

Research Article

Studies on neuropharmacological and analgesic effects of *Periploca aphylla* extract in mice

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Abstract

The current study was carried out on a crude extract of stem of *Periploca aphylla*. It is found in the regions of Jhal magsi, Khuzdar and Pujgur in Balochistan. *P. aphylla* has numerous therapeutic uses in traditional medicine. It is used for the relief of pain, ulcer, skin disorders, tumors and for extensive range of other diseases. In neuropharmacological studies mice treated with the crude extract of *P. aphylla* showed sedative effects in open field, cage crossing, traction and rearing test particularly at the dose of 500 mg/kg as compared with standard drug diazepam. In force induced swimming test increase in immobility time was observed at the dose of 250 and 500 mg/kg. Results were significantly comparable with standard drug diazepam. The administration of the crude extract of *P. aphylla* produced significant analgesic effects particularly at the dose of 500 mg/kg in the acetic acid induced writhing test and the formalin test. In conclusion, results suggests that the methanolic extract of *P. aphylla* possesses sedative effects and potent analgesic effects.

keywords: Analgesic; *Periploca aphylla*; Sedative

Introduction

Plants are vital components of healthy life since they deliver us food and medicines that are safe and effective. Medicinal plants play an important role due to the presence of

wide variety of bioactive compounds in them and having various biological and pharmacological activities [1]. About 6000 medicinal plant species exist in Pakistan, and Balochistan which is the largest

province of Pakistan, has many medicinal plants [2]. *Periploca aphylla* is a medicinal plant of Balochistan and is used by the local people to cure various diseases. It is found in the regions of Jhal Magsi, Pujgur and Khuzdar in Balochistan [3].

P. aphylla belongs to the genus *Periploca* (Asclepiadaceae), which has twelve species. In Pakistan three species have been found. Genus *Periploca* is medicinally very important [4]. *P. aphylla* is called as “Bata” or “Barara.” Locally. Part used is milky juice from the stem and is used for swellings and tumors. It is also employed to treat flu, cough, and swollen joints. *P. aphylla* is also used for the treatment of constipation, ulcer and skin diseases. Numerous chemical compounds isolated from *P. aphylla* have shown antibacterial activities and α -glucosidase (type VI) inhibitory effect [5, 6]. Studies on phytochemical constituents of *P. aphylla* show the occurrence of steroids [7], flavonoids, terpenoids, cardiac glycoside, tannins, betacyanin, coumarin and amino acids. Current research work was carried out to explore the effect of *P. aphylla* on central nervous system (CNS) and its ability to inhibit the pain as previously no data was available on neuropharmacological and analgesic activities of the plant.

Material and methods

Plant material

Plant material (stem) was collected from Jhal magsi District of Balochistan, Pakistan. Plant was identified by Mrs. Bushra Aziz Khan, Assistant Professor, Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan. Voucher specimen No. J-213 was submitted in herbarium of Pharmacognosy department. Plant material was dried under shade and soaked in methanol for 15 days under reduced pressure by using rotary evaporator. After evaporation of solvent, dark green residue was obtained.

Animals

Male and female (both) albino mice, weighing about 20-25 gram were collected from the CASVAB (Centre of advance studies in vaccinology and biotechnology) University of Balochistan, Quetta. Animals were kept under standard environments, temperature was maintained at 25 ± 1 °C, in 12 hours light/ 12 hours dark cycle. The animals had free access to water and feed. Prior to experimentation, the animals were adjusted to Lab environment for 7 days.

Phytochemical tests

Phytochemical tests were carried out for the presence of Alkaloids, Terpenoids, Saponins, Glycosides, Tannins and Flavonoids [8].

Neuropharmacological studies

Neuropharmacological studies were carried out by Open field activity, cage crossing activity, rearing activity, traction test and forced swimming test on mice. Mice were divided in 4 groups, Group A= Control (Distilled water treated), Group B= Crude extract 250 mg/Kg treated group, Group C= crude extract 500mg/ Kg treated group and Group D= Standard drug Diazepam treated group. Each group consisted of 5 mice.

Open field activity

The total area of apparatus was 76×76 cm², and the height of the walls was 42 cm. Floor of apparatus was separated by means of lines into twenty five equal squares. Mice were placed in center of the apparatus (one at a time). Observation was to count (for 10 minutes) the number of squares crossed by the mice with four paws. Open field activities of control group, crude drug treated group and standard drug treated group were observed in balanced design to avoid order effect [2, 9].

Rearing test

Rearing test was conducted by using 1000 ml beaker. Bottom was covered with white paper. Animals were observed for 10 minutes [2, 9] for the count of upward

movements during their erect position [10, 12].

Cage crossing test

Cage crossing test was carried out by using transparent plastic cage (26x 26 x26 cm). Mice were placed in the cages and number of cage crossings were noted for 10 minutes [13].

Forced swimming test.

Forced swimming test (FST) was used to determine the antidepressant activity [13, 14]. Test was conducted by placing the mice individually in a cylindrical tank (18.5 cm height, 12.5 cm diameter) containing clean water at 25°C (13.5 cm depth). Swimming was noted when horizontal and large movements of forepaws were made, that lead to dislocation of the mice body around the apparatus. After six minutes, mice were taken out of the apparatus and permitted to dry beneath a lamp and returned back to the home cage [15, 16].

Traction test

Traction test describes the sedative or stimulant activity of the drug. In this test the observation was to determine the time consumed by mice to travel an iron rod (of one-meter length). Before experiment, mice were trained to walk on the iron rod. Any decrease or increase in time taken by the mice were recorded and compared with control and standard drug treated animals [9, 10, 17].

Analgesic activity

Acetic acid induced writhing test

In this test abdominal constriction was induced in mice (weighing about 25–28 g). Acetic acid (0.6% in 0.9% saline) 0.1ml/10g was administered I/P after 30 minutes of test drug administration. Control animals received 2ml of Distilled water and positive control received standard analgesic drug Aspirin (300 mg/kg). Total numbers of abdominal constrictions were counted for 30 minutes [18].

Formalin test

Twenty microliter of 2.5% formalin, made in distil water, was injected beneath surface of the right hind paw of the mice. Observation (number and time spent on licking and biting) was made for 30 minutes. In first phase (neurogenic pain), mice were observed from 0 to 5 minutes. In second phase mice were observed form 15-30 minutes [18].

Statistical analysis

All the calculations were recorded as mean with \pm SEM. The Dunnett's test was used to determine the significance of difference between the means and values of $P < 0.01$ were considered as highly significant and $P < 0.05$ significant [2].

Results

Phytochemical tests

Results were positive for the presence of tannins, terpenoids, saponins and flavonoids in phytochemical tests (Table 1).

Table 1. Preliminary phytochemical test of methanolic extract of *P. aphylla*

Chemical Constituents	Stem extract of <i>P. aphylla</i>
Alkaloids	Absent
Terpenoids	Present
Saponins	Present
Gglycosides	Absent
Tannins	Present
Flavonoids	Present

Open filed activity

For control group, squares crossed by the mice was 191.6 ± 3.18 taken as mean, and

142 ± 2.12 and 134.6 ± 2.5 for 250mg/kg and 500mg/kg of mice treated with crude extract of *P. aphylla* respectively and 94.4 ± 1.57 for

diazepam. Crude extract of *P. aphylla* significantly ($p < 0.05$) decreased the open field activity (Table 2).

Table 2. Assessment open field activity

Treatment	Dose mg/kg orally	Mean No. of observations ±S.E.M
Control	0.5ml Distilled water	191.6 ± 3.18
Crude extract of <i>P. aphylla</i>	250 mg/kg	142 ± 2.12*
	500mg/kg	134.6 ± 2.5*
Diazepam	2mg/kg	94.4 ± 1.57**

Values are the mean number of open field activities in 10 minutes. All values are mean ± SEM; n=5; * = Significant results ($P < 0.05$), ** = highly significant results ($P < 0.01$)

Cage crossing test

Cage crossing test showed increased depressant activity ($p < 0.05$) at oral dose of 500mg/kg *P. aphylla* as compared with the Diazepam 2mg/kg that produced 22.8 ± 0.8 number of cage crossing activities. At

250mg/kg and 500mg/kg of crude extract *P. aphylla*, the average number of cage crossing activities were 35.4 ± 1.5 and 29.4 ± 0.92 respectively. Whereas the average number of cage crossing activities for the control group were 48.2 ± 0.86 (Table 3).

Table 3. Assessment of cage crossing activity

Treatment	Dose mg/kg orally	Mean No. of observations ±S.E.M
Control	0.5ml Distilled water	48.2 ± 0.86
Crude extract of <i>P. aphylla</i>	250 mg/kg	35.4 ± 1.5*
	500mg/kg	29.4 ± 0.92*
Diazepam	2mg/kg	22.8 ± 0.8**

Values are the mean number of cage crossing activities in 10 minutes. All values are mean ± SEM; n=5; * = Significant results ($P < 0.05$), ** = highly significant results ($P < 0.01$)

Rearing test

In rearing test a reduction was seen in exploratory activity as well as in open field activity. The results show that rearing activity was depressed significantly ($p < 0.05$) at the higher doses of the drug i-e 500mg/kg. Diazepam 2mg/kg produced an average of 14.1 ± 0.85 rearing activities. On the

contrary, when the oral dose of *P. aphylla* was administered at 250mg/kg and 500mg/kg, the average number of rearing activities were 28.6 ± 1.03 and 23.4 ± 0.81 respectively. Whereas the average number of rearing activities for the control group was 39.4 ± 0.92 (Table 4).

Table 4. Assessment of rearing activity

Treatment	Dose mg/kg orally	Mean No. of observations ±S.E.M
Control	0.5ml Distilled water	39.4 ± 0.92
Crude extract of <i>P. aphylla</i>	250 mg/kg	28.6 ± 1.03*
	500mg/kg	23.4 ± 0.81*
Diazepam	2mg/kg	14.1 ± 0.85 **

Values are the mean number of rearing activities in 10 minutes. All values are mean ± SEM; n=5; * = Significant results ($P < 0.05$), ** = highly significant results ($P < 0.01$)

Traction test

In traction test it was observed that, when the oral dose of 500mg/kg was given, the time to travel on iron rod was decreased. Average 11.2 ± 0.37 seconds were taken by control group to travel the iron rod. When the dose of 250 and 500mg/kg crude extract

of *P. aphylla* was given, the average time taken to travel the iron rod by mice was 13.4 ± 0.68 and 14.8 ± 1.39 seconds respectively. While it was 19.4 ± 0.92 seconds when the mice were treated with standard drug Diazepam (Table 5).

Table 5. Assessment of traction test

Treatment	Dose mg/kg orally	Mean No. of observations +S.E.M
Control	0.5ml Distilled water	11.2 ± 0.37
Crude extract of <i>P. aphylla</i>	250 mg/kg	$13.4 \pm 0.68^*$
	500mg/kg	$14.8 \pm 1.39^*$
Diazepam	2mg/kg	$19.4 \pm 0.92^{**}$

Values represent the mean \pm SEM. Statistically significant from control and standard drug. * Significant at $p < 0.05$, ** highly significant at $p < 0.005$.

Forced swimming test

The results of forced swimming test show that the mean mobility time of control (Distilled water treated) animals was 3.54 ± 0.01 minutes and immobility time was 2.06 ± 0.01 minutes, For mice treated with crude extract of *P. aphylla* at 250mg/kg oral dose mobility time was 2.56 ± 0.004 , immobility time was 3.04 ± 0.01 minutes. For mice treated with 500mg/kg oral dose mobility

time was 2.25 ± 0.004 and immobility time was 3.35 ± 0.005 minutes. For standard drug treated group, at the dose of 2mg/kg the mobility time was 1.29 ± 0.001 and immobility time was 4.31 ± 0.005 . The crude extract shows significant ($p < 0.05$) results as compare to standard drug. Findings indicate that the crude extract of *P. aphylla* possesses both sedative and muscle relaxant activities (Table 6).

Table 6. Effect of crude extract of *P. aphylla* on forced swimming test in mice

Treatment	Dose mg/kg orally	Mobility time Mean No. of observations \pm S.E.M	Immobility time Mean No. of observations \pm S.E.M
Control	0.5ml Distilled water	3.54 ± 0.01	2.06 ± 0.01
Crude extract of <i>P. aphylla</i>	300 mg/kg	$2.56 \pm 0.004^*$	$3.04 \pm 0.01^*$
	500mg/kg	$2.25 \pm 0.004^*$	$3.35 \pm 0.02^*$
Diazepam	2mg/kg	$1.29 \pm 0.001^{**}$	$4.31 \pm 0.005^{**}$

All values are mean \pm SEM; n=5; * = Significant results ($P < 0.05$), ** = highly significant results ($P < 0.01$)

Analgesic activity

Acetic acid induced writhing test

In this test aspirin (300mg/kg) oral dose was used as reference drug. Significant ($p < 0.05$)

dose related inhibition of writhes were observed after administration of crude extract of *P. aphylla* (Table 7).

Table 7. Effect of methanolic extract of *P. aphylla* on acetic acid induced writhing test in mice

Treatment	Dose mg/kg orally	Mean No. of observations ±S.E.M
Control	0.5ml Distilled water	84.2± 0.37
Crude extract of <i>P. aphylla</i>	250 mg/kg	54.6±1.5
	500mg/kg	43.2±0.86
Aspirin	300mg/kg	35.6±1.03

Value represent the mean ± SEM. Statistically significant from control and standard drug. * Significant at p< 0.05, ** highly significant at p< 0.005

Formalin test

In this test *P. aphylla* crude extract showed significant (p<0.05) analgesic effect (as decrease in number of licking and biting and

time spent on licking and biting were observed). Results were more significant at 500mg/ kg oral dose as compared with aspirin (Table 8).

Table 8. Effect of crude extract of *P. aphylla* formalin induced inflammatory pain in mice

Treatment	Dose mg/kg orally	First Phase Mean No. of observations - ±S.E.M		Second Phase Mean No. of observations - ±S.E.M	
		Number of Licking & Biting	Time Spent (Seconds)	Number of Licking & Biting	Time Spent (Seconds)
Control	0.5ml Distilled water	60.4±2.20	80.8±1.72	76.2±1.99	185.2±1.28
Crude extract of <i>P. aphylla</i>	250 mg/kg	43.4 ±0.81*	64.1±2.89*	67.6±1.16*	110.8±2.91*
	500mg/kg	33.8±1.65*	40.8±5.25*	43.8±1.24*	74.2±1.11*
Aspirin	300mg/kg	23.8±1.59**	34.8±1.16**	25.4±1.21**	40.8±2.27**

All values are mean ± SEM; n=5; * = Significant results (P<0.05), ** = highly significant results (P<0.01)

Discussions

There was a positive evidence of presence of tannins and terpenoids as the phytochemical tests were carried out. Along with tannins and terpenoids, saponins and flavonoids were also present. As for as the results of neuropharmacological activities are concerned, rearing, cage crossing, open field and traction test showed significant decreased (p<0.05) values which is indicative of the sedative and passive activity of the extract. The results were more significant at 500mg/kg oral dose of *P. aphylla*. Alertness is suggestive of increase in loco-motor activity whereas sedation shows decrease in loco-motor activity [19,

21]. Depressed loco-motor activity of crude extract of the plant shows CNS depressant activity. Gamma-aminobutyric acid (GABA) is considered as inhibitory neurotransmitter in central nervous system. Most of the muscle relaxant, sedative and hypnotic drugs exert their action through this neurotransmitter. So it has been postulated that crude extracts of the *P. aphylla* may increase the inhibitory effect of this neurotransmitter by initiating membrane hyperpolarization which afterwards decrease the rate of firing of critical neurons in CNS. Another hypothesis about the above mentioned phenomena is that the plant extract directly activates the Gama

aminobutyric acid receptors in the central nervous systems [20, 22, 23]. Tannins and flavonoids were also detected in phytochemical test. It has been seen that the plants containing saponins, flavonoids and tannins are useful for treating mental ailments [23, 24]. Extensive research has shown that there are certain flavonoids and neuroactive steroids that basically function as ligands for the gamma aminobutyric acid receptors in the central nervous system. Therefore, it is obviously understandable that these can act like Benzodiazepines [20, 23].

In animal models, for assessment of anti-depressant activity, forced swimming test is applied. In such models anti-depressant activity is calculated by shortening of immobility time. On the other hand, if immobility duration is long, it reflects the depressant activity of the plant extract [21, 25]. Crude extract of the plant showed a marked central nervous system depressant activity which was dose dependent, and it may linked to the presence of flavonoids [25].

When acetic acid was administered intraperitoneally, it caused pain by abdominal muscle contraction which progressed into hind limb extension and body part elongation. All these effects are mediated by receptor located in peritoneum, [27] it is clear that the sensation of pain was produced by the intra peritoneal injection of acetic acid which causes the release of endogenous substances supposed to initiate the pain sensation. Prostaglandin E₂, Prostaglandin F_{2α}, Prostaglandin I₂, certain other prostaglandins and peritoneal mast cells have accumulated due to this process which have shown by many studies [27] in peritoneal fluids treated with acetic acid. Capillary permeability is another mechanism by which acetic acid further increases the pain sensation [20].

Crude extracts of *P. aphylla* has the ability to reduce the writhing in mice induced by acetic acid. This is indicative of the fact that the plant possesses the anti-nociceptive activity [28].

There are two distinct phases in Formalin test which reflect different stages in the process of pain. The first phase shows its direct effect on nociceptors which are mainly responsible for non-inflammatory pain. Contrary to this, the second phase exerts its effect on inflammatory pain [29]. A significant (P<0.05) analgesic effect was produced in both phases. This indicates that the anti-nociceptive effect of the extract was produced by both inflammatory and neurogenic mechanisms [30].

Conclusion

Current study reveals that crude extracts of *P. aphylla* possess CNS depressant and analgesic activity. It may be used as alternative medicine for depression and as analgesic agent, however detail studies are required for isolation of active constituents responsible for its pharmacological effect.

Authors' contributions

Conceived and designed the experiments: S Muhammad & G Razaque, Performed the experiments: A Jabbar & M Younis, Analyzed the data: A Qadir, Contributed reagents/materials/analysis tools: N Ahmad & IA Baloch, Wrote the paper: A Jabbar & G Mustafa.

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