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Antiparkinsonian-like effects of *Plumbago scandens* on tremorine-induced tremors methodology

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Abstract

Tremorine-induced tremors model is used to evaluate antiparkinsonian drugs because rest tremor is a sign that distinguishes Parkinson's disease (PD) from other diseases. The effects of crude ethanolic extract (CEE) and total acetate fraction (TAF) of *Plumbago scandens* were investigated at several doses. These extracts at doses of 125 and 250 mg/kg i.p. failed to reduce tremors in tremorine-treated mice. TAF showed significant effects only at a dose of 500 mg/kg. Both CEE and TAF at doses of 1000 and 2000 mg/kg i.p. suppressed the tremors in a dose-dependent fashion for 60 min. Biperiden, an anticholinergic drug, was used as standard at a dose of 3 mg/kg i.p. This study suggests that *P. scandens* is a plant with possible therapeutic value for PD.

Keywords: Parkinson's disease; L-DOPA; Tremor; *Plumbago scandens*; Tremorine; Mice

1. Introduction

Parkinson's disease, a progressive disorder of the Central Nervous System (CNS), is caused by the degeneration of dopaminergic neurons in the substantia nigra of the midbrain and is characterized by tremor, muscular rigidity, bradykinesia, difficulty with balance and walking, depression and dementia (Young, 1999). The rest tremor is a sign that distinguishes Parkinson's disease from other diseases and its medical treatment while initially effective later may become ineffective (Carr, 2002). Experimental animal models of tremor have predominantly been applied to investigate drugs with possible therapeutic value for PD tremor. Tremorine, of which the active metabolite is oxotremorine, has been used to produce tremor in mice. Oxotremorine is a selective agonist of the muscarinic acetylcholine receptor, and systemic application of tremorine stimulates acetylcholine receptors both in the periphery and also cholinergic receptors in the basal ganglia in the CNS (Gerlanch and Riederer, 1996).

In recent years, there has been increased interest in drugs extracted from plants, and this is due to several reasons, namely, conventional medicine can be inefficient, abusive and/or incorrect use of synthetic drugs results in side effects and a large percentage of the World's population does not have access to conventional pharmacological treatment (Rates, 2001). In addition, natural products are viewed as templates for structure optimization programs designed to develop new drugs. (Newman et al., 2003; Raskin et al., 2002). The exploration of ethnopharmacological treatments may be a cost effective alternative, since naturally occurring compounds have been used in the treatment of Parkinson's disease. Based on Ayurvedic principles, a concoction in cow's milk of powdered *Mucuna pruriens*, *Hyoscyamus reticulates* seeds, *Withania somnifera* and *Sida cordifolia* roots is prescribed for treating Parkinson's disease and commercial preparations of *M. pruriens* are also available in India (Nagashayana et al., 2000).

Plumbago scandens L (Plumbaginaceae), commonly called "louco", "folhas de louco", "herva do diabo" and "Jasmim azul", is a plant used popularly to treat stomach infections, ulcers, heart disease and against intermittent fever. Antitumor (Moraes et al., 1997), antibacterial and antifungal

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activity (Gutierrez-Lugo et al., 1996) has been reported and MsC work carried out in our laboratory showed a possible antiparkinson effect in a preliminary pharmacological screening (data not published).

This paper reports the effects of crude ethanolic extract and total acetate fraction of *P. scandens* on tremors induced by tremorine.

2. Material and methods

2.1. Animals

Male Swiss albino mice (25–32g) were used. They were kept in polypropylene cages at room temperature of 21±2 °C with 12 h light/dark cycles and had free access to food and water. The experiments were always conducted between 11:00 and 17:00 h.

The animal experiments were performed according to internationally followed ethical standards and approved by the Laboratório de Tecnologia Farmacêutica/UFPB Animal Care and Use Committee.

2.2. Plant material

P. scandens was collected throughout August and September 2002 and a voucher specimen (Agra et al. 5607) prepared by Professor Maria de Fátima Agra of the Botany Section is deposited in the JPX Herbarium of the Laboratório de Tecnologia Farmacêutica, Federal University of Paraíba. The whole plant was dried and the powdered plant material was extracted exhaustively with 90% EtOH in Sohxlet for 24 h. The extract was evaporated under reduced pressure and the residue yield was approximately 22%. This residue was suspended in the MeOH/H_2O (3:7) with mechanical agitation for 30 min. Next it was partitioned with hexane, chloroform and ethyl acetate which were concentrated under vacuum to obtain the fractions free of solvents.

The resulting fractions were analyzed by commercial TLC aluminium sheets with Merck silica gel 60 F254 and the visualization of the spots was made by spraying diphenylboryloxyethylamine (NP) solution (1%) in MeOH and also $AlCl_3$-EtOH solution (1%), observing the presence of flavonoids under UV light at 366 nm.

Extracts and the fractions were dissolved with cremophor drops and distilled water. The pH of the extract was neutral using Litmus paper before the administration.

2.3. Drugs

Tremorine (1,4-dipyrrolidino-2-butyne) dihydrochloride, an cholinergic muscarinic agonist, was bought from Sigma (St. Louis, MO) and Biperiden lactate (anticholinergic) obtained from Akineton (Knoll). Drugs were injected intraperitoneally (i.p.) in a dose volume of 1 ml/100 g/mouse.

2.4. Preliminary psychopharmacology screening

Crude ethanolic extract (CEE) and total acetate fraction (TAF) of *P. scandens* (1000 mg/kg, i.p.) were administered to groups of mice (n=10) and a wide variety of grossly observable changes were quantified (e.g. behavioral, neurological, autonomic and toxic effects). After the treatment with CEE and TAF, mice were observed every 30 min up to 4 h for studying behavioral changes (Cifuentes et al., 2001). Control group received the vehicle (distilled water and cremofor).

2.5. Effects of P. scandens on rotarod test

Mice were placed on a horizontal rotating rod at a rate of 6 rpm. Animals able to remain on the rod for longer than 3 min were selected and divided into five groups (n=10). CEE and TAF of *P. scandens* (1000 and 2000 mg/kg, i.p.) were injected to the test groups; the control received the vehicle (distilled water and cremofor). After 30 min, animals were placed on the rod at intervals of 30 min, up to 3 h. If an animal failed more than once to remain on the rod for 3 min, the test was considered positive, namely, motor incoordination was present (Perez et al., 1998).

2.6. Effects of P. scandens on tremorine-induced tremors

The mice were divided into groups of 10 animals each and pretreated with the CEE and TAF of *P. scandens* (125, 250, 500, 1000 and 2000 mg/kg, i.p.) and biperiden lactate (1, 2 and 3 mg/kg, i.p.). The control group was treated with vehicle (distilled water and cremofor). Tremorine, at dose of 6 mg/kg i.p., was administered 30 min after the pretreatment. Tremors were scored visually in individual animals at intervals of 10–60 min after tremorine administration using a rating scale of 0 to 3, as described by Coward et al. (1977) and Fukuzaki et al. (2000): 0=no tremor; 1=occasional isolated twitches; 2=moderate or intermittent tremor associated with short periods of quiescence; 3=pronounced continuous tremor.

2.7. Statistical analysis

The data obtained were evaluated by Kruskal–Wallis test with Dunn's Multiple comparison tests in GraphPad Prism 3.02, GraphPad Software. Significance was accepted for values of $p<0.05$.

3. Results

CEE and TAF (1000 mg/kg) produced effects on gross behavior. Decreased locomotor activity, catalepsy (reversible by stimuli) and parpebral ptosis were observed. In the rotarod test, no motor incoordination was found at doses of 1000 and 2000 mg/kg i.p. of CEE and TAF of *P. scandens*.

Biperiden (3 mg/kg i.p.) significantly reduced tremorine-induced tremor to 60 min. The doses of 125 and 250 mg/kg

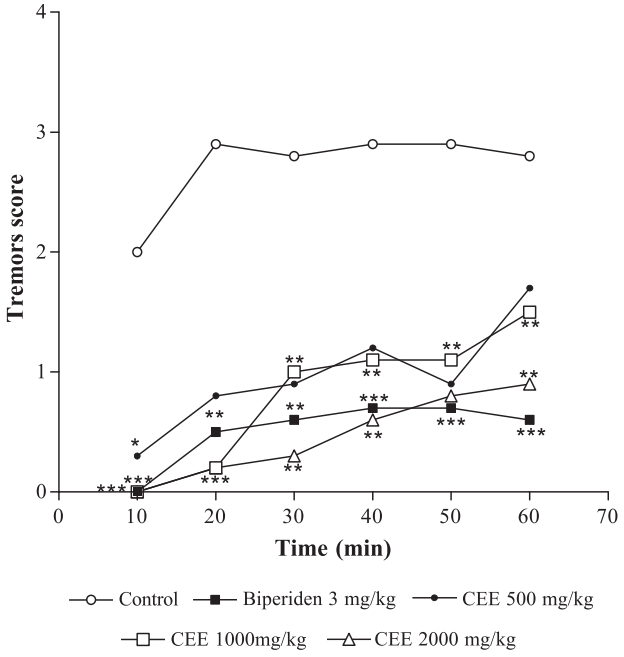

Fig. 1. Effects of biperiden (3 mg/kg i.p.) and CEE 500, 1000 and 2000 mg/kg on the tremorine 6 mg/kg i.p. induced tremor in mice. Each point represents the mean (N=10). *p<0.05, **p<0.01 and ***p<0.001 compared to vehicle-treated animals. Kruskal–Wallis/Dunn's test.

i.p. of CEE or TAF failed to reduce tremors in tremorine-treated mice. Although, at dose of 500 mg/kg i.p., CEE failed to reduce tremors, TAF, at the same dose level, showed the similar effects obtained with higher doses. Both CEE and TAF of *P. scandens*, at doses of 1000 and 2000 mg/kg i.p., suppressed the tremors dose dependently during the observation time (Figs. 1 and 2). The reduction of tremors was associated with the decrease of salivation and piloerection (data not showed).

4. Discussion

Tremors can be produced by oscillation in sensorimotor loops, so-called mechanical-reflex tremors, and produced by the oscillatory properties of central neuronal networks. The tremors are classified in physiologic, essential, Parkinson (resting tremor) and cerebellar tremors, being also verified unusual forms of tremors (Elble, 1996). Tremorine, via oxotremorine, a cholinergic muscarinic agonist induces its effects by stimulation of neurons of basal ganglia (Llinás and Yarom, 1981; Llinás and Yarom, 1986) and produced tremors that resemble to the rest tremor that is characteristic of patients with PD (Slater and Dickinson, 1982) and (Wang and Fowler, 2001). The brain areas that initiate cholinergic tremor are uncertain although the striatum with its very high density of muscarinic cholinergic receptors is a favoured area (Kobayashi et al., 1978).

Parkinson's disease (PD) results from the degeneration of dopamine neurons in the substantia nigra and this depression of dopaminergic function promotes an increase of cholinergic action. Therefore, drugs with both anti-muscarinic and anti-nicotinic activity are used for treatment of PD (Cousins et al., 1997; Gao et al., 1998).

Anticholinergic drugs, such as Biperiden (Brocks, 1999) and Budipine (Klockgether et al., 1996) may be used as initial treatment, particularly the tremor, of PD.

Biperiden by antagonization of cholinergic neurons reverts the tremorine induced-tremors. The tremors score shows that 3 mg/kg i.p. of biperiden significantly reduced the effects of tremorine (6 mg/kg i.p.), in mice for 60 min, and thus the effect of biperiden was compared with the results obtained with CEE and TAF of *P. scandens*. Dose of 1 mg/kg i.p. was ineffective to reduce the tremors by a period of 60 min. In the dose of 2 mg/kg i.p., the biperiden's effects appear only after 30 min.

Administration of tremorine, within 5 or 10 min, produced tremors, profuse salivation, urination and piloerection. These effects are the result of its conversion to oxotremorine in the body (Sharif and Ali, 1994), which relates the enhancement of the tremor (Shinozaki et al., 1987). Tremorine is a prodrug that is activated in vivo (Sethy and Francis, 1990) and whose effects are antagonized by therapeutically effective antiparkinson drugs being used as a model in the screening of drugs with perspectives for the treatment of PD (Dickinson et al., 1981). It is reasonable to assume that these effects produced by tremorine originate in brain areas that have muscarinic receptors and a motor function. For these reasons and because cholinomimetics produced tremor when injected intrastriatally in the rat, the site of tremor production may be the neostriatum (Dickinson and Slater, 1982).

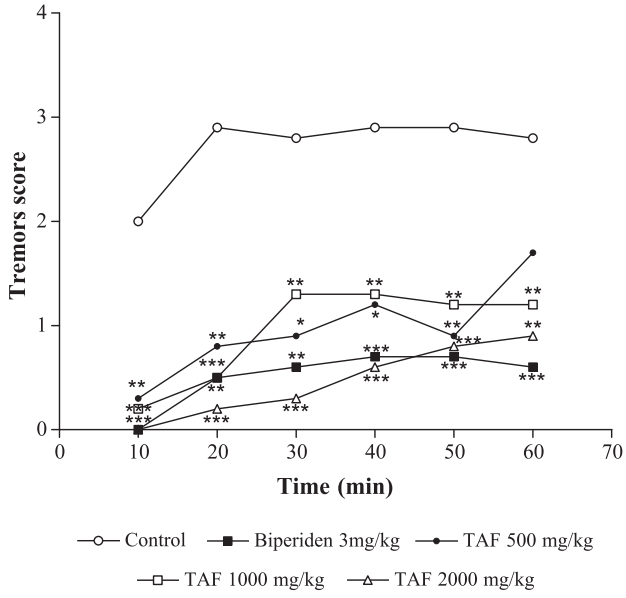

Fig. 2. Effects of biperiden (3 mg/kg i.p.) and TAF 500, 1000 and 2000 mg/kg on the tremorine 6 mg/kg i.p. induced tremor in mice. Each point represents the mean (N=10). *p<0.05, **p<0.01 and ***p<0.001 compared to vehicle-treated animals. Kruskal–Wallis/Dunn's test.

The seeds of the Datura plant have an anticholinergic effect and have been employed for several decades as antiparkinsonian drug (Hussain and Manyam, 1997). Pimprinine, an extracellular alkaloid produced by *Streptomyces*, inhibited tremorine induced pharmacological effects in mice, thereby indicating that this substance may have an antiparkinsonian-like activity (Naik et al., 2001).

Previous phytochemical studies of *P. scandens* resulted in the isolation of flavonoids (Agra and Barbosa-Filho, 1990) and plumbagin, a quinoid, which seems to be the major constituent of this plant (Harbone, 1967; Bhattacharyya and De Carvalho, 1986).

The significant reduction observed with 1000 and 2000 mg/kg (i.p.) of CEE and TAF on tremors in tremorine-treated mice is a sign of need for more complete studies to investigate the possible therapeutic value of *P. scandens* for PD tremor. In addition, the active compounds are probably present in the total acetate fraction because the effects observed were more pronounced. The reduction of tremors score with 500 mg/kg i.p. of TAF and the absence of effects with CEE at same dose reinforce the observation that the total acetate fraction contains the active substances.

Decrease locomotor activity, the presence of catalepsy and palpebral ptosis are indicators of the depressant activity of Central Nervous System. The action over the tremors is associated to central effects since no motor incoordination was observed with CEE and TAF in rotarod test.

5. Conclusion

In conclusion, *P. scandens* has shown activity on tremorine-induced tremor. Both TAF and CEE showed an antitremor activity with some potency differences. Further phytochemical and pharmacological studies need to be carried out with these fractions.

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References

Agra MF, Barbosa-Filho JM. Levantamento da flora medicinal da Paraíba e triagem fitoquímica. Rev Bras Farm 1990;71(3):72–6.

Bhattacharyya J, De Carvalho VR. Epi-isoshinanolone from *Plumbago scandens*. Phytochemistry 1986;25(3):764–5.

Brocks DR. Anticholinergic drugs used in Parkinson's disease: an overlooked class of drugs from a pharmacokinetic perspective. J Pharm Pharm Sci 1999;2(2):39–46.

Carr J. Tremor in Parkinson's disease. Parkinsonism Relat Disord 2002;8:223–34.

Cifuentes CM, Gómez-Serranillos MP, Iglesias I, Villar del Fresno AM. Neuropharmacological profile of ethnomedicinal plants of Guatemala. J Ethnopharmacol 2001;76:223–8.

Cousins MS, Carriero DL, Salamone JD. Tremulous jaw movements induced by the acetylcholinesterase inhibitor tacrine: effects of antiparkinsonian drugs. Eur J Pharmacol 1997;322:137–45.

Coward DM, Doggett NS, Sayers AC. The Pharmacology of *N*-carbamoyl-2-(2-6-dichlorophenyl)acetamidine hydrochloride (LON-954) a new tremorogenic agent. Arzneim-Forsch/Drug Res 1977;27:2326–32.

Dickinson SL, Slater P, Longman DA. Effects of naltrexone and prolyl-leucyl-glycinamide on drug-induced tremor and rigidity in the rat. Neuropharmacology 1981;20:757–62.

Dickinson SL, Slater P. Effect of lesioning dopamine, noradrenaline and 5-hydroxytryptamine pathways on tremorine-induced tremor and rigidity. Neuropharmacology 1982;21:787–94.

Elble RJ. Central mechanisms of tremor. J Clin Neurophysiol 1996;13(2):133–44.

Fukuzaki K, Kamenosono T, Nagata R. Effects of Ropinirole on various parkinsonian models in mice, rats and cynomolgus monkeys. Pharmacol Biochem Behav 2000;65(3):503–8.

Gao ZG, Liu BY, Cui WY, Li LJ, Fan QH, Liu CG. Anti-nicotinic properties of anticholinergic antiparkinson drugs. J Pharm Pharmacol 1998;50:1299–305.

Gerlanch M, Riederer P. Animal models of Parkinson's disease: an empirical comparison with the phenomenology of disease in man. J Neural Transm 1996;103:987–1041.

Gutierrez-Lugo MT, Barrientos-Benitez T, Luna B, Ramirez-Gama RM, Bye R, Linares E, et al. Antimicrobial and cytotoxic activities of some crude drug extracts from Mexican medicinal plants. Phytomedicine 1996;2(4):341–7.

Harbone JB. Comparative biochemistry of the flavonoids: IV. Correlations between chemistry, pollen morphology and systematics in the family plumbaginaceae. Phytochemistry 1967;6:1415–28.

Hussain G, Manyam BV. *Mucuna pruriens* proves more effective than L-DOPA in Parkinson's disease animal models. Phytother Res 1997;11:419–23.

Kobayashi RM, Palkovits M, Hruska RE, Rothschild R, Yamamura HI. Regional distribution of muscarinic cholinergic receptors in rat brain. Brain Res 1978;154:13–23.

Klockgether T, Wullner U, Steinbach JP, Petersen V, Turski L, Loschmann PA. Effects of the antiparkinsonian drug budipine on central neurotransmitter systems. Eur J Pharmacol 1996;301:67–73.

Llinás R, Yarom Y. Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurons in vitro. J Physiol 1981;351:569–84.

Llinás R, Yarom Y. Oscillatory properties of guinea-pig inferior olivary neurons and their pharmacological modulation: an in vitro study. J Physiol 1986;376:163–82.

Moraes MO, Fonteles MC, Moraes MEA, Machado MIL, Matos FJA. Screening for anticancer activity of plants from the northeast of Brazil. Fitoterapia 1997;68(3):235–9.

Nagashayana N, Sankarankutty P, Nampoothiri MRV, Mohan PK, Mohanakumar KP. Association of L-DOPA with recovery following Ayurveda medication in Parkinson's disease. J Neurol Sci 2000;176(2):124–7.

Naik SR, Harindran J, Varde AB. Pimprinine, an extracellular alkaloid produced by *Streptomyces* CDRIL-312: fermentation, isolation and pharmacological activity. J Biotechnol 2001;88:1–10.

Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003;66:1022–37.

Perez GRM, Perez LJA, Garcia DLM, Sossa MH. Neuropharmacological activity of *Solanum nigrum* fruit. J Ethnopharmacol 1998;62:43–8.

Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, et al. Plants and human health in the twenty-first century. Trends Biotechnol 2002;20(12):522–31.

Rates SMK. Plants as source of drugs. Toxicon 2001;39:603–13.

Sethy VH, Francis JW. Pharmacokinetics of muscarinic cholinergic drugs as determined by ex vivo (3H)-oxotremorine-M binding. J Pharmacol Methods 1990;23:285–96.

Sharif SI, Ali BH. Effects of grapefruit juice on drug metabolism in rats. Food Chem Toxicol 1994;32(12):1169–71.

Shinozaki H, Hirate K, Ishida M. Modification of drug-induced tremor by systemic administration of kainic acid and quisqualic acid in mice. Neuropharmacology 1987;26(1):9–17.

Slater P, Dickinson SL. Effects of lesioning basal ganglia nuclei and output pathways on tremorine-induced tremor in rats. J Neurol Sci 1982; 57:235–47.

Wang G, Fowler SC. Concurrent quantification of tremor and depression of locomotor activity induced in rats by harmaline and physostigmine. Psychopharmacology (Berl) 2001;158:273–80.

Young R. Update on Parkinson's disease. Am Fam Phys 1999;59(8): 2155–67.