

## Brief Communication

## Anti-anxiety Activity Studies on Homoeopathic Formulations of *Turnera aphrodisiaca* Ward

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*Turnera aphrodisiaca* Ward (Turneraceae) has been traditionally used for the treatment of anxiety neurosis, and as an aphrodisiac. Mother tinctures (85% ethanol extracts) of *T. aphrodisiaca* have also been used for the treatment of central nervous system disorders. In the present investigation, *T. aphrodisiaca* mother tinctures formulated by three reputed manufacturers of homoeopathic medicines (NLK, DWSG and SBL) were evaluated for their anxiolytic activity. Dried mother tinctures of *T. aphrodisiaca* were subjected to anxiolytic activity evaluation at various doses, i.e. 50, 75, 100, 125 or 150 mg/kg p.o. in mice using elevated plus maze apparatus. Dried mother tinctures exhibited significant anxiolytic activity at 50 mg/kg (NLK), 75 mg/kg (DWSG) and 125 mg/kg (SBL), respectively, with reference to control as well as standard (diazepam, 2 mg/kg p.o.). Mother tinctures of *T. aphrodisiaca* available in the market, have significant anxiolytic activity. Amongst the three mother tinctures of *T. aphrodisiaca* analyzed, the dry residue of NLK possesses the highest amount of anxiolytic constituent(s). To ensure uniformity and consistency of biological effects in herbal formulations, these should be standardized on the basis of bioactive markers. The authors are actively involved in isolating the bioactive constituent(s) from *T. aphrodisiaca* so that the plant can be standardized on the basis of biologically active constituent(s).

**Keywords:** anxiolytic – aphrodisiac – flavonoids – homoeopathy – mother tincture

### Introduction

*Turnera aphrodisiaca* Ward (synonym *Turnera diffusa* Willd., family Turneraceae) is commonly known as ‘Damiana’. The leaves of *T. aphrodisiaca* have been used traditionally as a stimulant, aphrodisiac, tonic, diuretic, nerve tonic, laxative, and in kidney, menstrual and pregnancy disorders (1,2). The British Herbal Pharmacopoeia (3) lists specific indications for Damiana as anxiety neurosis associated with impotency, and includes other indications such as depression, nervous dyspepsia, atonic constipation and coital inadequacy. Damiana has achieved some repute in the treatment of sexual impotence where it is used in conjunction with strychnine, phosphorus or some other stimulants in homoeopathic formulations (4). The leaf infusion of Damiana has been used as a traditional

remedy in diseases related to the gastrointestinal and respiratory systems (5), reproductive organs (6), and for the treatment of gonorrhoea in Latin American societies (7). Mother tincture (85% ethanol extract) of Damiana is an important homoeopathic medicine for the treatment of sexual debility and nervous prostration (8).

Phytochemical reports on *T. aphrodisiaca* indicate that the plant contains tetraphyllin B (a cyanoglycoside) (9); gonzalitosin I (a flavonoid) (10); arbutin (a phenolic glycoside) (11); damianin (12); tricosan-2-one, hexacosanol (hydrocarbons) (13); a volatile oil containing  $\alpha$ -pinene,  $\beta$ -pinene, *p*-cymene and 1,8-cineole (11); and  $\beta$ -sitosterol (a phytosterol) (10).

A survey of the literature on *T. aphrodisiaca* revealed only three pharmacological reports on the plant. Aqueous extract of *T. aphrodisiaca* whole plant has been reported to exhibit significant hypoglycaemic activity in alloxan-diabetic male mice (14). Aguilera *et al.* (15) have reported that a decoction of *T. aphrodisiaca* leaves possesses significant hypoglycaemic activity in rabbits upon oral administration. Aqueous extract of

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the plant was reported to exhibit aphrodisiac activity in sexually sluggish male rats at a dose of 1 ml/kg (16).

*Turnera aphrodisiaca* has a long tradition of use as a homoeopathic medicine in central nervous system (CNS) disorders. Moreover, mother tinctures of *T. aphrodisiaca* are marketed for CNS depressant effects by reputed manufacturers. Still, no systematic work has ever been carried out to evaluate CNS activities of *T. aphrodisiaca* mother tinctures. Thus, it was considered worthwhile to subject marketed *T. aphrodisiaca* mother tinctures to anti-anxiety screening studies.

## Materials and Methods

### Test Materials

Mother tinctures of *T. aphrodisiaca* from three reputed manufacturers, namely, National Laboratory, Kolkata, India (batch No. 304) (NLK), Dr Wilmar Schwabe, DHU-Arzneimittel, Germany (batch No. 3821002) (DWSG) and SBL Private Limited, Sahibabad, Ghaziabad, India (batch No. MT321) (SBL) were procured from the local market. Thirty millilitre samples of each of the three mother tinctures were dried under reduced pressure using Buchi 461 Rotary vacuum evaporator and were preserved in a vacuum desiccator containing anhydrous silica gel blue. All the dried mother tinctures were subjected to phytochemical screening (17).

### Vehicle and Standard

Simple syrup I.P.+Tween 80 (5%) was used as vehicle for preparing the suspension of various test doses of different mother tinctures. Diazepam (2 mg/kg p.o.) (Triko Pharmaceuticals), suspended in vehicle, was used as the standard anxiolytic drug.

### Preparation of Doses

Dried mother tinctures were suspended in the vehicle in such concentrations as to administer 50, 75, 100, 125 or 150 mg/kg doses to mice in a volume ranging from 0.20 to 0.24 ml per oral route.

### Mice

Laca mice (either sex), bred at the Central Animal House, Panjab University, Chandigarh, were fed a standard pellet diet (Ashirwad, Chandigarh) and water *ad libitum*. Groups of five mice (20–24 g) were used in all sets of experiments. Mice were fasted for 18 h before use. Approval from the Institutional Animal Ethical Committee of Panjab University, Chandigarh was taken before carrying out the anti-anxiety studies.

### Elevated Plus-maze Model of Anxiety

The plus-maze apparatus, consisting of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor used to observe anxiolytic behaviour in mice (18,19). Each mouse was placed at the center of the elevated plus maze with its head

facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as: (i) preference of the mouse for its first entry into the open or closed arms, (ii) the number of entries into the open or closed arms, (iii) average time spent by the mouse in each of the arms (average time = total time spent in open arms/number of entries in arms). Dried mother tinctures of *T. aphrodisiaca* were administered orally using a tuberculin syringe fitted with oral canula. Dose administration schedule was adjusted so that each mouse took its turn on the elevated plus-maze apparatus 45 min after administration of the dose. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety.

### Statistics

The results have been expressed as mean ± standard error of mean (S.E.M.). The test doses were compared with diazepam and control by analysis of variance (ANOVA) followed by Studentized Tukey's test (20). Control group was also compared with the standard group.

## Results and Discussion

The percentage yields (w/v) of NLK, DWSG and SBL dry residues were 0.54, 2.27 and 2.03. All the three dried mother tinctures of *T. aphrodisiaca* tested positive for the presence of flavonoids, alkaloids, steroids, cyanogenic glycosides, carbohydrates, proteins and tannins.

The mean number of entries and time spent by the mice in open arms after oral administration of various doses of the mother tinctures of *T. aphrodisiaca* are shown in Table 1.

Anti-anxiety activity of the mother tinctures of *T. aphrodisiaca*, marketed by reputed manufacturers of homoeopathic medicines, was evaluated employing a widely used model, i.e. elevated plus-maze. A mother tincture is essentially an alcoholic extract prepared according to the procedure described in Homoeopathic Pharmacopoeia (21). Despite being a homoeopathic formulation, a mother tincture is as good as an alcoholic extract commonly employed by researchers during phytochemical or biological studies. The elevated plus-maze model was chosen since it is effective, cheap, simple, less time-consuming, requires no preliminary training for the mice, and does not cause much discomfort to them while handling. The model is principally based on the observations that exposure of mice to an elevated and open maze results in approach–avoidance conflict, which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) induces anxiety in mice when placed on the elevated plus-maze. The ultimate manifestation of anxiety and fear then is exhibited by decrease in motor activity, which is measured by the time spent by mice in the open arms.

Dried *T. aphrodisiaca* mother tinctures of NLK, DWSG and SBL, separately suspended in a suitable vehicle, were administered orally to mice. The activity was compared with

**Table 1.** Anti-anxiety activity of various dried mother tinctures of *Turnera aphrodisiaca* using elevated plus-maze apparatus

Treatment	Dose (mg/kg)	Mean <sup>a</sup> number of entries $\pm$ S.E.M.	Mean <sup>a</sup> time <sup>b</sup> (s) $\pm$ S.E.M.
Control diazepam	Vehicle	2.6 $\pm$ 0.51 <sup>+</sup>	3.58 $\pm$ 0.40 <sup>+</sup>
	2.0	6.6 $\pm$ 1.03*	12.67 $\pm$ 0.54*
NLK	50	7.0 $\pm$ 0.71*	12.42 $\pm$ 0.79*
	75	4.4 $\pm$ 0.81	7.52 $\pm$ 0.48**
	100	3.6 $\pm$ 0.93 <sup>+</sup>	6.76 $\pm$ 0.67**
	125	2.0 $\pm$ 0.32 <sup>+</sup>	2.43 $\pm$ 0.37 <sup>+</sup>
	150	0.0 $\pm$ 0.00 <sup>+</sup>	0.00 $\pm$ 0.00**
DWSG	50	3.4 $\pm$ 0.75 <sup>+</sup>	7.24 $\pm$ 0.93**
	75	6.2 $\pm$ 1.07*	11.90 $\pm$ 1.01*
	100	4.2 $\pm$ 0.74	9.07 $\pm$ 0.96**
	125	2.8 $\pm$ 0.86 <sup>+</sup>	4.80 $\pm$ 0.59 <sup>+</sup>
	150	1.2 $\pm$ 0.37 <sup>+</sup>	1.09 $\pm$ 0.34**
SBL	50	2.8 $\pm$ 0.66 <sup>+</sup>	4.13 $\pm$ 0.95 <sup>+</sup>
	75	2.8 $\pm$ 0.58 <sup>+</sup>	4.82 $\pm$ 0.32 <sup>+</sup>
	100	4.8 $\pm$ 0.86	7.16 $\pm$ 0.72**
	125	6.4 $\pm$ 0.98*	11.18 $\pm$ 0.88*
	150	4.4 $\pm$ 0.87	8.33 $\pm$ 0.39**

<sup>a</sup>5; <sup>b</sup>average time each animal spends in open arms = total duration in open arms/number of entries in open arms; \* $P < 0.05$  versus control; <sup>+</sup> $P < 0.05$  versus diazepam; ANOVA followed by Studentized Tukey's test. S.E.M., standard error of the mean; NLK, National Laboratory, Kolkata, India; DWSG, Dr Wilmar Schwabe, DHU-Arzneimittel, Germany; SBL, SBL Private Limited, Sahibabad, Ghaziabad, India.

that observed in the control group as well as with the group treated with the standard anxiolytic drug diazepam. Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze (Table 1). Significant anxiolytic activity was observed in NLK (50 mg/kg), DWSG (75 mg/kg) and SBL (125 mg/kg), respectively, which was on a par with that of diazepam as is evident from statistical equivalence between the results of these doses and these manifested by diazepam (Table 1). However, the activity decreased at higher doses, probably due to a sedative effect.

From the above findings, clearly that all three mother tinctures of *T. aphrodisiaca* have significant anxiolytic activity. Dried NLK mother tincture exhibited significant anxiolytic activity at 50 mg/kg followed by DWSG (75 mg/kg) and SBL (125 mg/kg). DWSG and SBL mother tinctures yielded similar percentages (about 2%) of dry residue while NLK mother tincture yielded one-quarter of them. Despite this one-quarter yield, NLK exhibited significant activity at a lower dose in comparison to DWSG and SBL. This could possibly be due to: (i) addition of anxiolytic compound(s) as no effective official control is applicable to these OTC products, or (ii) qualitative and/or quantitative variability in the chemical constituents of *T. aphrodisiaca* depending on the collection sites, season, processing, storage conditions, etc. Phytochemical screening showed that all the mother tinctures have similar classes of

phytoconstituents. Flavonoids, alkaloids or steroids might be responsible for the anxiolytic activity of *T. aphrodisiaca*.

Bearing in mind the variations in biological effects of homeopathic formulations of the three reputed manufacturers, the authors opine that these formulations should be standardized on the basis of bioactive markers. The authors are currently involved in bioactivity-directed isolation of anxiolytic constituent(s) from *T. aphrodisiaca* so that its formulations can be standardized on the basis of biologically active constituent(s).

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