Psychopharmacology (1999) 143 : 15–19

ORIGINAL INVESTIGATION

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Stimulating property of *Turnera diffusa* and *Pfaffia paniculata* extracts on the sexual behavior of male rats

Received: 13 January 1998/Final version: 15 September 1998

Abstract Sexually potent and sexually sluggish/impotent male rats were treated orally with different amounts of *Turnera diffusa* and *Pfaffia paniculata* fluid extracts (0.25, 0.50, 1.0 ml/kg). While having no effect on the copulatory behavior of sexually potent rats, both plant extracts – singly or in combination – improved the copulatory performance of sexually sluggish/impotent rats. The highest dose of either extract (1 ml/kg) (as well as the combination of 0.5 ml/kg of each extract) increased the percentage of rats achieving ejaculation and significantly reduced mount, intromission and ejaculation latencies, post-ejaculatory interval and intercopulatory interval. Neither extract affected locomotor activity. These results seem to support the folk reputation of *Turnera diffusa* and *Pfaffia paniculata* as sexual stimulants.

Key words Male sexual behavior · Sexual impotence · Plant extracts · *Turnera diffusa* · *Pfaffia paniculata* · Rat

Introduction

Sexual incompetence, including overt sexual impotence, is a problem of increasing concern (Krane et al. 1989; NIH Consensus Development Panel on Impotence 1993). It is not shown only in humans: for example, a variable percentage of male rats is sexually impotent, i.e. is unable to show consummatory responses such as ejaculation, or even to initiate sexual intercourse with a receptive female. Sexual inadequacy may be the consequence of endocrine diseases, typically diabetes (Kolodny et al. 1973) and hyperprolactinemia (Franks et al. 1978). However, a substantial percentage of impotent subjects (either humans or rats) have no endocrine disturbance. The incidence of sexual inadequacy in human males, and the concern it causes in the affected subjects, are also indirectly indicated by the great number of available treatments (drugs or combinations of drugs for systemic administration, topical application, or intracavernous injection) (Montorsi et al. 1995).

Several plant extracts have been widely used for many years in order to improve sexual performance (Lewin 1931; Gay et al. 1975; Hollister 1975; Kirkorian 1984; Nishimoto et al. 1984; Susset et al. 1989; Popik et al. 1995). In spite of this, pharmacologists have generally disparaged this use, and few experimental studies have been performed in order better to define claims of efficacy (Clark et al. 1984; Taha et al. 1995).

Turnera diffusa and *Pfaffia paniculata* have a wide popular reputation as aphrodisiacs in Latin America, and have been used for a long time as folk medicines to stimulate male sexual drive and performance (Berger 1950). However, no controlled studies with either animals or humans on such supposed sexual stimulatory effects are available.

Pfaffia paniculata is a shrubby vine indigenous to the Amazon basin area and other tropical regions of South America (De Oliveira 1986). The part used is the root, which contains the nortriterpene pfaffic acid, six pfaffic acid saponins named pfaffosides A, B, C, D, E and F, a mixture of stigmasterol and sitosterol and their glycosides, pterosterone, ecdysterone and three ecdysteroid glycosides, for an overall amount corresponding to about 11% by weight of the root (Takemoto et al. 1983; Nakai et al. 1984; Nishimoto et al. 1984,1988). The indigenous peoples of the Amazon region have used the root of *Pfaffia* for centuries as a tonic and an aphrodisiac (De Oliveira 1986).

Turnera diffusa (also named *Damiana aphrodisiaca*) is a small shrub found mainly throughout Mexico, the Caribbean, Central and South America. The

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medicinal parts are the leaves, harvested during the flowering season (Martinez 1959). They contain triacontane, β-sitosterol, hexacosanol, and 5-hydroxy-7,3′,4′-trimethoxyflavone (0.4–0.5% in all) (Dominguez and Hinojosa 1976), α and β-pinene, *p*-cymene, and 1,8 cineole (0.5–0.9% in all), tannins (ca. 4%), resins (ca. 7%), hydroquinone glycosides (ca. 0.2–0.7%), and cyanogenic glycosides (Steinegger and Hänsel 1992). Since the ancient Maya civilization, *Turnera* has been used as an aphrodisiac (Martinez 1959).

The aim of our present study was therefore to evaluate the influence of *Turnera diffusa* and *Pfaffia paniculata* extracts on the copulatory performance of male rats, either sexually impotent or sexually potent, in a well validated animal model.

Materials and methods

Subjects

A total of 324 Sprague-Dawley rats of either sex (274 males and 50 females) were obtained from Charles River (Calco, Como, Italy). They were housed in groups of four or five, males and females separately, in Plexiglas cages (40 × 25 × 15 cm), in climatized colony rooms (21 ± 1°C; 60% humidity) on a 12-h light/dark cycle, with lights off at 7 a.m. The rats were 3-months old on arrival, and weighed 240–260 g (males) and 180–200 g (females). Food in pellets (MIL, Morini, S. Polo d'Enza, Reggio nell'Emilia, Italy) and tap water were available ad libitum. Housing conditions and experimentations were in accordance with the European Community regulations on the care and use of animals for scientific purposes (CEE Council 86/609, included in the Italian D.L. 27/01/92, no. 116). The animals were accustomed to our housing conditions for at least 1 week before being used.

The females were ovariectomized under ethyl ether anesthesia and brought into estrous by the subcutaneous (SC) injection of 15 μg estradiol benzoate followed, 48 h later, by the SC injection of 500 μg progesterone. They were screened with non-experimental sexually experienced males and only those exhibiting good sexual receptivity (solicitation behavior and lordosis in response to mounting) and no rejection behavior, were used.

Behavioural testing procedure

The sexual behavior of males was tested during the period of darkness (between 1000 and 1400 hours) in a sound-proof room, under a dim red light, according to the standard procedure (Dewsbury 1972; Clark et al. 1987). After a 10-min adaptation period in a rectangular glass observation cage (60 × 50 × 40 cm), a stimulus female was presented to the male by dropping it gently into the cage. The following behavioral parameters were recorded or calculated: mount latency and intromission latency, the time from introduction of the female to the occurrence of the first mount or intromission; ejaculation latency, the time from the first intromission to ejaculation; post-ejaculatory interval, the time from ejaculation to the subsequent intromission; mount and intromission frequencies, the number of mounts and intromissions preceding ejaculation; intercopulatory interval, the average interval between successive intromissions (calculated as ejaculation latency divided by intromission frequency); copulatory efficacy, a measure of intromissive success (calculated as intromission frequency divided by mount frequency + intromission frequency). Mount latency, intromission latency, ejaculation latency, post-ejaculatory interval and intercopulatory interval are considered to be inversely proportional to arousal/motivation, while intromission frequency and copulatory efficacy are considered to be indicative of performance/potency (Beach 1956). Tests were terminated immediately after the first post-ejaculatory intromission; or if intromission did not occur within 15 min of female introduction; or if ejaculation latency exceeded 30 min; or if postejaculatory interval exceeded 15 min. Rats were trained with sexually receptive females 7 times, at 5-day intervals, before the experimental test. After the seventh pre-experimental training test, 65% (178 out of 274) satisfied the above criteria of sexual vigor in at least the last three pre-experimental tests (sexually potent). The remaining 96 rats, who failed to achieve ejaculation in one, two or all the last three pre-experimental tests, were considered sexually sluggish/impotent.

Preparation of extracts

Fluid extracts were prepared according to the standard procedure (European Pharmacopoeia 1997) from air-dried *Turnera* leaves and *Pfaffia* roots, identified and kindly supplied by Professor Milton L. Brazzach, Sao Paulo, SP, Brasil. Briefly, *Pfaffia* roots and *Turnera* leaves, separately, were crushed and then mixed thoroughly with 30% ethanol in water (v:v), and allowed to stand in closed percolators overnight. Thereafter, the percolate was allowed to flow slowly, the residue was pressed out, and the expressed fluid was combined with the percolate. The liquid so obtained was concentrated under reduced pressure and at room temperature so that one part by volume was eventually equivalent to one part by weight of the original dried materials.

Treatments

Eight groups of sexually potent, and nine groups of sexually sluggish/impotent rats were assigned by using random, stratified blocking design, so that the means of all groups of potent rats and of all groups of sluggish/impotent rats were roughly the same (in the respective category) on each single measure. Then, the eight groups of potent and the first eight groups of sluggish/impotent rats were randomly assigned to one of the following treatments, by the oral route (PO), 1 h before testing: (1) solvent, 1 ml/kg; (2) *Turnera* extract (T), 0.25 ml/kg; (3) T, 0.5 ml/kg; (4) T, 1 ml/kg; (5) *Pfaffia* extract (P) 0.25 ml/kg; (6) P, 0.5 ml/kg; (7) P, 1 ml/kg; (8) T, 0.5 ml/kg plus P, 0.5 ml/kg. The ninth group of sluggish/impotent rats was treated with T, 1 ml/kg, 2 h before testing. Each group consisted of eight to ten rats. The sexually potent rats left over were discarded from this study (and used for other purposes).

In order to rule out the possibility that modifications of copulatory performance may in fact merely reflect an extract-induced modification of motor activity, the influence of *Turnera* and *Pfaffia* extracts on locomotion was measured, in separate experiments, by means of an ultrasound apparatus (Cibertec, Barcelona, Spain) placed on the lid of the home cage of rats. The activity counts were printed by an external, timer-controlled counter. Locomotor activity was measured for two 10-min periods, separated by a 5-min interval, starting 1 h after extract administration. Only the highest dose was used (i.e., 1 ml/kg fluid extract). Each group consisted of eight rats, and each rat was tested only once and received only one treatment. Rats used for locomotor activity measurement were not used for sexual behavior study.

The experiments were performed in a soundproof room, between 1000 and 1400 hours.

Statistics

The data are presented as means ± SEM; the behavioral ones were analyzed using Kruskal-Wallis analysis of variance (ANOVA)

followed by Mann-Whitney *U*-test; percent data were analyzed using Fisher's exact probability test.

Results

As shown in Table 1, neither *Turnera diffusa* nor *Pfaffia paniculata* extracts, alone or in combination, had any influence on the copulatory behavior of sexually potent rats. On the other hand, as shown in Table 2, both extracts, alone or in combination, significantly improved the copulatory performance of sexually sluggish/impotent rats. In particular, the highest dose of either extract (1 ml/kg) (as well as the combination of 0.5 ml/kg of each extract) increased the percentage of rats achieving ejaculation and significantly reduced mount, intromission and ejaculation latencies and post-ejaculatory interval. The effect was much more evident when treatment was made 2 h, instead of 1 h, before the behavioral test.

Since intromission frequency was not modified by the above treatments, intercopulatory interval came out to be significantly shortened.

Finally, neither *Turnera diffusa* nor *Pfaffia paniculata* extracts, at the dose of 1 ml/kg PO, had any influence on the locomotor activity of adult male rats: the motility counts were 303 ± 2 in saline-treated and 286 ± 30 and 309 ± 21 in *Turnera*- and *Pfaffia*-treated animals, respectively (means ± SEM; eight rats per group).

Discussion

The present data show that fluid extracts of *Turnera diffusa* and *Pfaffia paniculata*, singly or in combination, certainly improve the copulatory performance of sexually sluggish/impotent male rats, while having no significant effect in the case of sexually potent animals.

Indeed, both extracts, alone or in combination, dose-dependently shortened mount, intromission and ejaculation latencies, post-ejaculatory interval and intercopulatory interval; the effect being particularly impressive 2 h after the administration of the highest dose of *Turnera* extract (1 ml/kg). Mount and intromission latencies and post-ejaculatory interval are

Table 1 Influence of *Turnera diffusa* (T) and *Pfaffia paniculata* (P) fluid extracts on male sexual behavior. Groups of eight to ten sexually potent rats. Each value is the mean + SEM. *ML* mount latency; *IL* intromission latency; *EL* ejaculation latency; *MF* mount frequency; *IF* intromission frequency; *PEI* post-ejaculatory interval; *CE* copulatory efficacy; *ICI* intercopulatory interval; *%M* % mounting; *%E* % ejaculating

Treatment (ml/kg PO, 1 h prior to the test)	ML (s)	%M	IL (s)	MF (no.)	IF (no.)	EL (s)	%E	PEI (s)	CE	ICI (s)
Saline 1.0	145 ± 34	100	226 ± 48	4.2 ± 1.4	11.7 ± 2.2	721 ± 85.0	100	568 ± 59	0.74	61.6
T 0.25	195 ± 48	100	250 ± 52	4.9 ± 0.6	14.7 ± 2.4	789 ± 117	100	488 ± 72	0.75	53.7
T 0.50	158 ± 40	100	189 ± 34	4.5 ± 0.3	13.0 ± 1.8	687 ± 106	100	519 ± 62	0.74	52.8
T 1.0	190 ± 36	100	231 ± 68	3.8 ± 0.4	15.2 ± 1.9	643 ± 90.0	100	450 ± 53	0.80	42.3
P 0.25	109 ± 70	100	194 ± 62	4.4 ± 0.4	13.3 ± 2.1	810 ± 109	100	491 ± 71	0.75	60.1
P 0.50	152 ± 43	100	208 ± 48	3.5 ± 0.6	15.0 ± 1.9	740 ± 63.0	100	502 ± 68	0.81	49.3
P 1.0	118 ± 39	100	171 ± 36	3.9 ± 0.4	14.8 ± 2.0	698 ± 42.0	100	463 ± 81	0.79	47.2
T 0.50 + P 0.50	121 ± 58	100	102 ± 28	4.0 ± 1.1	15.2 ± 1.4	590 ± 66.0	100	480 ± 63	0.79	38.8

Table 2 Influence of *Turnera diffusa* (T) and *Pfaffia paniculata* (P) fluid extracts on male sexual behavior. Groups of eight to ten sexually sluggish/impotent rats. Each value is the mean + SEM. *ML* mount latency; *IL* intromission latency; *EL* ejaculation latency; *MF* mount frequency; *IF* intromission frequency; *PEI* post-ejaculatory interval; *CE* copulatory efficacy; *ICI* intercopulatory interval; *%M* % mounting; *%E* % ejaculating

Treatment (ml/kg PO, 1 h prior to the test)	ML (s)	%M	IL (s)	MF (no.)	IF (no.)	EL (s)	%E	PEI (s)	CE	ICI (s)
Saline 1.0	371 ± 48	100	616 ± 90	1.7 ± 1.2	13.6 ± 2.5	1352 ± 132	50	978 ± 79	0.89	99.4
T 0.25	280 ± 60	100	564 ± 84	2.4 ± 1.1	13.8 ± 1.9	1176 ± 89	60	760 ± 58	0.85	85.2
T 0.50	215 ± 33	100	466 ± 71	3.6 ± 0.9	15.3 ± 2.0	1135 ± 96	75	586 ± 71	0.81	74.4
T 1.0	152 ± 37*	100	284 ± 28*	2.5 ± 1.3	15.1 ± 3.2	855 ± 39*	75	512 ± 33*	0.86	56.4*
P 0.25	331 ± 83	100	609 ± 46	2.7 ± 1.5	11.6 ± 2.4	1250 ± 101	50	925 ± 81	0.81	107.8
P 0.50	300 ± 46	100	438 ± 58	3.2 ± 0.8	13.0 ± 2.5	875 ± 85	75	604 ± 64	0.80	67.3
P 1.0	207 ± 36*	100	279 ± 32*	2.8 ± 0.9	15.2 ± 3.2	742 ± 43*	75	550 ± 27*	0.84	48.8*
T 0.50 + P 0.50	131 ± 29*	100	268 ± 18*	4.2 ± 1.8	14.4 ± 2.0	715 ± 51*	75	505 ± 36*	0.77	49.6*
T 1.0, 2 h	46 ± 22*	100	70 ± 24**	4.0 ± 0.7	15.7 ± 1.9	802 ± 69*	100	461 ± 31*	0.80	51.1*

*$P < 0.05$ and **$P < 0.01$, compared with saline-treated rats (Mann-Whitney *U*-test)

considered especially to be inversely proportional to arousal or motivational effects, while between intromissions interval is considered to be inversely proportional to performance or potency (Beach 1956). The shortening of ejaculation latency cannot be considered, in our present case, equivalent of "ejaculatio praecox", because the number of intromissions required to achieve ejaculation was not reduced; simply, treated rats copulated more vigorously than controls. These results thus provide experimental support to the folk reputation of *Turnera diffusa* and *Pfaffia paniculata* as sexual stimulating drugs. It is of special interest that these drugs did not further increase the activity of sexually potent animals, but normalized sexual motivation and performance of sexually sluggish/impotent animals.

Male sexual behavior is regulated by a range of redundant mechanisms involving several neuropeptides (oxytocin and melanocortins, with stimulating activity; opioids, galanin and NPY, with inhibitory activity) and neurotransmitters (mainly dopamine, serotonin, noradrenaline and NO).

With respect to our present results, the following data may be of interest. The stimulatory effect of oxytocin on male sexual behavior is proportionately greater in sexually sluggish than in sexually potent rats (Arletti et al. 1990). Similarly, the opioid antagonist naloxone typically improves the copulatory activity of sexually sluggish/impotent rats (Gessa et al. 1979). An imbalance between opioids and oxytocin in brain structures involved in the control of male sexual activity, with a prevalence of opioids, may underlie a condition of copulatory inadequacy, because in sexually impotent rats there is reduced expression of oxytocin mRNA and increased expression of pro-enkephalin and prodynorphin mRNAs in the paraventricular nucleus of hypothalamus (Arletti et al. 1997). Low, non-stereotypy-inducing doses of direct or indirect dopaminergic drugs improve the copulatory performance of sluggish/impotent males (Gessa and Tagliamonte 1975; Da Prada et al. 1977; Benassi-Benelli and Ferrari 1979; Bertolini et al. 1979; Ferrari et al. 1985), while a further improvement of the sexual behavior of vigorous copulators is not always clearly apparent (Hyyppä et al. 1971; Whalen et al. 1975; Benassi-Benelli and Ferrari 1979; Ferrari et al. 1985; Ferrari and Giuliani 1996). Finally, facilitation of central noradrenergic transmission, either by blockade of alpha 2-adrenoceptors or by stimulation of beta 2-adrenoceptors, while having either no effect or a worsening effect in sexually potent rats, improves copulatory behavior in sexually sluggish rats (Benelli et al. 1989, 1993).

A close relationship exists between these neuropeptidergic and monoaminergic systems involved in the regulation of male sexual behavior: central oxytocinergic transmission is also increased by catecholaminergic agonists (for review, see Argiolas 1992).

So, from our present data, it would appear that the plant extracts used in this study, which selectively improve the sexual behavior of sluggish/impotent rats, while being ineffective in potent rats, might act mainly by increasing central noradrenergic and dopaminergic tone, and possibly (indirectly) oxytocinergic transmission.

Acknowledgements This work was supported in part by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Roma, Italy.

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