Pharmacology

Neurobehavioral Effects of *Phoenix dactylifera* in Mice

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ABSTRACT

The present study deals with the investigation of the neurobehavioral effects of the methanolic extract of *Phoenix dactylifera* (PD) fruits, studied on locomotor activity, exploratory behavior, motor coordination, hot-plate test, haloperidol-induced catalepsy, sodium nitrite–induced respiratory arrest, maximum electroshock–induced convulsions using mice. The results revealed that the extract in mice considerably reduced the exploration time in closed arms with an increased exploration in open arms, increased the time spent in mirrored chamber, increased the discrimination index, potentiated the haloperidol-induced catalepsy, reduced the onset of death in sodium nitrite–induced respiratory arrest. These results suggest that the methanolic extract of P. dactylifera fruits possess a wide range of CNS activities, which need further investigation.

Key words: Anxiety, convulsions, locomotor activity, nootropic, *Phoenix dactylifera*

INTRODUCTION

During the last two decades, pharmacotherapy with psychoactive drugs has been increasingly recognized as most effective in the management of anxiety, stress and psychosomatic disorders. However, prolonged use of tranquilizers and psychotropic drugs leads to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. Moreover, such agents primarily relieve the symptoms and offer a palliative relief of a temporary nature.[1]

Numerous herbal medicines are recognized as active on the central nervous system (CNS), and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches or epilepsy, that do not respond well to conventional treatments.[2]

Phoenix dactylifera Linn. (date palm), called "nakhla" and

the "Tree of Life" by the Arabs, is considered as one of the oldest cultivated fruit trees. It is a member of the monocotyledon family Arecaceae.[3] It is believed to be indigenous to the countries around the Arabian Gulf.[4] Date palm female trees bear fruits by the age of 3 to 5 years and are fully mature at 12 years. The fruit is a nutritious source of sugar, minerals and vitamins. *P. dactylifera* is concentrated between latitudes 10° and 30° north of the Equator, mainly in arid regions of the Middle East and North Africa, where it is thought to have been cultivated for over several thousands of years.[5] The various parts of this plant are widely used in traditional medicine for the treatment of various disorders, which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders, etc.[6,7]

In traditional medicinal practices, dates are considered as "tonic" and "aphrodisiac."[8] Date fruit extracts have been reported to possess antiulcer, anticancer,

antidiarrheal, hepatoprotective, antimutagenic, antioxidant, aphrodiasiac, antiinflamatory, antimicrobial, antigenotoxic, antihyperlipidemic and nephroprotective activities. [9-25]

P. dactylifera (date palm) has been traditionally claimed for its use in treatment of various nervous disorders and memory complaints but not yet scientifically documented so far. In the present study, we evaluated the activities of *P. dactylifera* on the CNS to investigate whether there is any scientific basis for the use of the plant in traditional medicine for the treatment of CNS disorders.

MATERIALS AND METHODS

Plant material

Fresh fruits of *P. dactylifera* were collected from local market and authenticated by Botanical Survey of India (Voucher specimen number: BSI/WC/Tech/2009/674). The methanolic extract of dried fruit was prepared in the approved laboratories of Green Chem, Bangalore, India, using the procedure mentioned below.

Preparation of extract

The *P. dactylifera* fruits were manually separated from the pits and dried at room temperature and ground into powder using a stainless-steel blender. This powder was then extracted with methanol–water (4:1, v/v), at room temperature (20°C for 5 h using an orbital shaker). The extracts were then filtered and centrifuged at 4000g for 10 min, and the supernatant was concentrated under reduced pressure at 40°C for 3 h using a rotary evaporator to obtain the methanolic extract.

Chemicals and drugs

Haloperidol (Rajesh chemicals, Mumbai) and sodium nitrate (Loba chemicals, Mumbai) were purchased from the respective vendors. Diazepam (Calmpose) and Pentazocin injection (Fortwin), Piracetam suspension (Nootropil) and Phenytoin (Eptoin) tablets were purchased from the local market.

Animals

Swiss albino mice weighing 25-30 g were used. They were caged in a room under standard laboratory conditions (temperature 23 ± 1°C, relative humidity 55% ± 5% and lighting 08:00-20:00 h). The animals were fed on a pelleted diet (Chakan Oil Mills, Pune, India) and water *ad libitum*. The animals were transferred to the laboratory at least 1 h

before the start of the experiment. The experiments were performed during the day (08:00-16:00 h).

Ethical clearence

All studies were carried out in accordance with the guidelines provided by the Indian Council for Medical Research and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and the Institutional Animal Ethical Committee approved the study (Approval No.: CPCSEA/IAEC/PC-04/07-2K8).

Preliminary acute toxicity test

Healthy adult male albino mice (18-22 g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic cooperation and development (OECD-2000). The mice were observed continuously for 2 h for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days.[26]

General pharmacological observation

Behavioral effects of PD extract (30, 100 and 300 mg/kg) were assessed by the method described by Irwin *et al.* (1968). The mice were divided into three groups (n = 6) and treated with PD extract at a dose of 30, 100 and 300 mg/kg, respectively. The animals were then placed in an observation cage and observed after 30 min of administration up to 2 h for behavioral changes. The observation parameters consisted of body position, locomotion, rearing, respiration, righting reflex and lacrimation. The observation parameters consisted of body position, alertness, reactivity to touch stimuli, righting reflex and lacrimation.[27]

Effect on motor coordination

Digital rotarod appatatus (Inco - Ambala, India) was used to evaluate the muscle relaxing and sedative effects in the extract and vehicle-treated mice. The animals were trained to remain for 3 min on the rod rotating at a speed of 25 rpm. investigation. Only animals performing up to the required parameter were included in the test and devided into five groups. Group I served as control and received only vehicle. Groups II, III and IV were treated orally with PD extract at a dose of 30, 100 and 300 mg/kg, respectively. All animals were subsequently assessed for their performance on the rotarod after 60 min. Group V recieved reference standard diazepam at a dose of 1.0

mg/kg ip 30 min before the test. The fall-off time from the rod was noted for each animal.[28]

Effect on locomotor activity

Locomotor activity was recorded with a using a digital activity cage (Actophotometer Space-lab, India). The animals were devided into five groups (n = 6). Each mouse was indiviually placed in the actophotometer for 5 min. Four groups of animals were orally treated with vehicle, PD extract at a dose of 30, 100 and 300 mg/kg, respectively, and after 60 min the mice were placed individually in the actophotometer for recording the basal activity score. Group V recieved reference standard diazepam at a dose of 2.0 mg/kg (i.p.) 30 min before the test. Mean change in the locomotor activity was recorded for each group.[29]

Analgesic activity

Digital hot-plate method was carried out to study the analgesic effect. Animals were divided into five groups (n = 6). Four groups of animals were orally treated with vehicle, PD extract at a dose of 30, 100 and 300 mg/kg, respectively. The fifth group received reference standard Pentazocin 30 mg/kg (i.p.). The heated surface of a digital hot-plate analgesia meter (Columbus- USA) was maintained at 55°C ± 01° C. The mice were gently placed on the heated surface of the plate and the time required for paw licking or jumping (reaction time) was recorded at 0, 60 and 120 min. To minimize damage to the animal paw, the cut-off time for latency of response was taken as 15 s.[30]

Elevated plus-maze test

This test was used to assess anxiolytic activity in the extract- and vehicle-treated mice (Lister, 1987). Locally fabricated apparatus (VJ Instrments, India) consisted of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) elevated to the height of 40 cm. Animals were divided into five groups (n = 6) and pretreated with vehicle or PD extract (30, 100 and 300 mg/kg) 60 min before and reference standard diazepam (1 mg/kg, i.p.) 30 min, respectively, before the test. The mice were placed individually in the center of the maze, head facing toward open arm and the number of entries in open and closed arms and time spent in open and closed arms, respectively, were recorded for a period of 5 min. Entry into an arm was defined as the point when the animal places all four paws onto the arm.[31]

Object recognition test

This paradigm was used to evaluate effects of *P. dactylifera* extract on cognition based on the spontaneous exploratory behavior of mice. The task took place in a white-colored plywood box (70 × 60 × 30 cm) with a grid floor that was illuminated by a 60-W lamp suspended 50 cm above the box. The object to be discriminated was also made of plywood in two different shapes with 8 cm height and colored black. Animals were devided into five groups (n = 6). On the day before test, were submitted to a habituation session, freely exploring the object free box for 2 min. On the day of test animals were treated with vehicle or PD extract (30, 100 and 300 mg/kg) 60 min before and reference standard Piracetam (50 mg/kg, i.p.) 30 min, respectively, before the first trial. In the first trial (T1), two identical objects were presented in two opposite corners of the box, and the amount of the time taken by each mouse to complete 20 s of object exploration was recorded. Exploration was considered as sniffing or touching the object with the nose and/or forepaws at a distance less than 2 cm. During the second trial (T2, 90 min after T1), a novel object replaced one of the objects presented in T1, and mice were left individually in the box for 5 min. The time spent for exploring the familiar (F) and the new object (N) was recorded separately, and discrimination index (D) was calculated as $(N\_F)/(N + F)$. Care was taken to avoid place preference and the influence of olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T2 and cleaning the apparatus with hydrogen peroxide.[32]

Double-unit mirrored chamber test

This test was used to assess the anxiolytic effect of the PD extract. The locally fabricated apparatus consisted of a mirrored cube (30 × 30 × 30 cm), open on one side and placed in a square box. The container box (40 × 40 × 30 cm) had a white floor and black wall making 5 cm corridor completely surrounding the mirrored chamber. A sixth mirror was placed on the wall of the box, positioned to face the open side of the mirror chamber. Animals were devided into five groups (n = 6) and treated with vehicle, PD (30, 100 and 300 mg/kg). The animals were placed at the back side of mirrored cube and the latency to enter the mirror chamber and time spent in mirror chamber during 5 min observation period was recorded 60 min after the drug administration. Diazepam (1 mg/kg, i.p.) was used as a reference standard administered 30 min before the test. These mice were not exposed to the apparatus before the test and evaluated only once to avoid habituation problem. The apparatus was washed after each evaluation to eliminate potential cues such excreta, urine left by the previous occupant.[33]

Haloperidol-induced catalepsy

Animals were divided into five groups (n = 6). The control

drug and reference standard-treated animals recieved vehicle or PD extract (30, 100 and 300 mg/kg) 60 min before, and the standard group recieved piracetam 100 mg/kg (i.p.) 30 min before haloperidol (1 mg/kg, i.p.). The duration of catalepsy after injecting haloperidol was noted at 0, 15, 30, 60, 90, 120 and 150 min with cut-off time of 300 s by means of "Bar test" (Ferre *et al.* 1990). The phenomenon was measured as the duration of an imposed position maintained by the mouse with both forelimbs extended and resting on 2.5-cm high wooden bar (0.9 cm diameter). To better measure the catalepsy (avoiding false results) the animals were tested twice at each time interval and only the greater duration of time was recorded.[34]

Sodium nitrite–induced respiratory arrest

Sodium nitrite induces chemical hypoxia by reducing the oxygen-carrying capacity of blood by converting hemoglobin to methemoglobin and produces death due to respiratory arrest at a dose of 250 mg/kg (i.p.) in vehicle-treated mice. This test was used to determine the effect of *P. dactylifera* extract on the onset of respiratory arrest. Animals were devided into five groups (n = 6). Control and test group animals recieved vehicle or PD extract (30, 100 and 300 mg/kg, respectively) 60 min before and the standard group recieved piracetam 50 mg/kg (i.p.) 30 min before sodium nitrite. The time between injection of sodium nitrite and death was recorded.[35]

Maximal electroshock–induced seizures (MES)

Animals were divided into five groups (n = 6). The animals were pretreated with vehicle, PD extract (30, 100 and 300 mg/kg) 60 min before or reference standard Phenytoin (20 mg/kg, i.p.) 30 min before delivery of electroshock. An electric shock (42 mA, 0.2 s) using an electroconvulsiometer (INCO, Ambala, India) was applied through the crocodile ear clip and duration of hindlimb extension was recorded for each animal.[36]

Statistical analysis

The observations are reported as mean ± SEM. Differences between group means were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test to assess the significance of differences between individual groups. $P > 0.05$ were considered insignificant.

RESULTS

Preliminary acute toxicity test

All mice were free of any toxicity up to the dose of 2 g/kg

without any mortality. From this data, three different doses, *viz.*, 30, 100 and 300 mg/kg, were selected for further study.

General pharmacological observation

Mice orally treated with the PD extract (30, 100 and 300 mg/kg) and submitted to the general observations did not show any difference in their behavior and parameters determined during the observation periods. They were alert, with normal grooming, touch response and pain response. Alertness, limb tone and grip strength were normal and the animals did not show staggering gait or contractions.

Effect on motor coordination

The PD extract (30, 100 and 300 mg/kg) did not produce any significant effect on the motor coordination as determined by the rotarod performance. All mice were able to maintain equilibrium on the rotating rod for more than 5 min, whereas diazepam showed a significant decrease in motor coordination (data not shown).

Effect on locomotor activity

The PD extract (30, 100 and 300 mg/kg) did not produce any significant reduction in locomotor activity as compared with the control animals receiving only the vehicle. However, the diazepam-treated group revealed a statistically significant decrease in locomotor activity as compared with the control [Table 1].

Analgesic activity

The PD extract at doses of 100 and 300 mg/kg produced significant increase in the reaction time in the hot plate at 60 and 120 min after administration ($P < 0.01$), which was most marked at a dose of 300 mg/kg, whereas PD extract 30 mg/kg showed a significant increase in reaction time at 60 min ($P < 0.05$) , but was insignificant at 120 min after the administration [Figure 1].

Elevated plus-maze test

The subgroups treated with PD extract at the doses of

Table 1: Effect of PD extract and Diazepam on mean change in locomotor activity

Treatment (mg/kg)	Mean change in locomotor activity
Vehicle (10 ml/kg)	23.5 ± 4.09
PD 30	29.83 ± 9.81
PD100	22.6 ± 3.63
PD 300	16.83 ± 2.50
Diazepam 2	75.5 ± 5.83**

n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

100 ($P < 0.05$) and 300 mg/kg, and diazepam ($P < 0.01$) exhibited a significant decrease in the time spent in the open arm and also a showed significant increase in the time spent in open arms, whereas PD extract at the dose of 30 mg/kg was found ineffective in this regard [Table 2].

Object recognition test

The subgroups treated with PD extract at doses 100 ($P < 0.05$) and 300 mg/kg ($P < 0.01$) exhibited a significant increase in discrimination index when compared against vehicle-treated mice. PD extract at 300 mg/kg was more significant in this regard. Piracetam (50 mg/kg) was found to be most significant in increasing the discrimination index [Figure 2].

Double-unit mirrored chamber

None of the doses of PD extract produced any significant alteration in latency to enter the mirrored chamber but the doses 100 and 300 mg/kg significantly increased the time spent in the mirrored chamber in PD extract–treated subgroups when compared with vehicle-treated control group, whereas diazepam significantly decreased the latency to enter the mirrored chamber and increased the time spent in the mirrored chamber [Table 3].

Haloperidol-induced catalepsy

Maximum catalepsy was noted after 120 min of haloperidol administration. The catalepsy was significantly intensified by PD extract. The dose 100 mg/kg significantly increased
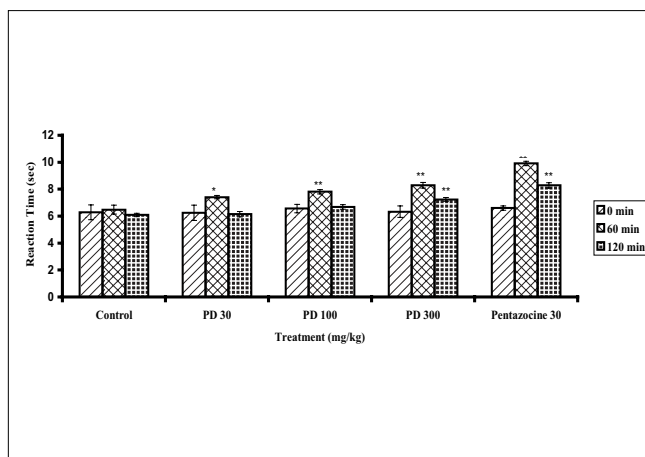

Figure 1: Effect of PD extract and Pentazocine on reaction time in hot plate test. n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
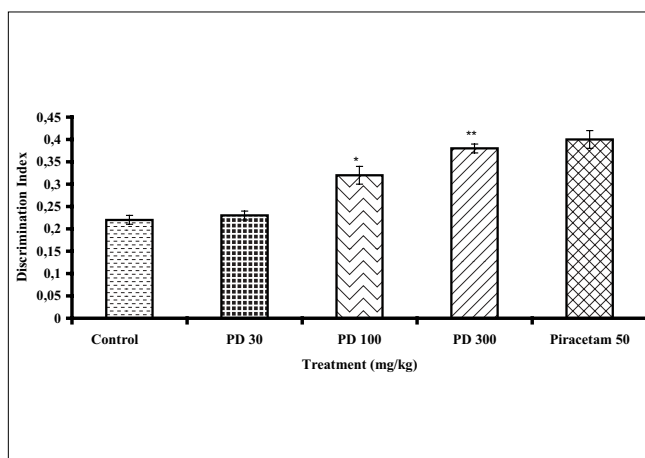

Figure 2: Effect of PD extract and Piracetam on discrimination index in object recognition test. n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 2: Effect of PD extract and Diazepam on anxiety induced using elevated plus-maze apparatus

Treatment (mg/kg)	Time spent (Mean ± SEM)	
	Open arm	Enclosed arm
Vehicle (10 ml/kg)	40.24 8.07	221.06 ± 11.01
PD 30	44.46 ± 6.11	203.96 ± 16.12
PD100	83.27 ± 11.60*	155.70 ± 17.35*
PD 300	92.05 ± 7.55**	150.68 ± 17.43**
Diazepam 2	111.76 ± 16.40**	135.61 ± 11.89**

n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3: Effect of PD extract and Diazepam on anxiety induced using double-unit mirrored chamber

Treatment (mg/kg)	Latency to enter (s)	Time Spent (s)
Vehicle (10 ml/kg)	194.55 ± 19.17	40.33 ± 2.96
PD 30	191.33 ± 16.71	35.32 ± 3.23
PD100	193.59 ± 13.28	64.72 ± 5.16*
PD 300	205.33 ± 10.14	72.53 ± 9.89**
Diazepam 2	125.25 ± 4.27**	76.64 ± 8.29**

n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
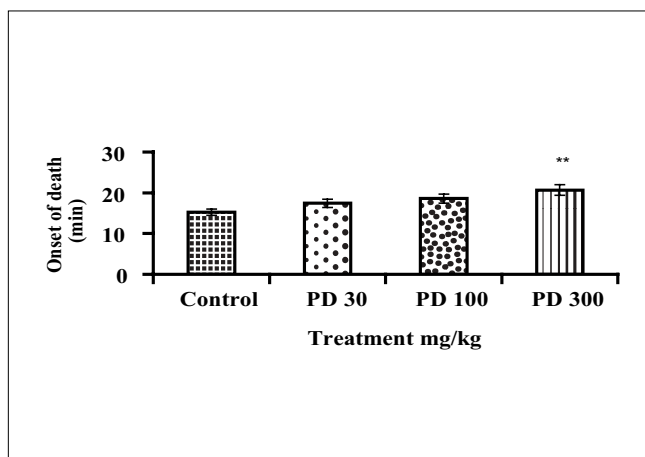

Figure 3: Effect of PD extract on the onset of respiratory arrest induced by sodium nitrite in mice. n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 4: Effect of PD extract on haloperidol induced catalepsy

Duration of catalepsy	Treatment (mg/kg)			
(min) (Mean ± SEM)	Vehicle (10 ml/kg)	PD30	PD100	PD300
0 min	3.19 ± 0.28	3.38 ± 0.29	3.68 ± 0.23	3.75 ± 0.41
15 min	16.45 ± 1.47	16.13 ± 2.52	17.55 ± 2.26	18.44 ± 2.81
30 min	69.21 ± 6.40	70.13 ± 4.53	68.49 ± 6.82	75.32 ± 6.09
60 min	132.91 ± 5.29	131.80 ± 3.78	143.15 ± 4.54	162.75 ± 4.15**
90 min	161.74 ± 4.48	165.5 ± 5.70	180.66 ± 3.67*	186.56 ± 3.33**
120 min	219.65 ± 7.52	211.30 ± 6.84	217.83 ± 11.07	215.08 ± 4.68
150 min	136.45 ± 6.47	152.30 ± 4.50	148.11 ± 7.82	158.69 ± 6.96

n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 5: Effect of PD extract on MES induced convulsions

Treatment (mg/kg)	Duration of hind limb extension (s)	Mice convulsed/ mice used
Vehicle	19.52 ± 1.13	6/6
PD 30	16.30 ± 2.24	6/6
PD 100	15.94 ± 0.73	6/6
PD 300	14.9 ± 1.45	6/6
Phenytoin (25)	Nil	0/6

n = 6 Data were analyzed by one-way ANOVA followed by Dunnett's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

the duration of catalepsy 90 min after haloperidol administration ($P < 0.05$), whereas the dose 300 mg/kg significacntly potentiated the catalepsy 60 and 90 min after haloperidol administration ($P < 0.01$). The dose 30 mg/kg was found ineffective in either increasing or decreasing the catalepsy [Table 4].

Sodium nitrite–induced respiratory arrest

The dose 300 mg/kg of PD extract showed significant increase in the onset of death ($P < 0.01$), whereas the doses 30 and 100 mg/kg were unable to increase the onset of sodium nitrite–induced respiratory arrest [Figure 3].

Maximal electroshock–induced seizures

None of the doses of the PD extract (30, 100, 300 mg/kg) significantly reduced the duration of electroconvulsometer-induced hindlimb extension but a nonsignificant dose-dependent reduction was seen, whereas almost no hindlimb extension was seen in phenytoin-treated animals [Table 5].

DISCUSSION

In this work, the effects of different doses of methanolic extract of *P. dactylifera* fruits were studied in several neuropharmacological models. The results of the study provided evidence that the methanolic extract of *P. dactylifera* fruit possesses a wide spectrum of CNS activity. Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity.[37]

However, none of the doses of PD extract were found to have any effect on the locomotor activity. Moreover, the lack of effect on locomotor activity works to the advantage of the plant demonstrating nootropic activity.[38] Additionally, the PD extract did not demonstrate any effect on the muscle coordination, as indicated by the findings with respect to the rotarod model, suggesting that the inhibitory effect of the extract might be elicited via central mechanisms, not by peripheral neuromuscular blockade, and also ruled out the possibility of neurotoxicity.[39,40] The significant increase in the discrimination index by PD extract exhibited that the plant possesses nootropic activity in the absence of cognitive deficit.[41] This suggests the usefulness of extract in improving the performance in competition and to promote poor learners. The elevated plus-maze (EPM) is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans. An anxiolytic agent increases the time spent in open arms and decreases the time spent in enclosed arm of the EPM.[42] In the present study, oral administration of PD extract demonstrated an anxiolytic-like effect in mice, as it significantly increased the time spent in open arms and decreased the time spent in closed arms. The anxiolytic effect of the PD extract was further confirmed by the findings of mirror chamber test in which the PD extract significantly increased the time spent in the mirrored chamber.[33] As per the earlier reports, increase in central serotonergic activity invariably leads to anxiety, whereas decrease in brain 5- hydroxytryptamine activity results in anxiolysis. The findings of the anxiolytic study of the extract suggested the antiserotonergic activity.[43] This further supported the nootropic activity as the increase in serotonin level has been reported to interfere with learning acquisition and memory consolidation.[44] The PD extract also significantly increased the reaction time in hot-plate test, indicating that the extract possesses significant analgesic activity.[30] It is well documented that the drugs effective against tonic hindlimb extension induced by electroshock generally have proven to be effective against

partial and tonic clonic seizures in human beings.[45] The PD extract was unable to reduce the duration of maximum hindlimb extension in maximum electroshock-seizures model, indicating the ineffectiveness of the extract against tonic-clonic seizures. It is well established that haloperidol induced catalepsy occurs due to the blockade of dopaminergic neurotransmission.[34] In our experimental findings, the PD extract significantly potentiated the haloperidol-induced catalepsy, which indicated that the extract possesses antidopaminergic activity and which suggests its use in the treatment of psychosis.[46] Sodium nitrite induces respiratory arrest by converting hemoglobin into methemoglobin, thereby reducing oxygen-carrying capacity and cholinergic transmission and ultimately leading to death.[35] In this study, PD extract significantly increased the onset of death due to the sodium nitrite–induced respiratory arrest, indicating that the PD extract increases the cholinergic transmission in the brain, which is known to play a vital role in cognition and which further support the nootropic findings of the extract. [47]

CONCLUSION

From the findings of the present study it can be concluded that the methanolic extract of *P. dactylifera* possesses significant anxiolytic, analgesic, nootropic and antipsychotic activities, which may be attributed to various mechanisms such as decreased serotonergic and dopaminergic transmission and increased cholinergic transmission. These findings scientifically validated the traditional claim and suggested its valuable role in the treatment of various CNS disorders. The study further revealed that the extract is devoid of any neurotoxicity and CNS-depressant effect. As the present study is based upon the behavioral models without any associated neurochemical estimations, it becomes necessary to carry out the specific binding studies and estimations of the neurotransmitter levels in the brain to understand the exact mechanism of action and extend these results further.

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