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ANALGESICS AND ANTIBACTERIAL ACTIVITIES OF *BERBERIS LYCIUM* ROOTS COLLECTED FROM DISTRICT ZIARAT, BALOCHISTAN

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Abstract:

Berberis lycium (berberidaecae) is a conventional medicinal plant of Balochistan province in Pakistan. It is traditionally used as an antipyretic, anti inflammatory, anti hypercholesterolemia, antidiabetic and for wound healing. This present study has evaluated the pharmacological (analgesic) and antibacterial activities of the crude ethanoic extract of the roots of Berberis lyceum. Disc diffusion and Well methods were used for antibacterial activities where as acetic acid induced Writhing and Formalin tests were used to determine analgesic activities. Berberis lycium crude extracts showed significant antibacterial activity on Gram +ive anaerobe Clostridium perfringens and Gram -ive Escherichia coli. However, it did not show analgesic activity in albino mice at both 250 and 500 mg/kg oral doses. It is therefore concluded that B. Lycium has a very positive effect on both Clostridium perfringens and Escherichia coli.

Keywords: *Analgesics, antibacterial, Berberis Lycium, Ziarat, Balochistan*

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INTRODUCTION:

Plants are utilized in ancient medication for many years. Since ancient, the meditative data of various plants is being collected in many systems such as Siddha etc [1] Regarding WHO, more than 10 percent out of 258,650 species of plants are used for treatment. Majority of Pakistani population still rely on plants based therapy. Even most known drugs such as morphine have also been derived from plants [2].

The present developmental setup however focuses on the synthetic approach of medicines. Slight modification in the functional groups brings about changes in the activities of the drugs but still more stress is given on the natural resources [3]. Reported by WHO, a huge population depends on plant remedy. Such population uses the extracts of plants [4]. In Asia, herbal medicines have a long lasting history. The Hamalian herbs have been prominent for such purpose since ancient. Around 600 B.C Vedas reported the first use of plant as a medicine. This shows the archaic source data, which includes sixty seven plant species [5]. The use of Unani drugs which were first introduced by Greece and then were brought to Indo Pak territory by Muslim scholars is at large. Many people of different region of Pakistan still rely on plants' extracts for the Ailment. Pakistan has different climates therefore is rich in medicinal plants [6].

Berberis lycium (commonly called Ishkeen in Urdu) hail from family Berberidaceae grows in many areas of Pakistan including Himalaya and Balochistan. The plant as a whole or several parts such as root, stem are used for medicinal purpose. This plant has additionally gained wide acceptance for its healthful price in Ayurveda medication. This plant is thought to forestall many disorders of the body [7]. Balochistan is rich in medicinal plants however these have not been evaluated sufficiently. Most of the medicinal plants are yet to be checked for its medicinal usage. Most of the inhabitants still benefits from crude extracts of plants [8]. The genus Berberis Lycium is employed by the social group people in J&K, India, since the past times. The experimental investigation showed that there are many medicinally important substances such as tannins; terpenoids, flavonoids etc are present in it. Its roots are yellow in color, wealthy in alkaloids Berberine etc.

Pharmacological activities of Berberis lycium

Medical specialty investigation has shown that it exhibits medicinal properties, hepatoprotective, pesticidal, antimutagenic; wound healing, anticancer, antimicrobial, antihyperlipidemic and antifungal characteristics, additionally edible specifics [9]. However, present study aimed to analyze analgesic activities of the plant's root collected from Ziarat, Balochistan. This, to the best of our knowledge was not evaluated earlier. Moreover, the antibacterial activity on Clostridium Perfringens and E.Coli was evaluated first time in Balochistan. Furthermore, ethanol, for the first time in Balochistan was used for the extraction of the crude drug from the roots of Berberis Lycium.

MATERIAL AND METHODS:

Collection and identification of Plant

Roots of Berberis Lycium were collected from District Ziarat, Balochistan; Mr. Qasim Shah (lecturer in botany at college side in Local Government of Balochistan) identified the plant.

Preparation of methanol extract

After collection, the roots were dried under shade for 15 days. The roots after 15 days were then minced into a powder by a mincer. After drying plant was converted into fine powder with the help of mincer. Later on the minced powder was soaked in ethanol in an air tight glass jar for 7 days. After that the Solvent was filtered and evaporated by using Rotary evaporator, Dark brown semi-solid extract was obtained.

Experimental Animals

Albino mice (25- 30 grams) of either sex were used. And these mice were acquired from CASVAB (a research center of University of Balochistan).

Analgesic activity

Acetic acid induced writhing test

Writhing induced by acetic acid in mice, the test was used to determine the analgesic activity [10]. 6% acetic acid solution was administered intra-peritoneal to the mice later on, after 30 minutes of the administration of the saline treated (control group), B. Lycium 250 & 500 mg/kg treated group and Aspirin 300mg/kg treated group. Meanwhile the writhings were counted for 30 minutes. Decrease in the writhings is deemed to have analgesic activity of the plant in contrast to control (aspirin). However, the plant did not show any decrease in the writhings [11].

Formalin test

Mice were treated with formalin for the sake of pain induction. 20 µl of 1% formalin in distilled water was subcutaneously injected to the paw (dorsal hind) of the mice witha microsyringe (26-gauge needle), after 30 minutes of administration of saline (control group), B.Lycium 250 & 500 mg/kg oral dose and Aspirin 300mg/kg orally. After injection the mouse was put into a chamber where the licking and

biting of the injected paw were counted. The time of licking and biting was divided in two phases the first phase was from 0-15 and the second was taken from 26-40 [12].

Bacteria for test

The bacteria in the test were collected from CASVAB (Centre for Advanced Studies in Vaccinology and Biotechnology). Gram positive anaerobic bacteria (Clostridium perfringens) and Gram negative facultative anaerobic bacteria (Escherichia coli) were selected based on the sudy objective. The microbes were cultured on RCM and Macconkey media respectively.

Antibacterial assay

Antibacterial activity was determined by Disc Diffusion Method [13] and Well Diffusion Method [14].

Preparation of 0.5 McFarland standards

The required standard was prepared by adding 0.5 ml. of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2 \cdot 2H_2O$) to 99.5 ml. of 0.18 M H_2SO_4 (1% w/v) while stirring constantly (Andrews, 2004).

Disc diffusion method

In 5ml of the normal saline the inoculum of each bacterium was prepared and such suspension was compared with 0.5 Mcfarland standards. The plates of hard RCM and Macconkey agar were inoculated thoroughly with sterile swabs of cotton. In DMSO (dimethylsulfoxide) solutions of CEE (500mg/ml) were prepared. While as 2mg/ml of penicillin for positive control was prepared in DMSO. For negative control simple pure DMSO was used.

Pure DMSO was used as negative control. 5mm diameter paper discs of Whatsman filter paper grade 5 (20µm) were dipped in the solution of CEE and were positioned on the inoculated media plates. Streptomycin and Penicillin were used as positive control while DMSO was used as negative control. The plates were incubated at 37ºC for 24-48 hours. Each test was repeated three times.

Preparation of stock solution

Stock solution of 500 mg/ml concentration of the extract was prepared in DMSO. Three twofold dilutions were made.

MIC determination by well diffusion method

25ml of media was poured in petri dishes and were let to solidify. The wells of 10mm diameter were made in the solidified media.1-2 µl of bacterial suspension (compared to 0.5 McFarland standard) was deposited on the solidified media with the help of sterile cotton swab. The plates were incubated for 24-48 hrs at 37ºC.

RESULTS:

Analgesic Activity (Writhing test)

Results show that in saline treated (control group) the number of writhes after administration of acetic acid in mice was 102±1.30. While in B. lycium 250mg/kg crude extract treated group numbers of the writhings were 100.2±1.59. In B. lycium 500mg/kg of crude extract numbers of the writhes were 99±1.24 and with standard drug (Aspirin) treated group the activity was 27.4±2.88 (Table 1).

Table 1: Analgesic activity of B. lycium

	dose	No of writhings
Control		102 ± 1.30
B.Lycium	250	100.2 ± 1.59
B.Lycium	500	99 ±1.24

Formalin test

1st phase

Results show that in saline treated (control group) the number of licking was 56.4±8.23 and time spent on licking was 96.6±12.17 seconds. While in 250mg/kg B.Lycium rude extract treated group numbers licking was 43.6 ±3.99 and time spent on this was 82.2±7.15 seconds. In 500mg/kg of crude extract numbers of licking was 42.8±0.73 and time spent was 84.8±2.44 seconds and with standard drug (Aspirin) treated group the number of licking was15.8±0.86 and time spent was32±2.12 seconds (Table 2).

2nd phase

Results have shown that in saline treated (control group) numbers of the licking were 11±1.58 and time spent on this was19.4±3.42 seconds. While in 250mg/kg M. lamellata crude extract treated group numbers the activity was 9.6±1.36 and time spent on this was14.4±1.50 seconds. In 500mg/kg of crude extract numbers the activity were 14±2.47and time spent was 18.2±2.80 seconds and with standard drug (Aspirin) treated group the activity was 0 (Table 2).

Antibacterial assay

Antibacterial activity was determined by Disc Diffusion Method [13] and Well Diffusion Method [15].

Well diffusion method

Table 3 elaborates that stock solution of B. Lycium showed an average inhibition zone of 22.66 mm while as dilution1 showed 19.33mm, dilution2

showed 16.33mm and dilution3 showed 13.66mm zone which declares the presence of antibacterial activity of B. Lycium against C. perfringens (an anaerobic Bacteria).

Table 4 explains that with 5mm of disc the stock solution gave a zone of 13.66mm however, dilution1 showed 9.33mm and dilution2 showed 7mm zone of inhibition but dilution 3 did not show any zone of inhibition.. This disc diffusion method declares the antibacterial activity of B. Lycuim against C. Perferingens in stock solution of 500mg/ml (w/v) in DMSO in its two-fold dilutions.

Table 2: Formalin induced analgesic activity

		Phase1 (0-15min)		Phase 2 (25-40 min)	
	Dose	No of licking & Biting	Time (sec)	No of licking & Biting	Time (sec)
Control		56.4± 8.23	96.6± 12.17	11±1.58	19.4±3.42
B. Lycium	250 mg/kg	43.6± 3.99	82.2± 7.15	9.6±1.36	14.4± 1.50
	500 mg/kg	42.8±0.73	84.8± 2.44	14±2.47	18.2±2.80
Aspirin	300 mg/kg	15.8±0.86	32±2.12	0	0

Table 3: Activity of B.Lycium on Clostridium Perferingis

	Disc No	Stock solution	Dilution 1	Dilution 2	Dilution 3
	1	23	19	16	14
	2	23	20	17	13
	3	22	19	16	14
Mean		22.66	19.33	16.33	13.66

Table 4: Activity of B. Lycium on Clostridium Perferingis

	Disc No	Stock solution	Dilution 1	Dilution 2	Dilution3
	1	14	9	7	0
	2	14	10	7	0
	3	13	9	7	0
Mean		13.66	9.33	7	0

Table 5: Activity of B.Lycium on E.Coli

	Disc No	Stock solution	Dilution 1	Dilution 2	Dilution 3
	1	25	20	17	14
	2	24	19	17	13
	3	23	18	15	13
Mean		24	19	16.33	13.33

Table 6: Activity of B.Lycium on E.Coli

	Disc No	Stock solution	Dilution 1	Dilution 2	Dilution 3
	1	15	12	8	0
	2	16	11	7	0
	3	14	10	7	0
Mean		15	11	7.33	0

The antibacterial activity of B.Lycium against E.Coli in well diffusion method is being explained in the table 5. The maximum zone of 24mm is given by stock solution where as the minimum activity is shown by dilution 3 which is 13.33 mm. However, Dilution1 gave a zone of 19mm and dilution 2 showed 16.33mm zone of inhibition. Thus, it is concluded that the B.Lycium has a significant antibacterial activity against E.Coli.

Disc diffusion method of antibacterial activity of B.Lycium against E.Coli is well elaborated in table 6. Stock solution gives 15mm zone and dilution1 gave a zone of 11mm while as dilution2 gave a zone of 7.33mm which is the least activity of B.Lycuim as dilution 3 didn't make any zone of inhibition. Hence stock solution and its first two-fold dilutions gave a significant result which shows the positive activity of B.Lycium against E.Coli.

DISCUSSION:

Nature has blessed a wide range of plant based active chemical substance that probably promote the health and these poly constituents increase action of each other [16]. Berberidaceae is a heterogeneous collection of 650 species and 17 genera of angiosperms. Berberis lycium is found through the temperate and subtropical regions of the world (apart from Australia). Berberis lycium is native to Nepal, globally distributed in various part of the world. It occurs in subtropical and temperate regions from Kashmir to Uttaranchal on the outer Northern-western Himalayas [13]. The present study has shown antibacterial activity of the plant collected from Ziarat District Balochistan, Pakistan. The has shown significant antibacterial activity against Clostridium Perferingens with 22.66mm of inhibition zone at the dose of 500mg/kg in well diffusion method [17]. While as in disc diffusion method the zone of inhibition given was 13.66mm. The zone of inhibition given by B.Lycium agans E.Coli in well diffusion method was 24mm while as the zone given by disc diffusion method was 15mm. This antibacterial activity against E.coli was earlier reported by Irsahd et al. The analgesic activity of B.Lycium against Albino mice in the present study was found negative as has shown in the table1 and table 2.

CONCLUSION:

From the stated tables and by the experiments performed this could be concluded that B.Lycium has positive activity against C.Perferingens and E.Coli. However it did not show significant analgesic activity against the pain induced in Albino mice.

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