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Behavioural effect of *Pavetta crassipes* extract on rodents

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Abstract

The effects of the ethanol extract of *Pavetta crassipes* on the central nervous system (CNS) and on actions of some selected centrally acting drugs were studied in mice and rats. These studies were carried out using the spontaneous motor activity (SMA), amphetamine-induced hyperactivity and stereotyped behaviour, pentobarbital-induced hypnosis and exploratory activity, apomorphine-induced climbing and haloperidol-induced catalepsy in rats. The results demonstrated that the extract of *P. crassipes* dose-dependently decreased SMA in mice and attenuated amphetamine-induced hyperactivity and the different episodes of stereotypic behavioural patterns induced by amphetamine. In addition, the extract decreased the number of head dips in the exploratory activity test and potentiated pentobarbital-induced sleeping time in rats. Furthermore, the extract inhibited apomorphine-induced climbing in mice and potentiated haloperidol-induced catalepsy in rats. Our results suggest that the extract of *P. crassipes* contains biologically active substance(s) that might be acting centrally through the inhibition of dopaminergic pathway or a system linked to this pathway to mediate the observed pharmacological effects.

Keywords: *Pavetta crassipes*; Dopaminergic pathways; Behaviour; Central nervous system; Sleep

1. Introduction

Recently, there has been increasing interest in the use of plants to treat diseases. The plant kingdom has become a target for the search of new drugs and biologically lead compounds by multinational drug companies and research institutes (Evans, 1996). The World Health Organization (WHO) has recommended, especially in developing countries, the initiation of programs designed to use medicinal plants more effectively in traditional health care systems (WHO, 1978). The resolution of the 31st WHO assembly requested a complete inventory, evaluation of the efficacy and safety and standardization of medicinal plants (Farnsworth, 1980). The plant *Pavetta crassipes* K. Schum (family: Rubiaceae) is a widely distributed shrub in the West African subregion. The leaves are eaten as food in West Africa and are used for the treatment of fevers and schistosomiasis (Irvine, 1961). It is called Gadu (Hausa) and Lolubo (Yoruba). Moreover, reports by Chhabra et al. (1991) and Gbeassor et al. (1989) indicated that the leaves of *P. crassipes* are used traditionally for the treatment of mental illness, convulsions, malaria and hookworms. Our laboratory has also reported some of the biological activities of *P. crassipes*, which include inhibitory effects on the gastrointestinal and uterine smooth muscles, its anti-inflammatory and muscle relaxant effect. (Amos et al., 1998a,b). There is no documented literature on the effect of *P. crassipes* on the central nervous system (CNS). It is on this basis that we investigated the possible neuroleptic potential of this widely used plant in the management of mental illness.

2. Materials and methods

2.1. Animals

Adult Wistar rats (180–220 g each), Swiss albino mice (20–25 g each) of either sex maintained at the Animal

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Facility Center (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used. All animals were housed under standard conditions of temperature and light (12-h light/12-h dark cycle) and fed on standard diet (Ladokun feeds, Ibadan, Nigeria) and water ad libitum. These animals were approved for use by the AFC committee after reviewing the protocol for good laboratory practice and animal handling, which is in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Publication No. 85-23, revised 1985).

2.2. Preparation of plant material

Fresh leaves of *P. crassipes* were collected from Minna, Niger State, Nigeria. The plant material was collected between the months of April and June 2001. The plant material was identified and authenticated by the late Mr. Abraham Ohaeri and Mallam Ibrahim Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja. Herbarium specimen (No. 4745) was deposited at NIPRD for future reference. The leaves were cleaned, air-dried and crushed into coarse powder using a pestle and mortar. Five hundred grams of the pulverized leaf was cold-macerated with 2.5 l of ethanol for 24 h with constant shaking using the GFL shaker (No. 3017 MBH, Germany), the resulting mixture was filtered using Whatman filter paper. The filtrate was then concentrated in vacuo at 40 °C using the rotary evaporator to give a mean yield of 20% w/w.

2.3. Pharmacological evaluation

2.3.1. Studies on spontaneous motor activity (SMA)

Adult mice of either sex were divided randomly into five groups of eight mice each. Three different groups were given the extract at doses of 50, 100 and 200 mg/kg po, another group received saline as control, while the last group received chlorpromazine (2 mg/kg ip) and served as positive control. Thirty minutes post drug administration, the animals were transferred individually to Letica activity cages (LE 886) consisting of four ventilated motor cages connected to a multicounter (LE 3806). Activity was automatically recorded after a 1-min latency period for 6 min at 30-min intervals for a period of 120 min (Amos et al., 2001a). In another set of experiments, adult mice of either sex were randomly divided into five groups of six mice. Animals in Groups 1, 2 and 3 were pretreated with graded doses of the extract (50, 100 and 200 mg/kg po), while animals in Groups 4 and 5 were pretreated with chlorpromazine (2 mg/kg ip) and normal saline (10 ml /kg po), respectively. Thirty minutes later, *d*-amphetamine (2 mg/kg ip) was administered to all the mice. Hyperactivity was measured using the Letica activity cages (LE 886) connected to a multicount (LE 3806); after a 1-min latency, activity was recorded for 6 min every 30 min up to 120 min.

2.3.2. Stereotype behavioural studies in mice

The methods of Randrup and Munkvad (1967) and Ellinwood et al. (1973) were used for the stereotyped

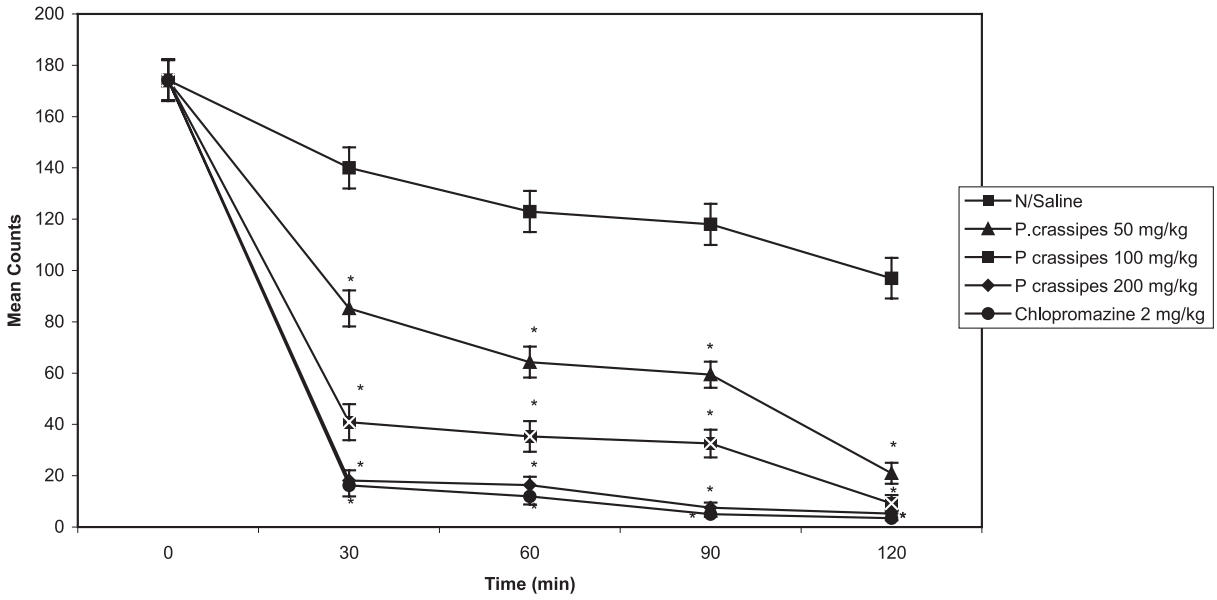

Fig. 1. Effect of the ethanol extract of *P. crassipes* on spontaneous motor activity in mice following different drug treatments. Saline 10 ml/kg (■), *P. crassipes* 50 mg/kg (▲), *P. crassipes* 100 mg/kg (●), *P. crassipes* 200 mg/kg (⊠) and chlorpromazine 2 mg/kg (♦) [$F(4,29) = 7.05$; $P < .05$]. * Statistical significance between treatment groups and control.

behavioural studies. The test was performed in five groups of eight mice each. The first group received saline as control, while animals in Groups 2, 3 and 4 received the extract of *P. crassipes* at doses of 50, 100 and 200 mg/kg po, respectively. The fifth group received chlorpromazine 2 mg/kg ip. Thirty minutes after saline and drug administration, the animals were injected with amphetamine (2 mg/kg ip). The signs of stereotype behaviour that included circling, jumping, sniffing and general locomotion were recorded by an observer unaware of the treatment (to remove bias) every 5 min for a period of 2 h by using a tally counter (Irwin, 1968).

2.3.3. Apomorphine-induced climbing studies in mice

Adult mice of either sex weighing between 20 and 22 g were randomly divided into four groups of eight mice each. The first group received normal saline (10 ml/kg po) and served as control. Groups 2, 3 and 4 received the extract at doses of 50, 100 and 200 mg/kg ip. Thirty minutes after treatment, all mice were injected with apomorphine (3 mg/kg sc). Readings were taken at 10, 20 and 30 min after apomorphine (3 mg/kg sc) administration. The mice were observed for climbing behaviour and scored as follows: 0 = four paws on the floor; 1 = forefeet holding the vertical bars; and 2 = four feet holding the vertical bars (Costall et al., 1978).

2.3.4. Measurement of catalepsy

Rats were treated with the extract at doses of 50, 100 and 200 mg/kg po. The severity of catalepsy was measured every 30 min thereafter up to a total of 3 h. Control groups received normal saline (10 ml/kg po). Catalepsy of an individual rat was measured in a stepwise manner by a scoring method as described by Khisti et al. (1997). The method assessed the ability of an animal to respond to an externally imposed posture:

Step I The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched gently on the back or pushed, a score of 0.5 was assigned.

Step II The front paws of the rat were placed alternately on a 3-cm-high block. If the rat failed to correct the posture within 15 s, a score of 0.5 for each paw was added to the score of Step I.

Step III The front paws of the rat were placed alternately on a 9-cm-high block. If the rat failed to correct the posture within 15 s, a score of 1 for each paw was added to the scores of Steps I and II.

Thus, for an animal, the highest score was 3.5 (cutoff score). The effect of the extract (50, 100 and 200 mg/kg) on

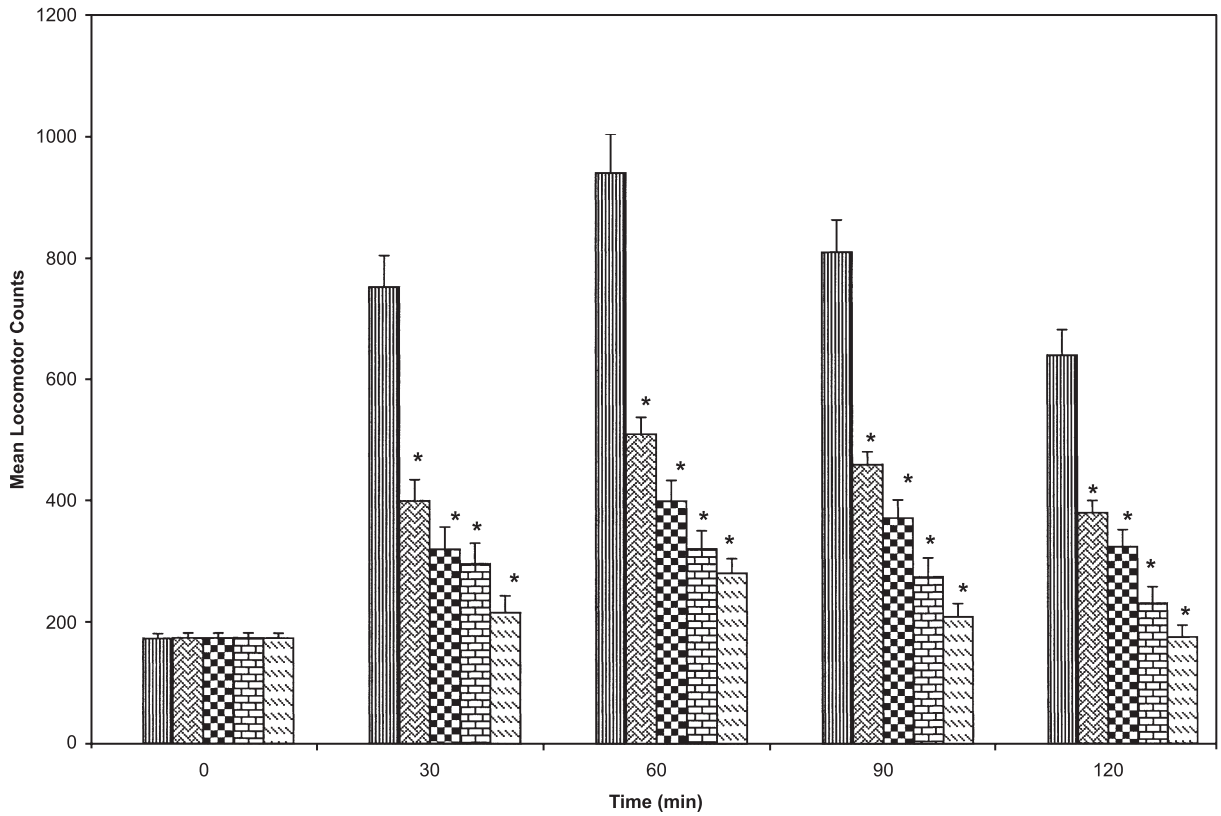

Fig. 2. Effect of the ethanol extract of *P. crassipes* on amphetamine (2 mg/kg)-induced hyperactivity in mice. Amphetamine 2 mg/kg + saline 10 ml/kg (▥), amphetamine 2 mg/kg + *P. crassipes* 50 mg/kg (▨), amphetamine 2 mg/kg + *P. crassipes* 100 mg/kg (▩), amphetamine 2 mg/kg + *P. crassipes* 200 mg/kg (▤) and amphetamine 2 mg/kg + chlorpromazine 2 mg/kg (▧) [$F(4,29) = 6.25$; $P < .05$]. * Statistical significance between treatment groups and control.

haloperidol-induced catalepsy was also investigated. The extract and normal saline (control) were administered intraperitoneally 30 min before haloperidol (Reavill et al., 1999).

2.3.5. Pentobarbital-induced sleeping time

The test was carried out in a total of 25 rats of either sex grouped into five ($n=5$) and treated as follows: animals in Group 1 received normal saline (1 ml/kg); animals in Groups 2, 3 and 4 received the extract at doses of 50, 100 and 200 mg/kg po, respectively; and animals in Group 5 received diazepam (1 mg/kg ip). Thirty minutes after treatment, all animals were given pentobarbital sodium (35 mg/kg ip). Each rat was observed for the onset and duration of sleep, with the criterion for sleep being loss of righting reflex (Wambebe, 1985; Amos et al., 2001b). The time from the loss of righting reflex to recovery was recorded as the sleeping time (Soulimani et al., 2001). In another series of experiments, rats were randomly divided into three groups ($n=5$) and were treated with the extract alone at doses of 50, 100 and 200 mg/kg. They were observed for onset and duration of sleep.

2.3.6. Exploratory behaviour in mice

This study was done using the head-dip test on the hole board (Amos et al., 2001a; Perez et al., 1998). Mice of either sex were divided into five groups of six mice each. Animals in Group 1 received normal saline and served as control, while those in Groups 2, 3 and 4 received the extract at doses of 50, 100 and 200 mg/kg po, respectively. The animals in Group 5 received clonazepam (0.20 mg/kg ip). Thirty minutes after treatment, mice were placed singly on an automatic Letica board with 16 evenly spaced holes and a counter (Letica LE 3333). The number of times the mice dipped their heads into the holes during the 5-min period was counted (File, 1973; Wolfman et al., 1994).

2.3.7. Test on motor coordination (Rota-Rod test)

The method used for the assessment of locomotor (forced motor) activity in mice was as described previously (Perez et al., 1998). The mice were placed on a horizontal rotating rod (Ugo Basile, Acceler Rota-Rod 7650, Jones and Roberts, Italy) for mice set at a rate of 16 rpm. Mice that were able to remain on the rod longer than 180 s were selected and divided into four groups ($n=5$). Animals in Group 1 received normal saline and served as control, while those in Groups 2, 3 and 4 received the extract at doses of 50, 100 and 200 mg/kg po. Thirty minutes after receiving the injection, animals were placed on the rod at

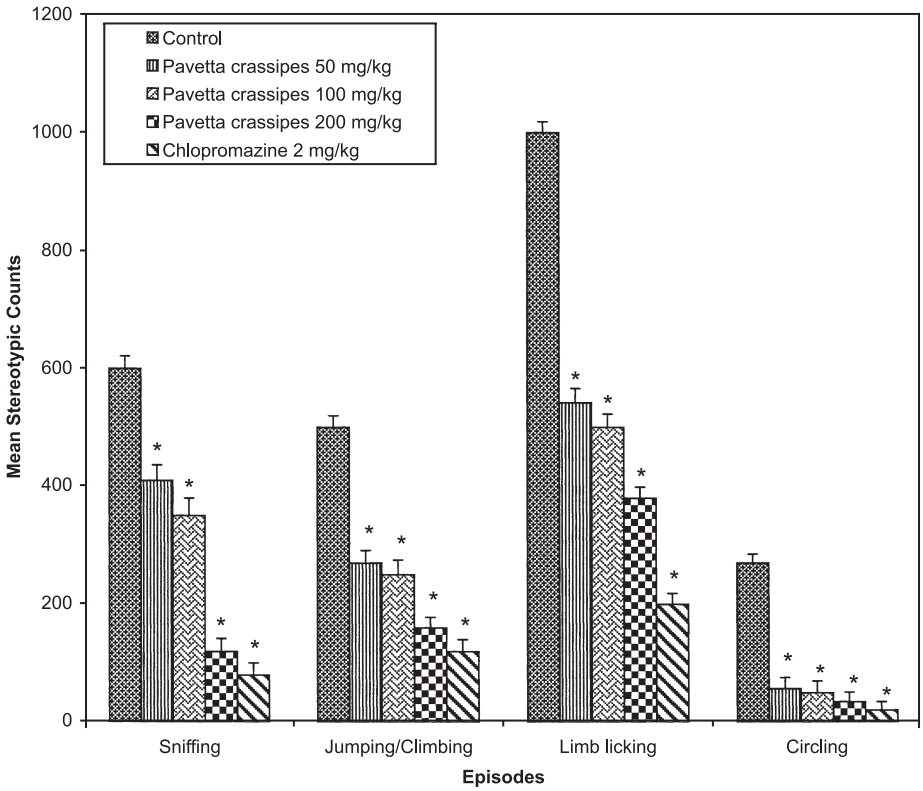

Fig. 3. Effect of the ethanol extract of *P. crassipes* on amphetamine-induced stereotype behaviour in mice following different drug treatments. Amphetamine 2 mg/kg + saline 10 ml/kg (▩), amphetamine 2 mg/kg + *P. crassipes* 50 mg/kg (▥), amphetamine 2 mg/kg + *P. crassipes* 100 mg/kg (▨), amphetamine 2 mg/kg + *P. crassipes* 200 mg/kg (▣) and amphetamine 2 mg/kg + chlorpromazine 2 mg/kg (▧) [$F(4,39)=8.42$; $P<.05$]. * Statistical significance between treatment groups and control.

intervals of 30 min up to 180 min. If an animal failed more than once to remain on the rod for 3 min, the test was considered positive, meaning that there was a lack of motor coordination (Fujimori and Cobb, 1965).

2.4. Statistical analysis

All the results were expressed as mean ± S.E.M., and differences in means were estimated by means of an ANOVA followed by Dunnet's post hoc test for multiple comparison. Results were considered significant at $P < .05$.

3. Results

3.1. Effect on SMA

Gross motor activity was investigated using the SMA cages. The extract (50, 100 and 200 mg/kg po) caused a significant ($P < .05$) dose-dependent decrease in SMA in mice. The decrease in activity was pronounced at 30 min and continued to decline with increase in time (Fig. 1). The extract was also found to attenuate amphetamine-induced hyperactivity in mice. Locomotion inhibition was present at 30 min and continued until 2 h., indicating that the onset to locomotion inhibition occurred between 0 and 30 min (Fig. 2).

3.2. Effect on amphetamine-induced stereotype behaviour and apomorphine-induced climbing

Amphetamine and apomorphine act centrally on dopamine to bring about stereotypic behaviours in laboratory animals. The extract at doses of 50–200 mg/kg attenuated the different stereotypic behaviour induced by amphetamine. The effect was dose dependent. Chlorpromazine, an agent used in psychosis, also protected mice against the stereotypic behaviour induced by amphetamine (Fig. 3). Similarly, the extract produced a dose-dependent inhibition of climbing induced by apomorphine in mice (Fig. 4).

3.3. Effect on haloperidol-induced catalepsy

Haloperidol is a neuroleptic agent that induces a trance-like state (catalepsy) in rodents. The extract (50, 100 and 200 mg/kg) was found to potentiate haloperidol-induced

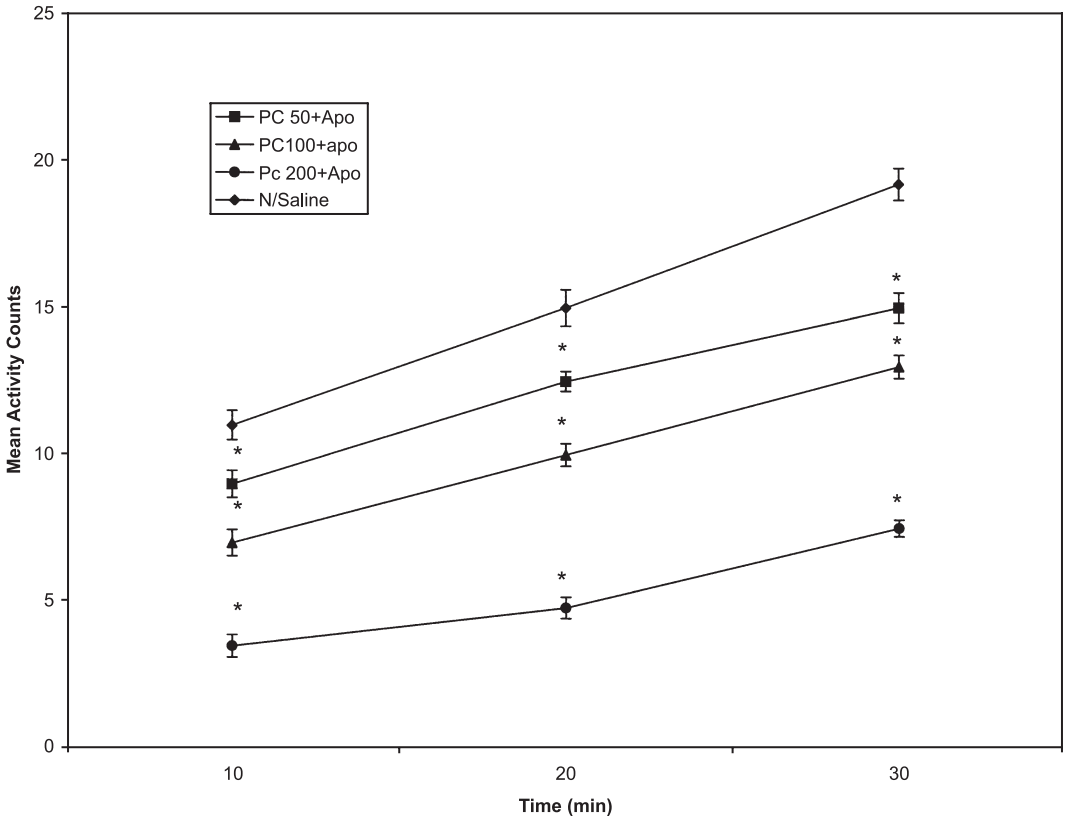

Fig. 4. Effect of the ethanol extract of *P. crassipes* on apomorphine-induced climbing behaviour in mice following different drug treatments. Apomorphine 3 mg/kg + saline 10 ml/kg (♦), apomorphine 3 mg/kg + *P. crassipes* 50 mg/kg (■), apomorphine 3 mg/kg + *P. crassipes* 100 mg/kg (▲), and apomorphine 3 mg/kg + *P. crassipes* 200 mg/kg (●) [$F(3,31) = 4.89$; $P < .05$]. * Statistical significance between treatment groups and control.

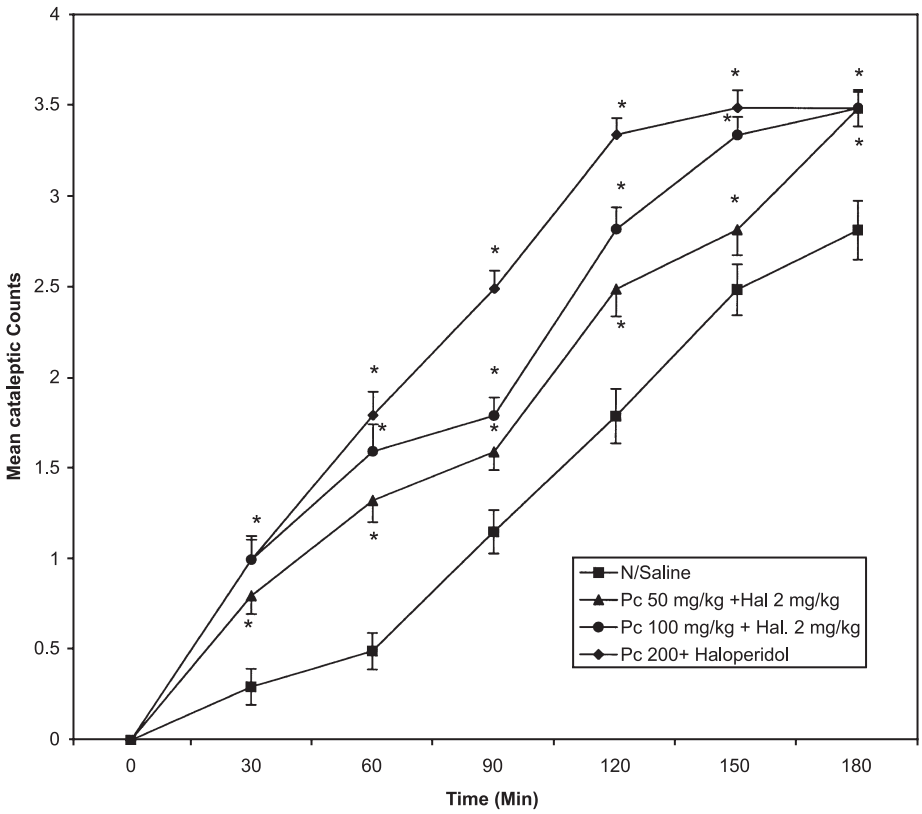

Fig. 5. Effect of the ethanol extract of *P. crassipes* on haloperidol-induced catalepsy in rats following different drug treatments. Haloperidol + saline 10 ml/kg (■), haloperidol + *P. crassipes* 50 mg/kg (▲), haloperidol + *P. crassipes* 100 mg/kg (●), haloperidol + *P. crassipes* 200 mg/kg (♦) [$F(3,23) = 5.01$; $P < .05$]. * Statistical significance between treatment groups and control.

catalepsy in rats. The effect was dose and time dependent, with the dose of 200 mg/kg producing the highest response (Fig. 5).

3.4. Effect on pentobarbital-induced sleeping time

Pentobarbital sodium is a barbiturate that induces sleep in both rodents and humans (Koch-Weser and Greenblatt, 1974). We induced sleep with pentobarbital (35 mg/kg ip) and tested the effect of the extract on both the onset and duration of sleep. The extract (50, 100 and 200 mg/kg) did not affect the onset of sleep, but significantly ($P < .05$) prolonged the duration of pentobarbital sleep at the doses tested. The increase in duration of sleep was found to be dose dependent. The extract given alone did not produce any sleep pattern in the treated groups using the criterion for sleep. Diazepam (1 mg/kg), the positive control used in this study, also potentiated pentobarbital hypnosis (Table 1).

3.5. Effect on exploratory activity in mice

The exploratory activity is used to differentiate between anxiolytics and sedatives. Sedatives decrease the number of head dips while anxiolytics increase the number of head dips in the hole board. The extract (50, 100 and 200 mg/kg) exhibited a significant ($P < .05$) dose-dependent decrease in the number of head dips in the hole-board experiments. Similarly, clonazepam at a sedative dose of 0.2 mg/kg attenuated the number of head dips in the hole-board experiment (Fig. 6).

3.6. Effect on motor coordination

This test was performed to investigate whether the extract was acting via the neuromuscular junction to mediate the observed effect rather than acting centrally. The extract

Table 1
Effect of the ethanolic extract of *P. crassipes* on pentobarbital (35 mg/kg)-induced sleeping time in rats

Treatment	Dose (mg/kg)	Duration of sleep (min)
Normal saline + pentobarbital	10 ml/kg	67.0 ± 6.8
P. crassipes + pentobarbital	50	95.2 ± 8.5 *
	100	142.5 + 10.8 *
	200	180.2 ± 15.4 *
Diazepam + pentobarbital	1	92.4 ± 3.6 *
P. crassipes (alone)	50	NS
	100	NS
	200	NS

Values are expressed as mean ± S.E.M.; $n = 5$. NS means no sleep observed.

* Significant difference from control (saline) group at $P < .05$ (ANOVA followed by Dunnett's test).

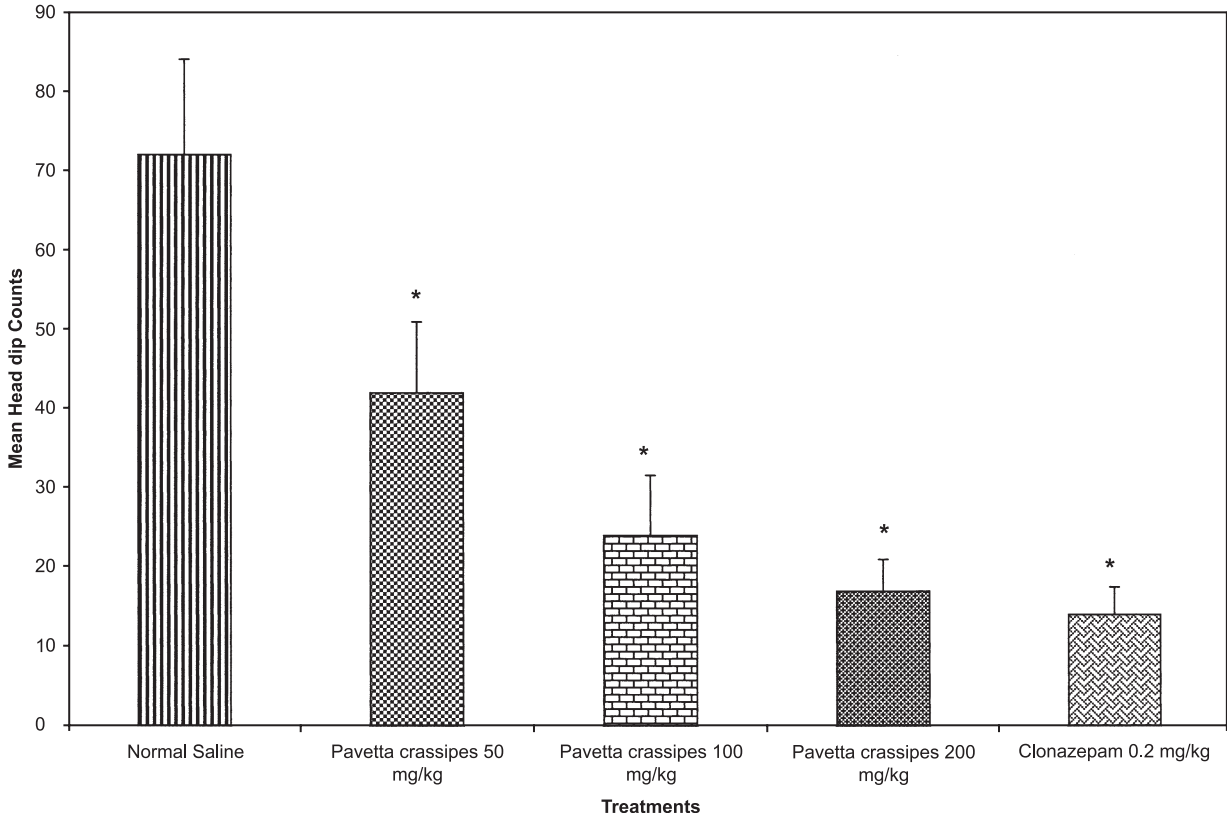

Fig. 6. Effect of the ethanol extract of *P. crassipes* on exploratory activity in mice following different drug treatments. Saline 10 ml/kg (▥), *P. crassipes* 50 mg/kg (▪), *P. crassipes* 100 mg/kg (▤), *P. crassipes* 200 mg/kg (▩) and clonazepam 0.2 mg/kg (▨) [$F(4,29) = 5.51$; $P < .05$]. * Statistical significance between treatment groups and control.

did not exhibit profound effect on motor coordination as determined by the Rota-Rod performance in mice (Table 2).

4. Discussion

This study has provided data on the effects of the ethanolic extract of *P. crassipes* on the CNS of rats and mice. Administrations of the extract caused a significant and dose-dependent decrease in SMA. SMA has been used in laboratory animals to evaluate gross behavioural effects of drugs (Carpendo et al., 1994). The SMA measures the level of excitability of the CNS (Mansur et al., 1971) and agents that suppress SMA may possess central sedative properties (Ozturk et al., 1996). The ability of the extract to decrease SMA may therefore suggest a central sedative effect. The extract also prolonged pentobarbital-induced sleeping time in rats. Prolongation of pentobarbital hypnosis is believed to be a sedative and/or hypnotic property (Fujimori, 1965) and could be attributed to inhibition of pentobarbital metabolism (Kaul and Kulkarni, 1978) or central mechanism involved in the regulation of sleep (N'Gouemo et al., 1994). The potentiation of pentobarbital sleep and the decrease in SMA strongly suggests central depressant activity (Perez et al., 1998).

Table 2
Effect of leaf extract of *P. crassipes* on rotarod (treadmill) performance

Time (min)	Cutoff time (s)	Endurance time (s)			
		Saline	Extract 50 mg/kg	100 mg/kg	200 mg/kg
30	180	180 ± 8.7	180 ± 10.2	180 ± 11.3	179 ± 8.6
60	180	180 ± 9.2	177 ± 8.1	178 ± 8.3	180 ± 9.5
90	180	178 ± 8.4	179 ± 7.9	180 ± 9.6	178 ± 8.2
120	180	179 ± 8.0	180 ± 9.5	177 ± 8.4	180 ± 9.5

Values are expressed as mean ± S.E.M.; $n = 5$.

The extract caused a significant reduction in the exploratory behaviour in mice. The hole-board test is a measure of exploratory behaviour (File and Wardill, 1975). An agent that decreases this parameter is considered a sedative (File and Pellow, 1985). Anxiolytics have been shown to increase the number of head dips in the hole-board test (Takeda et al., 1998). Therefore, the extract might be a sedative rather than an anxiolytic agent. The extract had no observable effects on motor coordination in the treadmill experiment at the doses tested, thus suggesting that the extract might not be acting through peripheral neuromuscular blockade to cause the observable effects. Rather, the extract may be a centrally acting neurosedative agent (Capaso et al., 1996).

Amphetamine induces hyperactivity by the release of dopamine from the dopaminergic nerve terminal particu-

larly at the striatal pathways in the mesolimbic system (Hoffman and Lefkowitz, 1996). The behavioural effect of amphetamine could be masked by dopamine D2 receptor antagonists (Anca et al., 1993). The effect of the extract against amphetamine-induced hyperactivity suggests a possible interference with central dopaminergic pathways, or it might be acting as GABA or NMDA antagonist. The ability of a drug to antagonize apomorphine-induced climbing in the mouse has been correlated with neuroleptic potential (Protais et al., 1976; Costall et al., 1978). In addition, inhibition of apomorphine-induced climbing in mouse is suggestive of D1 and D2 receptor inhibition (Moore and Axton, 1988).

The ability of an agent to induce catalepsy is widely used as an experimental indicator of neuroleptic potency (Gada et al., 1983). Although neuroleptic-induced catalepsy has been shown to be due primarily to blockade of dopaminergic neurotransmission (Baldessarini, 1990), a number of other neurotransmitter systems may indirectly influence cataleptic response, such as histaminergic (Muley et al., 1982), noradrenergic or GABAergic system (Balsara et al., 1984). Serotonergic neurotransmission modulates cataleptic effect (Balsara et al., 1979; Kalkman et al., 1998). Potentiation of haloperidol-induced catalepsy by the extract in this study may well be a simple synergistic effect of the extract and haloperidol.

Because we were unable to do binding studies, these effects do not necessarily suggest direct interaction with GABAergic or dopaminergic receptors. Rather, the data may be suggestive of indirect or physiological interactions with these receptor systems. Further work is required to demonstrate direct interactions with these receptor systems. The results of these studies generally indicate the sedative properties of the leaf extracts of *P. crassipes*, which provides for the first time, the rationale for its various applications in traditional medicine especially in the treatment of mental illness. Further studies are in progress in our laboratory to isolate and characterize the biologically active components in *P. crassipes* leaf extract.

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