

Analgesic and neuropharmacological activities of *Berberis lycium* Royle collected from district Sherani Balochistan, Pakistan

Samiullah¹, Javied Iqbal¹, Amanullah Khan³, Shafi Muhammad², Marvi¹,
Syed Umer Jan⁴ and Rahman Gul⁴

¹Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan

²Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan

³Provincial drug testing laboratory, Quetta, Balochistan, Pakistan

⁴Department of Pharmaceutics, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan

Abstract: *Berberis lycium* (family Berberidaceae) grows in district Sherani, Balochistan, Pakistan. It is used for the treatment of various disorders by the people of Balochistan. The present work was carried out to explore analgesic and neuropharmacological activities of crude methanolic extracts of *B. lyceum*. The analgesic activity was carried out by acetic acid induced writhing test and formalin test. Open field test, cage crossing test, rearing test, traction test and forced swimming test were carried out in neuropharmacological activities. The results reveal that crude methanolic extracts of *B. lyceum* showed significant ($P<0.05$) analgesic activity in acetic acid induced pain as well as with formalin test. In neuropharmacological activities, crude methanolic extracts of *B. lyceum* showed significant ($P<0.05$) central nervous system depressant activity and in forced swimming test it showed anxiolytic effects.

Keywords: Analgesic, anxiolytic, Balochistan, *Berberis lyceum*, Berberidaceae.

INTRODUCTION

Medicinal plants are acclimated for the ameliorative purposes or for the synthesis of advantageous drugs. Medicinal plants are easily accessible antecedent for health purpose in rural and affiliated area (Gupta *et al.*, 2015). Nowadays, plants are not the source of nutrients but also a potent medication (Shabbir *et al.*, 2012). Even as methods of medications better throughout the centuries plants play a basic role in the health disorder system (Irshad *et al.*, 2013). About (30%) yields existing in the market are prepared from plants (Gulfraz *et al.*, 2015). American customer paid 3 million dollars for drugs acquired from higher plants (Saranzai *et al.*, 2013). In China old treatment are centered for treating 90% of rural and 40% urban patients. In 1991, about 700,000 tons of plants acclimated in medicines out of which 80% were acquired from the land while in India, 400,000 registered acceptable medical consultants, compared to (332,000) registered doctors (Ahmad *et al.*, 1998). Pakistan is a country adored with array of climate, ecological zones and topographical region and accept different biodiversity, absolute of advanced kind of plant species. Nearly 6000 plants species with remedial properties are found in Pakistan (Gulfraz *et al.*, 2015). The plants species are quiet frequently used for therapeutic purposes by indigenous folks in their regular lives (Bibi *et al.*, 2015). A total of 1572 genera and 5521 species are known in Pakistan, out of which 400-600 are therapeutically significant. About 400 species are endemic to Pakistan (Bibi *et al.*, 2015). In Pakistan, the bounded communities

of altered regions accept centuries old awareness and traditional practices of a lot of the plants. This native knowledge of medicinal plants has been conveyed from generation through articulate advice and personal acquaintance (Alam *et al.*, 2011). Baluchistan is the largest province expressive 43.6 percent of the land accumulation of Pakistan. The rural residents of the Baluchistan are very much abased on biological assets for their nourishment. Among these rural populaces, remedial plants are the most suitable solution for most of the health problems (Jamal *et al.*, 2012, Ahmad *et al.*, 2014).

The highlands of N. Baluchistan (North) are the warm spots of healing and widespread plant in Pakistan, In Balochistan and numerous remedial plants have been collected and flogged in the native marketplace by local public so healing plants are limited only in endangered areas. Moreover, over misuse of medicinal plants affected serious risk to the existence and re-generation of countless curative plant species (Khatta *et al.*, 2013).

The widespread species that which are used in traditional medication in the region lack in pharmacological studies. In this regard present study is designed to examine the pharmacological properties of *Berberis lyceum*.

Genus *Berberis* (Berberidaceae) are prominent for its therapeutic value and are comprised in British and Indian pharmacopeias (Mashwani *et al.*, 2013). In Pakistan, *B. lycium* widely spread in Baluchistan province, NWFP, Punjab and in northerly areas at increase of 900 to 2900 m (Jabeen *et al.*, 2015). In Balochistan it is found in Sherani and Zirat districts, *B. lycium* roots have multipotential remedial properties and for the treatment of eye illnesses

*Corresponding author: e-mail: pharmacognosist59@yahoo.com

menorrhagia, chronic diarrhea, febrifuge and piles while leaves for jaundice and stem for diabetes, broken bones wounds, ulcers and sore eyes, stomachic antihistaminic, antipyretic, astringent, diaphoretic, antispasmodic and properties. Its fruit is used as a tonic against liver and heart diseases (Tamilselvi *et al.*, 2014). *B. lycium* is reported to contain various types of plant secondary metabolites including alkaloids diarylheptanoids, flavonoids terpenoids, steroids, phenols and tannins (Javadzadeh *et al.*, 2013).

MATERIAL AND METHODS

Plant material

The plant's fresh roots were collected from the village of Ahmadedergah near to Tahkhta Suleiman District Sherani Balochistan Pakistan.

Preparation of extract

The roots were wash with tap water kept for drying under shade then powdered in fine particles and extracted with methanol by maceration process at room temperature for (15) consecutive days with periodically shaking as well as stirring. The Methanolic extracts were filtered through Whatman filter paper and concentrated on rotary evaporator, dark brown semi-solid extract was obtained.

Animals

The male mice (Swiss Albino mice) of weight about (22-29 g) were used from Center for advance studies vaccinology and Biotechnology research (CASVAB), University of Balochistan Quetta. The animals were kept under standard protocol of relative humidity, temperature ($22\pm1^{\circ}\text{C}$), 12 hours light/dark cycles and provided with water and standard food at the Laboratory (CASVAB).

Drugs and chemicals

Diclofenac sodium (Hisun Pharma, Pakistan), Diazepam, acetic acid (Merck) and formalin (Merck) were used in experiments. All drugs and chemicals used were of analytical reagent grade.

Neuro-pharmacological activities

The methanolic extract (ME) was assessed for its neuropharmacological activities by using cage cross, traction test, open field, swimming test, and rearing test. Animals were arranged in four groups (control group treated with 0.5 ml/kg saline, *B. lycium* crude methanolic extract (CME) 250 and 500mg/kg and standard (Diazepam) drug treated groups).

Open field test (OFT)

The is classically used to evaluate anxiety and also be used for common assessment of animal basal locomotors activity and examination in rodent was carried out (Jabbar *et al.* 2016). The OFT of square meter was alienated into some squares. The device was surrounded a with 40 cm

top wall. After thirty minutes of oral administration of crude extract, the number of squares visited by the mice was noted 10 min.

Rearing test

1000ml glass beaker was used in this study. The observation was evaluated to count the upward movements of the mice locating the body in an erect posture in the beaker (Sanchez-Mateo *et al.*, 2002; Kasture *et al.*, 2002; Sakina *et al.*, 1990). The observation was analyzed for 10 minutes (Qureshi *et al.*, 2015).

Traction test

This test was to calculate the time spend by the mice to cover an iron rod of 1-meter length. At first, the mice were skilled to travel iron rod. Any difference in time taken by the treated mice compared to the control mice describes the calming or stimulating result of the drug, respectively (Sanchez-Mateo *et al.*, 2002; Kasture *et al.*, 2002; Debprasad *et al.*, 2003).

Cage crossing movement test

The study was made on animal in an accurately advised container of rectangular form. Both, drug treated and control animals were kept in cage and their cage crossing activities were commended (for 10 minutes). The investigation is significant for the motor action in an animal. This study was accomplished according to method reported by Crestani *et al.*, (2000).

Force swimming test (FST)

This test was performed as described by Turner (1965) and Sanchez *et al.* (2002). This test is used to determine CNS and muscle activity of the crude extract. Animals were positioned alone for period of six minutes in the glass tub filled with water at room temprature. Mice were placed alone for six minutes. Mouse when placed in the tub quickly starts to move its front and hind paws. The action time period of the animal is evaluated by a stop watch out of a total observation time of 6 minutes (Marvi *et al.*, 2016).

Analgesic activity

Formalin test

30 minutes after administration of vehicle, crude extract and standard drug, 20 μl of 5% formalin was introduced subcutaneously into the right hind paw of mice. The time spent (seconds) on biting and licking and number of biting and licking of the inserted paw for first phase (0-5 minutes) and second phase (16-30 min) were observed (Jabbar *et al.*, 2016).

Acetic acid-induced writhing test

The peripheral Analgesic activity of the *B. lycium* was evaluated by acetic acid-induced writhing test. The animals were distributed into, reference drug, control group and test groups ($n = 5$). Acetic acid (0.7%) was intraperitoneally (i.p) introduced to each mouse after 30

min of oral administration of the extract, vehicle and standard drug (Diclofenac sodium). Mice were observed for writhing (abdominal tightening, elongation of the body, and extension of the hind limb) for the 30 minutes (Vogel, 2007, Imam *et al.*, 2012, Ahmed *et al.*, 2017).

STATISTICAL ANALYSIS

All data were evaluated by using SPSS (version 22) and Dunnett's t-test was performed for determination of significance value between means of control, *B. lycium* crude methanolic extract (250 and 500mg/kg) and standard drug treated groups and $p < 0.05$ considered statistically significant (Marvi *et al.*, 2016).

Ethical Approval

The study was approved by the Ethical committee of Faculty of Pharmacy and Health Sciences (Protocol/Ref No:ERC/004/2017).

RESULTS

Open field test

The results reveal that mean number of squares crossed for control group were 215.2 ± 4.54 , for crude extract of *B. lycium* 250mg/Kg 193.6 ± 4.43 , for 500 mg/Kg 199.8 ± 3.22 and for standard drug (Diazepam) treated group 98.2 ± 4.13 . In this test significant ($p < 0.05$) result were observed in all of the drug doses and results were significant as compared with control group and standard drug.

Rearing test

The results of the test show that mean number of rearing activities of control group 45 ± 16.4 , for *B. lyceum* 250 mg/kg were 15 ± 1.79 , for 500 mg/Kg were 11.16 ± 1.72 and for standard drug group (Diazepam) 5.4 ± 2.75 . Results of experiment reveal that both doses of *B. lycium* produced significant ($p < 0.05$) sedative effects and results were comparable with control and standard drug diazepam.

Cage crossing test

The result of the test indicates that mean number of cage crossings were 29 ± 1.41 for control group, for *B. lyceum* 250 mg/Kg were 12.6 ± 0.81 , for 500mg/Kg were 17.6 ± 1.97 and for standard drug (Diazepam) treated group were 15.8 ± 1.39 . Results show that *B. lycium* at 250mg/Kg produced statistically significant results ($p < 0.05$) that causes reduced cage crossing activity as compared with control and standard drug.

Traction test

The result of the test indicates that mean time for crossings steel rod was 8 ± 9.06 seconds for control group, for *B. lycium* 250 mg/Kg value was 11 ± 1.7 , for 500 mg/Kg value was 13.6 ± 1.29 and for standard drug

(Diazepam) treated group was 18.4 ± 1.07 . Throughout the traction test administration of *B. lycium* increased the traveling time, which shows that the drug has sedative effects, results were comparable with control and standard drug (table 1).

Forced swimming test

The results of the experimentation signify that, for control group mean time consumed for mobility was 3.25 ± 0.009 minutes, and immobility time was 2.35 ± 0.009 minutes, for *B. lycium* 250 mg/Kg treated group, mobility time was 3.38 ± 0.01 minutes and immobility time was 2.22 ± 0.014 , for 500 mg/Kg treated group mobility time was 3.39 ± 0.02 and immobility time was 2.21 ± 0.01 minutes. For Diazepam (standard drug) group mobility time was 2.19 ± 0.02 minutes immobility time was 3.41 ± 0.02 . The results of the experiment signify that *B. lyceum* 250 and 500 mg/Kg produced anxiolytic effect (table 2).

Analgesic activity

Formalin test

The results of Phase-One (0-5 Minutes) of the test for control group reveals that, mean number of biting and licking were 40.2 ± 8.33 and time consumed on licking and biting was 48 ± 5.39 and in 2nd phase number of biting and licking were 60.6 ± 3.86 and time consumed on licking and biting was 124 ± 14.87 (seconds), for *B. lycium* 250 mg/Kg, number of biting and licking were 22.2 ± 3.4 and time consumed on licking and biting was 55.2 ± 3.11 (seconds), and in 2nd phase number of biting and licking were 37.6 ± 3.96 , time consumed on licking and biting was 71.6 ± 12.59 (seconds) for 500 mg/Kg number of biting and licking were 20.8 ± 3.22 and time consumed on licking and biting was 43 ± 4.42 (seconds) and in 2nd phase number of biting and licking were 57 ± 6.47 and time consumed on licking and biting was 43.2 ± 6.5 (seconds), for standard drug (Diclofenac Sodium) treated group number of biting and licking were 30 ± 2.16 and time consumed on licking and biting was 36 ± 1.03 (seconds), in 2nd phase number of biting and licking were 57.2 ± 0.8 and time consumed on licking and biting was 58.8 ± 0.37 (seconds). Results reveal that both doses of *B. lycium* showed significant ($p < 0.05$) results as compared with standard drug (table 3).

Writhing test

The average number of writhes for control group was 76.2 ± 2.645 , for *B. lycium* 250 mg/Kg were 26.2 ± 2.38 , for 500 mg/Kg were 23.4 ± 1.86 and for standard drug (Diclofenac Sodium) treated group were 21.8 ± 0.49 . Results reveal that *B. lycium* showed significant analgesic effects (table 4).

DISCUSSION

Current work represents the first step towards the understanding of the effects in the central nervous system of the crude extract obtained from the roots of the *B.*

lycium on experimental animals. The plant showed anxiolytic, CNS depressant and analgesic effects. Phytochemical literature reveals that *B. lycium* contain alkaloids flavonoids, terpenoids, phenols, diarylheptanoids, tannins and steroids (Javadzadeh *et al.*, 2013). CNS depressant effect is probably due to presence of alkaloids and flavonoids (Ahmad *et al.*, 2017), alkaloids are reported to produce CNS depressant effects. Many neuroactive steroids and flavonoids are GABA (Gamma aminobutyric acid) receptors ligands in the CNS that may act as benzodiazepine-like agent (Walf *et al.*, 2007, Detke *et al.*, 1995). Current study shows that *B. lycium* produced anxiolytic and sedative effects, this may be due to the phytoconstituents. In provision of this, it has been found that flavones bind with great affinity benzodiazepine location of the GABA receptor. The

locomotor test is a measure of the level of nervousness of the CNS and calm resulting from sadness of the CNS (Sharman *et al.*, 2014). The result showed that the extract pointedly reduced the locomotor activity as presented by the results of the open field test, cage crossing, rearing and traction test.

Immobility or despair behavior produced in forced swimming test were hypothesized to display animal's hopelessness and low mood (behavioral despair) and are taken as paradigm of depression. This simple behavioral procedure is widely used test for screening novel antidepressants (Dina *et al.*, 2010). The results reveal that immobility period with *B. lycium* crude extract was markedly short as compared to that of control at both (250 and 500mg/kg) doses. Phytochemical literature reveals

Table 1: Effect of *B. lycium* on neuropharmacological activities of mice

Treatment	Dose	Open field test Mean±SEM	Cage crossing test Mean±SEM	Rearing test Mean±SEM	Traction test Mean±SEM
Control	0.5ml saline/kg	215.2±4.54	29±1.41	45 ±16.4	8±9.06
<i>B. lyceum</i> Crude extract	250mg/kg	193.6±4.43*	12.6±0.81*	15±1.79*	11±1.70*
	500mg/kg	199.8±3.22*	17.6±1.97*	11.16±1.72*	13.6±1.29*
Diazepam	2mg/kg	98.2±4.13**	15.8±1.39**	5.4±2.75**	18.4±1.07**

Results are compared with control and standard drug. Values are the mean number of activities ±S.E.M, (n=5); * = Significant results ($P<0.05$), ** = highly significant results ($P<0.01$).

Table 2: Effect of *B. lycium* on forced swimming test of mice

Treatment	Dose	Mobility time Mean ±SEM (minutes)	Immobility time Mean ±SEM (minutes)
Control # 1	0.5ml/kg	3.25±0.009	2.35±0.009
<i>B. lycium</i>	250mg/kg	3.38±0.014*	2.22±0.014*
	500mg/kg	3.39±0.02*	2.21±0.01*
Diazepam	2mg/kg	2.19±0.02**	3.41±0.02**

Values are the mean number of activities ±S.E.M, (n=5); * = Significant results ($P<0.05$), ** = highly significant results ($P<0.01$).

Table 3: Effect of *B. lycium* on formalin test of mice

Treatment	Does mg/Kg	1st Phase (Mean ±S.E.M)		2nd Phase (Mean ±S.E.M	
		No of Licking	Time spent (seconds)	No of Licking	Time spent (seconds)
Control # 2	0.5ml/kg saline	40.2±8.33	48±5.39	60.6±3.86	124±14.87
<i>B. lycium</i>	250mg/kg	22.2±3.4*	55.2±3.11*	37.6±3.96*	71.6±12.59*
<i>B. lycium</i>	500mg/kg	20.8±3.22*	43±4.42*	57±6.47*	43.2±6.5*
Diclofenac Sodium	50 mg/kg, oral	30±2.16**	36±1.03**	57.2±0.8**	58.8±0.37**

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

Table 4: Effect of *B. lycium* on acetic acid induced writhing test of mice.

Treatment	Dose (mg/kg)	Mean Number of writhing
Control # 3	0.5ml/kg saline	76.2±2.64
<i>B. lyceum</i>	250 mg/kg	26.2±2.38*
<i>B. lyceum</i>	500 mg/kg	23.4±1.86*
Diclofenc Sodium	50 mg/kg	21.8±0.49**

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

that *B. lyceum* contain phenols (Javadzadeh *et al.*, 2013), previous reports indicated that polyphenolic compounds possess comparable antidepressant effect (Afsar *et al.*, 2017), with that of standard antidepressant drug which increases the level of noradrenaline and serotonergic transmission in brain of antidepressant models. The present study showed that *B. lycium* might have antidepressant and anxiolytic activity due to the existence of polyphenolic compounds.

Intense pain immediately starts after formalin injection (early phase). Besides inhibiting the production of prostaglandin, the findings suggest that the extract inhibited the activation of primary afferent fibers by formalin. The formalin test was used to determine, whether the analgesic effect of this extract occurs at the central or peripheral level. Formalin injection has been reported to produce a distinct biphasic analgesic response. The 1st phase (0 to 5 min) results from direct stimulation of nociceptors. The 2nd phase (15 to 30 min) is thought to be an inflammatory response related with inflammatory pain, a process in which several inflammatory mediators are involved, including histamine, serotonin, bradykinin and prostaglandins (Tjolsen *et al.*, 1992). The inhibitory effects of *B. lycium* extracts were observed in both phases. The literature shows that centrally acting drugs inhibit both phases of pain, while peripheral acting drugs mainly inhibit the second phase (Gutierrez *et al.*, 2017). Therefore, the results reveal that the *B. lycium* showed both peripheral and centrally action. The analgesic effects showed by the extracts might due to presence of phytochemicals including tannins and flavonoids, which are previously reported to possess analgesic activity (Waweru *et al.*, 2017).

The acetic acid induced writhing test is commonly used for peripherally acting drugs. The pain generation arises by liberating endogenous constituents and some other pain compound such as arachidonic acid metabolites via cyclooxygenases, such as prostaglandins (Tsong *et al.* 2007). Thus, *B. lycium* comprises two types of constituents having analgesic effect. The constituents in the *B. lycium* act peripherally by preventing endogenous substances deprived of any interruption in onset. Thus, the analgesic activity (peripherally) of *B. lycium* may be recognized by the existence of alkaloids probably belonging to various groups and polyphenolic compounds (Hijazi *et al.*, 2017). Therefore, it could be suggested that *B. lycium* might comprise pharmacologically dynamic constituents that can stop the release or the effect of endogenous substances responsible for the excitation of nerve terminations. These constituents may be qualitatively, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarone, triterpenes, sterols, anthraquinones and phenolic compounds are classes of compounds that known to have analgesic properties in various models of pain (Amoateng *et al.*, 2017).

Therefore, the phytochemicals found in the extract might have antagonized peripheral mediators of pain and thereby blocking transmission of pain (Koech *et al.*, 2017).

CONCLUSION

In light of the results of this study, it may be concluded that crude methanolic extract of *B. lycium* has CNS depressant, anxiolytic and analgesic activities, the current study also confirm the traditional usage of plant in pain. Extract may have several advantages such as cost-effectiveness and compatibility for biomedical and pharmaceutical applications, so it needs further investigation for quantitative and qualitative isolation of active constituents.

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