



Evaluation of Anti-anxiety activity of *Azadirachta indica* and *Ocimum sanctum* leaves in Rats

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Abstract

"Anxiety Disorders Association of America (ADAA)" stated anxiety disorder is the most common mental illness in the U.S.A. with 13.3% (19.1 million) of the adult population (ages 18-54) being affected. Anxiety disorders cause costs of more than \$42 billion a year, almost one third of the \$148 billion total mental health bill for U.S.A. Animal models of anxiety have been used in psychopharmacology typically in relative to the achievement or malfunction of a given model in predicting the clinical anxiolytic potency of pharmacological agents. The present paper deals with evaluation of anti-anxiety activity of *A. indica* & *O. sanctum* leaves.

Keywords: Anxiety, Leaves, Evaluation

Introduction

Anxiety is defined as an exaggerated feeling of apprehension, uncertainty and fear. It is an unpleasant state of tension with an anticipation of imminent danger. The somatic manifestation of anxiety includes fatigue, dizziness, palpitation, headache, insomnia and excessive perspiration. It is associated with almost all emotional disorder and frequently with physical illnesses. There is evidence that amygdala is responsible for expression of anxiety or fear and prefrontal cortex plays a role in fear extinction by regulating by the amygdala - mediated expression of fear although the molecular mechanisms underlying negative and positive regulation of the anxiety are not fully understood many genes have been reported to affect anxiety or fear. Anxiety is a pervasive phenomenon the common denominator in most

forms of mental disorder.^[3] Anxiety and mood disorders account for the vast majority of individuals suffering from mental health problems.¹

Azadirachta indica is a small to medium-sized tree, usually evergreen, up to 15 (30 max.) m tall, with a round, large crown up to 10 (20 max.) m in diameter; branches spreading; bole branchless for up to 7.5 m, up to 90 cm in diameter, sometimes fluted at base; bark moderately thick, with small, scattered tubercles, deeply fissured and flaking in old trees, dark grey outside and reddish inside, with colourless, sticky foetid sap.

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Leaves alternate, crowded near the end of branches, simply pinnate, 20- 40 cm long, exstipulate, light green, with 2 pairs of glands at the base, Otherwise glabrous; petiole 2-7 cm long, subglabrous; rachis channeled above; leaflets 8-19, very short petioluled, alternate proximally and more or less opposite distally, ovate to lanceolate, sometimes falcate (min. 2) 3.5-10 x 1.2-4 cm, glossy, serrate; apex acuminate; base unequal. Inflorescence an axillary, many-flowered thyrus, up to 30 cm long; bracts minute and caducous; flowers bisexual or male on same tree, actinomorphic, small, pentamerous, white or pale yellow, slightly sweet scented; calyx lobes imbricate, broadly ovate and thin, puberulous inside; petals free, imbricate, spatulate, spreading, ciliolate inside. Fruit 1 (max. 2)-seeded drupe, ellipsoidal, 1-2 cm long, greenish, greenishyellow to yellow or purple when ripe; exocarp thin, mesocarp pulpy, endocarp cartilaginous; seed ovoid or spherical; apex pointed; testa thin, composed of a shell and a kernel (sometimes 2 or 3 kernels), each about half of the seed's weight.²

Ocimum sanctum has a rich and fanciful history known since the Vedic age for its immense curative and multi-purpose utility. It has been the 'Herb royale' to the French, a sign of love by Italians, and a sacred herb in India. In the first century A.D. Roman naturalist Pliny reported that basil relieves flatulence, which had been subsequently proven true. In the Far East, the herb had been used as a cough medicine, and in Africa, it has been used to expel worms. American colonists considered holy basil is the essential ingredients in a snuff used to ease headaches. The plant grows all over India up to 2000 meters height. It is grown in houses, temples and gardens. An erect annual grows 0.5-1.5 meters in height and has red or purple quadrangular branches. The leaves are opposite, about 2-4 cm long, margins entire or toothed, hairy on both the surfaces, dotted with minute glands and are aromatic. The flowers are tiny, purple and inflorescence is a long spike or 12-14 cm in length. The fruits are small, smooth nut lets, reddish green in color.²

Material and Methods

Extraction

Extraction from leaves of *Azadiracta indica* and *Ocimum sanctum* leaves has performed using soxhlet apparatus.

The method of preparation of aqueous Neem and Tulsi leaf extract (NTLE) was followed as according to Mitra et al. The collected Neem and Tulsi leaves were shade dried and powdered. The dried leaf dusts were soaked overnight in double distilled water (15 g per 100 ml), filtered through loin cloth (fine cotton cloth). The filtrate was centrifuged at 5000 rpm for 10 min (using a REMI cold-centrifuge). The supernatant, thus obtained, was filtered again through loin cloth and the filtrate collected in sterile polypropylene tubes and frozen at -20 °C. The contents of the tubes were then lyophilized and the resulting lyophilized material therein, herein referred to as the aqueous Tulsi leaf extract (NTLE), was stored at -20 °C until further use. A definite amount of the NTLE (the lyophilized material) was always freshly dissolved in double distilled water to give a particular concentration and an aliquot of this solution (not more than 0.5 ml) was fed to rats with the help of a feeding needle. Any leftover of this solution was discarded. The yield of NTLE was 8.33±0.45% (w/w).

Preliminary phytochemical study

The Neem and Tulsi extracts were evaluated for the presence of alkaloids, steroids, and tannins flavonoids reducing sugars and saponin.

Evaluation of antianxiety activity

To evaluate the antianxiety activity of mixture of leaf extract of *azadirachta indica* and *ocimum sanctum* in rats, the plus maze apparatus model has been selected.³⁻⁵

Plus maze apparatus

For this study the 220 gm of albino Sprague Dawly rats (Either sex) were taken. There were three groups of rats. One for the control group, one group represented the positive control or standard group and one group was for test compound. There were 5 rats in each group. The tail of rats was labeled for each group i.e. for control, standard, and test group. The grouping and labeling is also there in table no 1.

Procedure: The plus-maze consists of two open arms, 43 × 15 cm (L × W), and two enclosed arms, 43 × 15.23 cm (L × W × H), open to the top,

arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 70 cm. The rats (200–250 g body weight) are housed in pairs for 10 days prior to testing in the apparatus. During this time the rats are handled by the investigator on alternate days to reduce stress. Groups consist of 6 rats for each dose. Thirty min after i.p. administration of the test drug or the standard, the rat is placed in the center of the maze, facing one of the enclosed arms. During a 5min test period the following measures are taken: the number of entries into and time spent in the open and enclosed arms; the total number of arm entries. The procedure is conducted preferably in a sound attenuated room, with observations made from an adjacent room via a remote control TV camera.

Table 1: Grouping and labeling of animal (Rats)

S. No	Group	Number of Rats in a cage
1	Control	5
2	Standard	5
3	Test, <i>A. Indica</i> and <i>O. sanctum</i> (200 mg/ kg)	5

Results and Discussion

With the help of method explained in experimental section the aqueous extract was collected as the test compound for the evaluation. The identification tests for the chemical constituents of were performed. The following results were there.

Table 2” Observation table for phytochemical test of Neem and Tulsi

S. No	Test for	Reagent/ Test	Observation	Result
1	Alkaloids	Mayer’s	White ppt	+
		Wagner’s	Brown color	+
		Dragondroff’s	Orange-red ppt	+
		Molish	Purple Color	+
2	Carbohydrate	Benedict	Orange ppt	-
		Fehling’s	Red-	+

			Orange	
3	Tannins	FeCl ₃	Green Color	+
4	Saponins	General	Reddish color	+

The experiment was performed using the Plus maze apparatus and the following results were obtained. Readings and data observed using plus maze apparatus (Mean Values, n=5)

Table 3: Antianxiety activity of mixture of leaf extract of *azadirachta indica* and *ocimum sanctum*

Activity	Group	At 15 min	At 30 min	At 1hr	At 3hr	At 6hr
Time Spent in Open Arm (Sec)	Control	2.9	1.8	3.1	1.1	1.3
	Standard (5mg/kg)	2.8	4.9	5.2	4.1	3.3
	Test (200mg/kg)	1.8	4.1	4.3	3.5	2.9
Time Spent in closed Arm (Min)	Control	5.2	10.2	8.5	10.8	9.6
	Standard (5mg/kg)	3.9	5.3	5.2	7.7	3.8
	Test (200mg/kg)	2.9	6.8	4.3	2.9	4.1
Entries in closed arm	Control	0.6	2.0	4.0	2.0	2.0
	Standard	0.5	0.6	0.9	2.5	1.9
	Test	1.0	0.8	1.3	0.8	1.2
Entries in Open Arm	Control	1.1	1.8	0.9	1.0	0.5
	Standard	2.2	3.5	4.5	3.2	2.1
	Test	1.8	2.7	3.8	2.1	1.3

Optimum Mean Values at t = 1hr are shown in the table where n = 5. The observations are Mean ± SD.

Conclusion

In the present study the anti-anxiety activity of *Azadirachta indica* and *Ocimum sanctum* leaves was evaluated in rats with the help of a pharmacological screening methods that is Elevated Plus Maze apparatus method. The data was obtained by performing the models and compared with data obtained from marketed preparation as a standard data. For the evaluation of anti anxiety activity of Neem and Tulsi leaves the standard drug etizolam was used, and for the test compound test -1 was used. In test -1, 200 mg/kg of the ethanolic extract of neem and tulsi

was used. The entries in open and closed arm, head dip in open arm by rat was observed. The results of the study shows that the standard drug was showing the maximum Antianxiety activity and the test compound (200 mg/ kg of leaves extract) was more near to the standard compound then control group. The data of study shows that the Neem and Tulsi leaves have a good antianxiety activity in the modern era of medical science we can convert the neem and tulsi extract in the form of capsule and other dosage form to treat the mental disorders with minimum adverse and side effects.

Table 4: Entries in Open and Closed arms, Head dips

Treatment/ Groups	Entries in Open and Closed arms, Head dips			
	Time spent in open arm (Sec)	Entries in open arms	Entries in closed arm	Number of head dips
Control	3.1 ± 0.43	0.9± 0.23	4 ± 1.8	0.0 ± 0.0
Standard(5 mg/kg)	5.2 ± 0.36	4.5 ± 0.38	0.9 ± 0.6	2.2 ± 0.73
Test, A. <i>Indica</i> and <i>O. sanctum</i> (200 mg/kg) (200 mg/kg)	4.3 ± 0.43	3.8 ± 0.23	1.3 ± 0.2	1.8 ± 0.80

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