There for behavioural alteration induced by higher dose DF2 (300 mg/kg) of aqueous extract.

The social interaction test was reported by File and Hyde as the first animal test of anxiety, which used a natural form of behaviour as the dependent measure [11]. An increase in social interaction, without a concomitant increase in motor activity, is indicative of an anxiolytic like effect. Because the behaviour of one rat influences that of other, it is important that the pair of rats is treated as unit, and only one score for the pair is used [17]. In present study, DF2 (300mg/kg) significantly increased the time of social interaction in LU condition without an increase in locomotory behaviour suggesting an anxiolytic-like effect.

Light/dark box is another widely used rodent anxiety model for screening anxiolytic or anxiogenic drugs. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors that is novel environment and light [13]. Drugs induced increase in behaviour in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity [18]. In this study, the time spent in light area, latency to enter dark chamber and tunnel crossing is an indices of anxiety. The DF2 (300mg/kg) had significantly increased the time spent in light area, latency to enter dark chamber and tunnel crossing, similar to standard drug, suggesting that anxiolytic activity of DF leaves extract as compare to control group.

CONCLUSION

The results obtained from these experimental models clearly confirmed that the anxiolytic activity of aqueous extracts of *Dolichandrone falcata* leaves. The acute treatment with DF2 (300mg/kg) clearly demonstrate a dose dependant anxiolytic effect comparable to diazepam (2mg/kg; i.p) in all experimental models of anxiety. The phytoconstituent like chrysin were reported for their anxiolytic effect [19] and these constituents were present in *Dolichandrone falcata* leaves [6] and bark [20] so this active principle might be responsible for anxiolytic effect. The mechanism of anxiolytic activity of *Dolichandrone falcata* leaves extracts is unclear hence further studies are needed to identify the anxiolytic mechanism(s) and the phytoconstituents responsible for the observe central effects of the aqueous extract of *Dolichandrone falcata* leaves.

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