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RESEARCH ARTICLE

Anxiolytic potential of *Tamarindus indica*

MILIND PARLE AND ISHA DHAMIJA

ABSTRACT

Tamarindus indica, commonly known as Imli, is a favorite condiment commonly used in south Indian dishes particularly in Rassam and Sambhar due to its rich nutritional value. The present study was undertaken to investigate the anxiolytic potential of *Tamarindus indica* in mice. A total of 150 Swiss mice divided in 25 groups were employed in this study. *Tamarindus indica* (2%, 4% and 8% w/w) was admixed in diet of mice for a period of 14 days. Elevated plus maze, Light dark model and Hole board test were used as behavioral models in this study. Effect of *Tamarindus indica* on MDA levels was also estimated. Mice prefer to stay in dark zone under normal conditions. Anxiolytics reduce this natural preference to darkness and increase the time spent in the lit compartment. *Tamarindus indica* produced significant ($P<0.01$) increase in the time spent in the lit compartment in light - dark model. *Tamarindus indica* enhanced significantly ($P<0.05$) the number of entries and time spent in the open arms, when tested using elevated plus maze model in mice. The hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess anxiety. *Tamarindus indica* significantly ($P<0.05$) increased the head dip counts in hole-board test at different concentrations (2%, 4% and 8% w/w) indicating its anxiolytic effect. *Tamarindus indica* significantly ($P<0.05$) reduced MDA levels in the brains of mice, thereby revealing its property of bringing about reduction in free radical generation. These findings reveal the anxiolytic potential of *Tamarindus indica*.

Key words : Elevated plus maze, Hole board test, Light- dark model, Tamarind

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INTRODUCTION

Anxiety is a normal human emotion in fast paced life, which encompasses behavioral, affective and cognitive responses to the perception of danger. In moderation, anxiety stimulates an anticipatory and adaptive response to stressful events. In severe form, anxiety destabilizes the individual interfering in his day-to-day activities. Anxiety is considered pathological, when it is out of proportion to the challenge and arises in the absence of stress. There are various types of anxiety disorders, such as generalized anxiety disorder (GAD), obsessive-compulsive disorder (OCD), post traumatic stress disorder, panic disorders and phobias (Parle *et al.*, 2010). Anxiety affects one eighth of the world's population and has become a very important area of research interest in psychopharmacology due to its high prevalence (Eisenberg *et al.*, 1998). Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite their undesirable side effects such as skeletal muscle relaxation, sedation, physical dependence and memory impairment (Parle and Chaturvedi, 2012). In recent years, the development of new anxiolytics from plant origin has been an area of interest. Various types of herbal medicines have been used as anxiolytics in different parts of the world. The root of the kava plant from the tropical Pacific region, St. John's wort from Europe, and saponin containing fraction of the leaves of *A. lebbeck* are known to have anxiolytic effects (Alqasoumi, 2012). Each and every part of the tamarind tree, especially the fruit is beneficial for the society. Tamarind is also reported to have significant antidepressant activity (Parle and Dhamija,

MEMBERS OF THE RESEARCH FORUM

Address for correspondence :
MILIND PARLE, Pharmacology Division, Department of Pharmaceutical Sciences, Guru Jambheshwara University of Science and Technology, HISAR (HARYANA) INDIA

Coopted auhors :
ISHA DHAMIJA, Pharmacology Division, Department of Pharmaceutical Sciences, Guru Jambheshwara University of Science and Technology, HISAR (HARYANA) INDIA

2012). Pulp of tamarind fruit (Imli) is an important component in chutneys, curries, jams, pickles, ice-creams, sharbat and tamarind fish (Parle and Dhamija, 2012). In the light of these considerations, the present study was undertaken to explore the anxiolytic activity of *Tamarindus indica* pulp using elevated plus maze model, light- dark model and hole-board test.

MATERIALS AND METHODS

Plant material:

The *Tamarindus indica* fruits were purchased from local market of Hisar and got authenticated from Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (Ref. no. NISCAIR/RHMD/Consult/-2011-12/1724/264).

Animals:

A total of 150 Swiss mice weighing around 20-25g divided in 25 groups were employed in the present study. Each group comprised of six animals. Mice were procured from disease free animal house of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). The animals had free access to food and water, and they were housed in a natural (12h each) light-dark cycle. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. The experimental protocol was approved by the Institutional Animals Ethics Committee and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number - 0436).

Drug protocol:

Mice in group I were employed for pilot study carried out to determine the effective concentrations of *Tamarindus indica* pulp (TIP). Mice belonging to group II to VI were subjected to light- dark model. Mice belonging to group VII to XI were subjected to elevated plus maze model, while group XII to XVI were employed in hole board test. Mice belonging to group XVII to XX were used in estimating MDA levels. Mice belonging to group XXI to XXV were exposed to photoactometer for assessing the locomotor activity. Diazepam (1 mg/kg), and *Tamarindus indica* pulp (2%, 4% and 8% w/w) were admixed in diet for 14 successive days to mice. Biochemical studies were carried out on 14th day after drugs/TIP administration. Effect on locomotor activity of mice was studied using photoactometer.

Experimental design:

Elevated plus maze (EPM):

The elevated plus-maze test has been widely validated for measuring anxiolytic and anxiogenic- activities in mice. The plus maze apparatus consisted of two open arms, 16 x 5 cm^2, and two closed arms, 16 x 5 x 12 cm^3, connected to a central platform 5 x 5 cm^2. The maze was elevated to a height of 25 cm above the floor. Each mouse was placed individually at the centre of elevated plus maze with its head facing towards an open arm and was observed for 5 min to record the number of entries in the open arm, closed arm and time spent in each arm (Lister, 1987).

Light and dark model:

Light and dark exploration test is commonly employed for evaluation of anxiolytic activity. The apparatus consisted of a rectangular box (45 x 27 x27 cm^3), partitioned into two compartments connected by a 7.5 x 7.5 cm^2 opening in the wall between compartments. An animal was placed in the center of the lit compartment and was observed for 10 min for time spent in this compartment (Bourin and Hascoet, 2003).

Hole board test:

The hole-board apparatus consisted of a wooden box (40 x 40 x 25 cm^3) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. For a period of 5 min the number of head dippings were counted by placing the animal in the centre of the apparatus (Yadav *et al.*, 2008).

Locomotor activity:

Locomotor activity was measured using photoactometer.

Collection of brain samples:

The animals were sacrificed by cervical decapitation under light anesthesia on the 14th day, 90 min after administration of the diet admixed with TIP or standard drugs. Immediately after decapitation, brain was carefully removed from the skull. For preparation of homogenate, the whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of brain malondialdehyde (MDA) levels.

Biochemical estimatons:

Estimation of thiobarbituric acid reactive substances or malondialdehyde:

Thiobarbituric acid reactive substances (TBARS) or malondialdehyde (MDA), a measure of lipid peroxidation, was estimated spectrophotometrically. The procedure was performed as described by Okhawa *et al.* (1979).

Statistical analysis:

All the results were expressed as mean ± standard error (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett's *t*-test. p-values < 0.05 were considered as

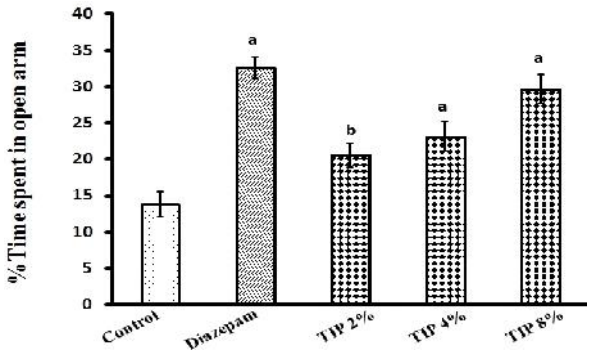
statistically significant.

RESULTS AND DISCUSSION

The results obtained from the present investigation have been discussed in the following sub heads:

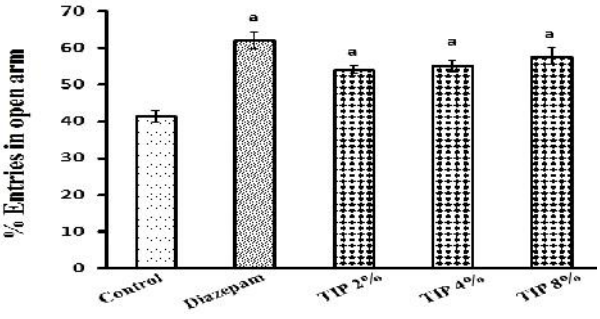
Anxiolytic effect of *Tamarindus indica* pulp using elevated plus maze:

Tamarindus indica pulp (TIP) at the concentrations of 2%, 4% and 8% w/w, when administered along with diet for 14 successive days, significantly ($p<0.05$) increased the time spent in open arm and enhanced the number of entries ($p<0.01$) in open arm of the Elevated plus maze. The effect of TIP was found to be comparable to that of diazepam (an established anxiolytic agent) (Fig. 1 and 2).



Values are expressed in mean ± S.E.M (n=6).
'a' denotes $p<0.01$ as compared to control group, 'b' denotes $p<0.05$ as compared to control group.
TIP: *Tamarindus indica* pulp

Fig. 1 : Effect of *Tamarindus indica* pulp on time spent in open arm by mice using elevated plus maze model

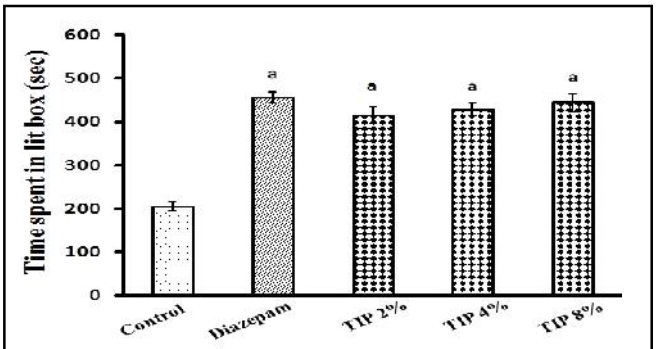


'a' denotes $p<0.01$ as compared to control group, 'b' denotes $p<0.05$ as compared to control group.
TIP: *Tamarindus indica* pulp

Fig. 2 : Effect of *Tamarindus indica* on number of entries of mice in open arm using elevated plus maze

Anxiolytic effect of *Tamarindus indica* pulp using light-dark model:

TIP at the concentrations of 2%, 4% and 8% w/w increased the time spent in lit box significantly ($p< 0.01$), in light and dark model. The effect of TIP was found to be comparable to that of diazepam (Fig. 3).

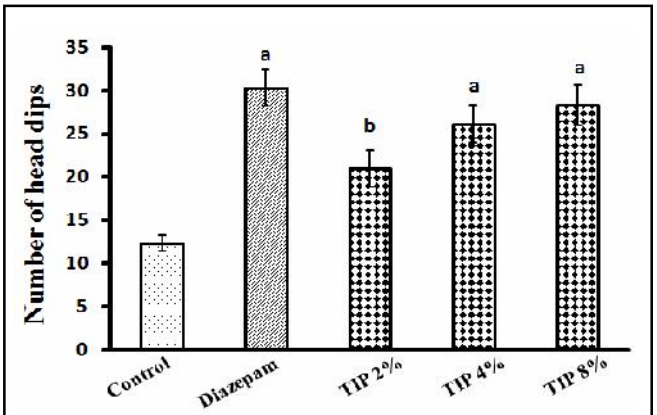


'a' denotes $p<0.01$ as compared to control group
Diazepam (1mg/kg, i.p.) was used as a standard drug

Fig. 3 : Effect of *Tamarindus indica* pulp on time spent in lit box of mice using light and dark model

Anxiolytic effect of *Tamarindus indica* pulp using hole board test:

TIP when administered with diet in different concentrations (2%, 4% and 8% w/w), produced a significant ($p<0.05$) increase in head dipping behavior of mice, when tested using hole board test (Fig. 4).



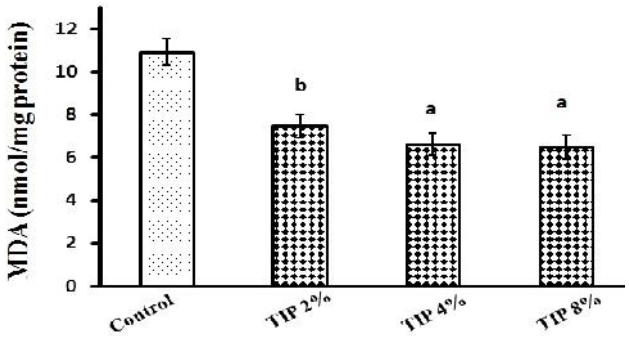
'a' denotes $p<0.01$ as compared to control group, 'b' denotes $p<0.05$ as compared to control group

Fig. 4 : Effect of *Tamarindus indica* pulp on number of head dips by mice using hole board test

Effect of *Tamarindus indica* pulp on brain MDA level:

The administration of TIP for 14 days in mice produced a

significant ($p<0.05$) fall in MDA levels, when compared to control group at all the concentrations (2%, 4% and 8% w/w). (Fig. 5).



Values are expressed in mean ± S.E.M (n=6).
'a' denotes $p<0.01$ as compared to control group, 'b' denotes $p<0.05$ as compared to control group

Fig. 5 : Effect of *Tamarindus indica* pulp on MDA level in mice

Anxiety may be regarded as a particular form of behavioral inhibition that occurs in response to novel environmental events. Anxiety is a mental disorder resulting from alterations in the levels of certain neuro-chemicals such as GABA, serotonin and dopamine. The common targets for the treatment of anxiety are GABA and Serotonin. Though several medicines are available for the management of anxiety disorder, most of them are associated with some or the other limitation. Benzodiazepines are the most commonly prescribed medicines, which act through GABAergic system. However, their consumption is associated with problems of sedation and dependence, while serotonin agonists like buspirone evoke adverse effects like dizziness, paresthesia and sedation. In the present study, anti-anxiety potential of *Tamarindus indica* fruit pulp (TIP) was tested in mice employing three experimental models *viz.*, light - dark model, elevated plus maze and hole board test. Chronic administration of *Tamarindus indica* pulp (TIP) for 14 successive days showed anti-anxiety potential in mice, as observed by increased time spent in lit box using light and dark model; increased time spent and number of entries in the open arm of elevated plus maze and increased number of head dips in the hole-board test. Head dipping behavior is sensitive to the changes in the emotional state of the animal and anxiolytic state may be reflected by increased head dipping behavior (Takeda *et al.*, 1998). The light-dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior in response to mild stressors. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of height and brightly-lit open space. Moreover, anxiolytic agents increase the frequency of entries and time spent in the open arm of the EPM (Radhakrishna *et al.*, 2011). The present study showed that tamarind fruit possessed potent anxiolytic activity, which was revealed using three different experimental models of anxiety.

Plants containing flavonoids and tannins are reported to have anxiolytic effect. Plants such as *Pulsatilla nigricans*, *Vitex agnus-castus*, *Ricinus communis*, *Cissus sicyoides* L. are found to have anti-anxiety activity due to the presence of flavonoids and tannins (Goyal and Kumar, 2010). Flavonoids like gossypin, naringin, quercitin, apigenin, hesperidin exhibited anxiolytic activity by binding on $GABA_A$ receptor site (Jager and Sabby, 2011). The presence of flavonoids and tannins in *Tamarindus indica* fruit may explain the anti-anxiety activity of tamarind observed in the present study. However the, involvement of serotonergic or GABAergic pathway in the mechanism of action of tamarind pulp needs to be explored.

MDA is an important marker for lipid peroxidation. Tamarind pulp decreased brain MDA levels in the present study suggesting that free radical generation had been diminished, thereby protecting the brains of mice. These results uniformly point out that tamarind fruit may be looked upon as a promising anti-anxiety agent.

Tamarind fruit **Tamarind pulp**

Conclusion:

In the present study, we observed that *Tamarindus indica* pulp (i) diminished brain MDA levels (ii) and reduced anxiety of mice, when tested using elevated plus maze, hole-board test and light-dark model.

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