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Research Article

Evaluation of Aphrodisiac Activity of Turnera aphrodisiaca

Suresh Kumar[1]*, Reecha Madaan[1] and Anupam Sharma[2]

[1]*S.D. College of Pharmacy, Barnala-148 101, Punjab, India*
[2]*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh*

Abstract
Turnera aphrodisiaca Ward (family Turneraceae) is reputed as an aphrodisiac throughout the world. Despite a long tradition of use as an aphrodisiac, no systematic work has ever been carried out on this plant to validate its traditional use. In the present investigation, various extracts (petroleum ether, chloroform, methanol and water), alkaloidal fraction, and volatile oil of *T. aphrodisiaca* were screened for aphrodisiac activity in mice. Mounting behaviour was taken as a parameter to screen aphrodisiac activity. Chloroform extract exhibited significant activity at a dose of 200 mg/kg, p.o. while methanol extract showed aphrodisiac activity at a lower dose, i.e., 50 mg/kg, p.o.. Volatile oil of *T. aphrodisiaca* was found to be devoid of aphrodisiac activity. Qualitative phytochemical screening showed the presence of alkaloids in chloroform and methanol extracts. Therefore, the alkaloidal fraction was isolated from aerial parts of *T. aphrodisiaca*, and tested for aphrodisiac activity at dose levels of 25, 50, 75, or 100 mg/kg, p.o. A dose dependent increase in activity was observed upon acute as well as subacute administration of alkaloidal fraction.

Keywords: Anti-anxiety; Aphrodisiac; Turneraceae; *Turnera aphrodisiaca*

INTRODUCTION

Turnera aphrodisiaca Ward (synonym *T. 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 (Hocking & Thomas, 1955; Parfitt, 1999). The British Herbal Pharmacopoeia (1983) lists specific indications for Damiana as anxiety neurosis associated with impotency, and includes other indications 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 stimulant (Osol et al., 1947). The leaf infusion of Damiana has been used in the diseases related to the gastrointestinal and respiratory system (Caceres, 1996), reproductive organs (Saggese, 1959), and for the treatment of gonorrhoea (Koch, 1936). Mother tincture of Damiana is an important homoeopathic medicine for the treatment of sexual debility, and nervous prostration (Boericke, 1988).

T. aphrodisiaca has been reported to contain cyanogenic glycoside tetraphyllin B (Spencer & Seigler, 1981); flavonoid gonzalitosin I (Dominguez & Hinojosa, 1976); arbutin (Auterhoff & Hackle, 1968); damianin (Steinmetz, 1960); tricosan-2-one, hexacosanol (Fryer, 1965); volatile oil containing α-pinene, β-pinene, p-cymene, and 1,8-cineole (Auterhoff & Hackle, 1968); and β-sitosterol (Dominguez & Hinojosa, 1976). Aqueous extract of *T. aphrodisiaca* whole plant has been reported to exhibit significant hypoglycaemic activity in alloxan-diabetic male mice (Perez et al., 1984).

***Corresponding author: Prof Suresh Kumar**
S.D. College of Pharmacy Barnala-148 101, Punjab, India
Ph. No.: 01679-243505
Fax : 01679-243505
E-mail: thakur_pu@yahoo.com

In another set of experiments, Aguilara et al. (1998) reported that a decoction of *T. aphrodisiaca* leaves possesses significant hypoglycaemic activity in rabbits upon oral administration. Aqueous extract of the plant has also been reported to exhibit sexual stimulating activity in sexually sluggish male rats at a dose of 1 ml/kg (Arletti et al., 1999).

Authors have reported that amongst various extracts (petroleum ether, chloroform, methanol and water) of *T. aphrodisiaca* aerial parts, only methanol extract (25 mg/kg, p.o.) exhibited significant anti-anxiety activity on elevated plus maze apparatus (Kumar & Sharma, 2005). An anxiolytic constituent apigenin has been isolated from methanol extract of *T. aphrodisiaca* aerial parts using bioactivity-guided fractionation (Kumar & Sharma, 2006).

Despite a long tradition of use of *T. aphrodisiaca* as aphrodisiac, only preliminary work was carried out by Arletti et al. on this plant. Therefore, various extracts viz., petroleum ether (60°-80°C), chloroform, methanol and water, alkaloidal fraction, and volatile oil of *T. aphrodisiaca* were evaluated for aphrodisiac activity in mice.

Materials and Methods

Plant Material

T. aphrodisiaca Ward aerial parts were procured from Rati Ram Nursery, Village Khurrampur, district Saharanpur (U.P.) in the month of August 2002. Identity of the plant was confirmed by the Head, NISCAIR, New Delhi.

Animals

Male and non-oestrous female Laca mice (either sex) were bred at the Central Animal House, Panjab University, Chandigarh. The animals were allowed a standard pellet diet and water *ad libitum*. Groups of five mice (20 – 24 g) were used in all sets of experiments. The animals were fasted for 18 h before use.

Table 2:Aphrodisiac activity of various extracts of T. aphrodisiaca aerial parts.

Treatment	**Dose (mg/kg, p.o.)**	**Average[n] no. of mounts**		
		9:15-9:30 a.m.	**10:30-10:45 a.m.**	**11:45 a.m.-12:00 p.m.**
Control [Distilled water + Tween 80 (5%)]	Vehicle	2.2 ± 0.45	3.0 ± 0.71	0.8 ± 0.45
Petroleum ether extract	50	2.0 ± 0.71	2.6 ± 0.55	0.6 ± 0.55
	100	1.8 ± 0.45	3.0 ± 0.71	0.8 ± 0.84
	200	1.8 ± 0.84	1.6 ± 0.89	1.0 ± 0.71
	400	2.0 ± 0.71	2.2 ± 0.84	1.0 ± 0.71
Chloroform extract	50	2.2 ± 0.45	4.4 ± 0.89	2.2 ± 0.84
	100	3.8 ± 0.84	7.0 ± 1.00*	2.4 ± 0.55
	200	4.6 ± 0.89*	9.2 ± 1.64*	4.2 ± 1.10*
	400	4.4 ± 1.14*	8.8 ± 1.30*	4.4 ± 1.14*
Methanol extract	50	3.2 ± 0.84	9.4 ± 1.34*	3.8 ± 0.84*
	100	3.0 ± 0.71	1.6 ± 0.89	0.0 ± 0.00
	200	3.2 ± 0.84	0.0 ± 0.00	0.0 ± 0.00
	400	2.4 ± 0.55	0.0 ± 0.00	0.0 ± 0.00
Water extract	50	2.6 ± 0.55	2.8 ± 0.84	0.8 ± 0.45
	100	2.0 ± 0.71	2.2 ± 0.45	1.0 ± 0.71
	200	2.0 ± 0.71	2.8 ± 1.64	1.4 ± 0.39
	400	1.8 ± 0.45	2.0 ± 0.71	1.2 ± 0.45

n = 5; *P<0.05 vs. control; ANOVA followed by Fischer's LSD test.

Solvents

***Fig.1: Turnera aphrodisiaca* Ward**

Table 1:Yield of various extracts of *T. aphrodisiaca* aerial parts.

Extract	**Yield (% w/w)**
Petroleum ether	3.61
Chloroform	2.19
Methanol	12.10
Water	5.50

Petroleum ether (60^o - 80^oC), chloroform (Ranbaxy Laboratory Chemicals) and methanol (S.D. Fine Chemicals Pvt.), all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material.

Recovery of solvents

Solvents from extracts were recovered under reduced pressure using Buchi 461 Rotary vacuum evaporator and were preserved in a vacuum desiccator containing anhydrous silica gel blue.

Mounting behaviour

To observe the libido-oriented mounting behaviour, non-oestrous female mice were paired with treated male mice (Subramoniam et al., 1997).

The male mouse assuming the copulatory position over the female was considered as a mount. After the administration of *T. aphrodisiaca* extracts, alkaloidal fraction, or volatile oil, the behaviour of the animals was observed for 3 h (9.00 a.m. - 12.00 p.m.). Males were placed individually after p.o. (i.p. in case of volatile oil) administration of *T. aphrodisiaca* in a clear aquarium (9.00 a.m.) and were allowed to acclimatize for 15 min. After that, a non-oestrous female was introduced into the arena. The number of mounts were recorded for 15 min (9.15 a.m. - 9.30 a.m.). Females were separated for next 60 min interval and were reintroduced again for 15 min, between 10.30 a.m. - 10.45 a.m., during which mounts were recorded. Similarly, the third interaction was done between 11.45 a.m. - 12.00 p.m., and mounts were recorded.

Vehicle

Distilled water + Tween 80 (5%) was used as vehicle for preparing the suspension of various test doses of different extracts. Test doses of volatile oil were prepared using olive oil as vehicle.

Statistics

The results have been expressed as mean ± standard deviation (S.D.). The test doses were compared with control by analysis of variance (ANOVA) followed by Fischer's LSD test (Scheffer, 1980).

Preparation of extracts

Powdered aerial parts (250 g) of *T. aphrodisiaca* were successively extracted in Soxhlet apparatus using the solvents in order of increasing polarity viz., petroleum ether (60^o - 80^oC), chloroform, and methanol. The marc was air dried, and water extract was obtained by boiling with distilled water for 2 h. The aqueous extract was filtered, concentrated and dried in an oven at $40^0 – 50^0C$. Table 1 shows the yield of various extracts. Every extract was then subjected to biological evaluation for aphrodisiac activity in

mice at various dose levels, i.e., 50, 100, 200, or 400 mg/kg, p.o. Mounting behaviour was taken as a parameter to evaluate aphrodisiac activity. Volatile oil was obtained from aerial parts (200 g) of the plant by water distillation using clevenger apparatus, and evaluated for aphrodisiac activity at the doses of 50, 100, 200, or 400 mg/kg, i.p. Since qualitative phytochemical screening showed the presence of alkaloids in chloroform and methanol extracts. Therefore, the alkaloidal fraction was also evaluated for aphrodisiac activity. The alkaloidal fraction was isolated from aerial parts of *T. aphrodisiaca* by following method: Aerial parts (2 kg) of *T. aphrodisiaca* were treated with lime, and then Soxhlet extracted with chloroform. The chloroform extract was concentrated to 1/4th of its original volume under reduced pressure. It was then partitioned in a separator using 5 × 50 ml of 2% acidulated water (HCl - water). The aqueous fraction was basified using NaOH solution to pH 8 – 9 followed by partitioning with chloroform (5 × 50 ml). The chloroform fraction was rich in alkaloids. This alkaloidal fraction was dried, and subjected to aphrodisiac activity upon acute administration of 25, 50, 75, or 100 mg/kg, p.o. Various doses, i.e., 25, 50, 75, or 100 mg/kg of alkaloidal fraction were also administered twice daily (9:00 a.m. and 5:00 p.m.) for nine days in mice. The aphrodisiac activity was observed on the 10th day of administration of respective dose.

Results

Tables 2-4 show the average number of mounts after acute administration (single dose) of various extracts viz., petroleum ether, chloroform, methanol and water (50, 100, 200, or 400 mg/kg, p.o.), volatile oil (50, 100, 200, or 400 mg/kg, i.p.) and alkaloidal fraction (25, 50, 75, or 100 mg/kg, p.o.) respectively. Table 5 shows the average number of mounts after subacute administration of alkaloidal fraction (25, 50, 75, or 100 mg/kg, p.o.).

Discussion

As is evident from table 2, chloroform and methanol extracts were found to be active. Chloroform extract exhibited maximum aphrodisiac activity at the dose of 200 mg/kg while methanol extract at the dose of 50 mg/kg during 2nd h of test with respect to control. The aphrodisiac activity declined with the passage of time, i.e., during 3rd h of the test as the bioactive constituent(s) seem to have undergone a metabolic degradation. Mice did not show any activity at higher doses of

Table 3: Aphrodisiac activity of volatile oil of *T. aphrodisiaca* aerial parts.

Treatment	**Dose (mg/kg, i.p.)**	**Average^{n} no. of mounts**		
		9:15-9:30 a.m.	**10:30-10:45 a.m.**	**11:45 a.m.-12:00 p.m.**
Control [Olive oil]	Vehicle	2.4 ± 0.55	3.2 ± 0.84	1.2 ± 0.45
Volatile oil	50	2.2 ± 0.45	2.0 ± 0.71	1.0 ± 0.71
	100	2.0 ± 1.22	1.6 ± 0.55	1.0 ± 0.71
	200	2.2 ± 1.30	2.6 ± 0.55	1.4 ± 0.89
	400	1.6 ± 0.55	1.8 ± 0.84	0.8 ± 0.45

n = 5; *P<0.05 vs. control; ANOVA followed by Fischer's LSD test.

Table 4:Aphrodisiac activity of alkaloidal fraction of *T. aphrodisiaca* upon acute administration.

Treatment	**Dose (mg/kg, p.o.)**	**Average^{n} no. of mounts**		
		9:15-9:30 a.m.	**10:30-10:45 a.m.**	**11:45 a.m.-12:00 p.m.**
Control [Distilled water + Tween 80 (5%)]	Vehicle	2.2 ± 0.84	2.8 ± 0.89	1.4 ± 0.89
Alkaloidal fraction	25	4.0 ± 0.71	6.0 ± 1.22*	2.2 ± 0.71
	50	4.2 ± 1.10	9.4 ± 1.14*	2.4 ± 0.55
	75	4.0 ± 1.22	10.0 ± 1.87*	3.2 ± 0.84
	100	4.4 ± 1.14	11.2 ± 1.92*	4.0 ± 0.89*

n = 5; *P<0.05 vs. control; ANOVA followed by Fischer's LSD test.

Table 5: Aphrodisiac activity of alkaloidal fraction of *T. aphrodisiaca* upon subacute (9 days) administration.

Treatment	**Dose (mg/kg, p.o.)**	**Average^{n} no. of mounts on 10th day**		
		9:15-9:30 a.m.	**10:30-10:45 a.m.**	**11:45 a.m.-12:00 p.m.**
Control [Distilled water + Tween 80 (5%)]	Vehicle	2.6 ± 0.84	3.8 ± 1.10	1.4 ± 0.89
Alkaloidal fraction	25	4.6 ± 1.67*	12.8 ± 2.59*	4.0 ± 0.89*
	50	5.2 ± 1.09*	14.8 ± 3.03*	3.8 ± 0.84*
	75	5.8 ± 0.84*	14.4 ± 2.30*	5.2 ± 0.71*
	100	6.0 ± 1.22*	15.0 ± 2.55*	5.6 ± 2.07*

n = 5; *P<0.05 vs. control; ANOVA followed by Fischer's LSD test.

methanol due to prevalence of sedative effects. Volatile oil did not exhibit aphrodisiac activity at any of the doses tested (Table 3). A dose dependent increase in aphrodisiac activity was observed in alkaloidal fraction upon acute as well as subacute administration during 2nd h of interaction (Table 4-5). Male mice showed maximum aphrodisiac activity upon sub-acute administration of alkaloidal fraction. This observation infers that T. aphrodisiaca improves reproductive organs upon long term use.

One more interesting observation is that crude methanol extract and purified alkaloidal fraction exhibited significant aphrodisiac activity at similar dose, i.e., 50 mg/kg. This observation suggests that one or more constituent(s) in combination with bioactive alkaloid possess synergistic beneficial effects on reproductive organs. Bioactivity directed fractionation, using various chromatography techniques, work is in progress to isolate bioactive alkaloid from T. aphrodisiaca.

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