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A STUDY ON ANALGESIC AND ANTIBACTERIAL ACTIVITIES OF RHAZYA STRICTA LEAVES COLLECTED FROM DISTRICT BOLAN, BALOCHISTAN PROVINCE, PAKISTAN

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ABSTRACT

Rhazya stricta is an inhabitant plant found in the deserts of Pakistan. The vegetations of this plant hold alkaloids, glycosides, triterpenes and tannins and it has a plentiful quantity of natural alkaloids. Natural alkaloids have many organic actions like as antihypertensive, antibiotic and anticarcinomious actions and also revealed as CNS activators. Phytochemical examination has acknowledged more than hundred alkaloids. For assessing Antibacterial and Analgesic effects of *Rhazya stricta*, the plants were collected in their respect season and dried under shade for 15 days and then the plants were converted in fine powder. A dark-brown semisolid was obtained by soaking the powder in ethanol for 7 days and subsequent evaporation of solvent. The analgesic activity was determined by induced writhing test by administration of 6% acetic acid solution. However minimal significant activity was observed. Antibacterial activity was determined by Disc Diffusion Method and Well Diffusion Method. Significance antibacterial activity was observed by using Disc Diffusion method cultures of *E.coli* bacteria. However, appearance of zone in the cultures of *C.perfringes* (an aerobic bacteria) when tested for antibacterial activity by Well Difusion method declares that *R.strichta* possesses such activity. Moreover, activity gains *E.coli* showed positive result when tested by Well Diffusion method.

From the stated tables and by the experiments performed this could be concluded that *R. stricta* has positive activity against *C.perferingens* and *E.coli*. However it showed minimal analgesic activity against the pain induced in Albino mice.

Keywords: *Rhaya stricta*, Antibacterial, Analgesic, Disc Diffusion method, Well Diffusion Method

INTRODUCTION

Certain medical conditions are still treated by utilizing the local and ethnic practices by using different plant extracts grown locally **[1]**. For the purpose, in a published study, 27 home-grown medications are studied which were reported to have a medicinal effect **[2]**. Similarly, in Pakistan there are so many plant with their extracts having medicinal usage have been reported as well **[3]**. Several diseases like intestinal problems, illnesses, liver grumbling, diabetes etc have been treated by using conventional medicines in Balochistan from the ages **[4]**.

One of these plants is *Rhazya stricta*, an perennial, noxious flora, small, vertical and glabrous. It is a important therapeutic herbal used in herbal medications to treat numerous disorders in central Asian states and Asian states, Qatar, Saudi Arabia and Dubai and other Arab countries. The specis Rhazya have its place to the order Gentianales, family Apocynaceae and subfamily Rauwolfioideae **[5]**. Rhazya species were named by Abu Bakr Mohammed bin Zakariya ArRazi, a muslim botanist and it is famous in European countries under the famous name of Rhazes **[6]**. Many parts of *Rhyza stricta* is used in common treatment against many diseases such as diabetes, foot injury, skin diseases, G.I.T pain etc. The shurb is used in Pakistan, in shape of extract, for many diseases that are antipyretic, anticarcinomas, different types of Diabetes, helminthiasis, inflammaton conditions, rheumatism, upper respiratory diseases, stomach problems, and skin ailments **[6]**. In the rural area of Balochistan, the vegetations of *R. stricta* are used in traditional medication as a dealing for syphilis, long-lasting rheumatism, and bodilyache. Pimples of face and acnes are cured by using powdered paste of Rhazya stricta. For burning foot disease its leaves are kept under foot. For tooth ache its branches are used as tooth brush. For the treatment of achenes and heat burn its paste soaked with butter is used **[1]**.

Distribution:

This plant is inhabitant of the desersts of Pakistan and many other parts of the world. It is plentifully found in the N.Western area of Pakistan and India. This

plant genera is well-known vegetations that breed in Pakistan and is well-thought-out unique of the utmost valuable therapeutic florae that are originate in the maximum deserty zones in the Region. *Rhazya stricta* cultivates in low lands by sediment having sand and stones. Yaghmoor., in 2015 said that its number is fast in sandy areas.

Phytochemistry:

The vegetations of this plant hold alkaloids, glycosides, triterpenes and tannins and it has a plentiful quantity of natural alkaloids. Natural alkaloids have many organic actions like as antihypertensive, antibiotic and anticarcinomious actions and also revealed as CNS activators. Phytochemical examination has acknowledged more than hundred alkaloids **[7]**. These alkaloids have several pharmacological properties. from the leaves and legunes of *Rhazya stricta* more than hundred Alkaloids are obtained. , a hefty figure of alkaloids from *Rhazya stricta* is not found and its extraction is more time devouring **[8]**.

MATERIALS AND METHODS

Collection of plant meterials

The *R. stricta* plants were collected in their repective season from Jhal Magsi area of Balochistan and were identified by Botany department University of Balochistan, Quetta.

Preparation of methanol extract

After collection, the plants were dried under shade for 15 days. The plants 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. 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), *R. strichta* 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. 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 with a microsyringe (26-gauge needle), after 30 minutes of administration of saline (control group), *R. stricta* 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.

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 study objective. The microbes were cultured on RCM and Macconkey media respectively.

Antibacterial assay

Antibacterial activity was determined by Disc Diffusion Method and Well Diffusion Method. Preparation of 0.5 McFarland standards The required standard was prepared by adding 0.5 ml. of 0.048 M BaCl2 (1.17% w/v BaCl2·2H2O) to 99.5 ml. of 0.18 M H2SO4 (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 37^{o}C 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 37^{o}C.

RESULTS and DISCUSION:

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 *R. strichta* 250mg/kg crude extract treated group numbers of the writhings were 100.2±1.59. In *R. strichta* 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)**.

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 *R. stricta* 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 *R. stricta* 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±2.47 and 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 **[9]** and Well Diffusion Method.

Well diffusion method

Table 3 elaborates that stock solution of *R. strichta* showed an average inhibition zone of 22.66 mm while as dilution1 showed 19.33mm, dilution 2 showed 16.33mm and dilution 3 showed 13.66mm zone which declares the presence of antibacterial activity of *R. strichta* against *C. perfringens* (an anaerobic Bacteria).

The antibacterial activity of *R. stricta* against *E.coli* in well diffusion method is being explained in the **Table 4**. 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 *R. stricta* has a significant antibacterial activity against *E.coli*.

Disc diffusion method

Disc diffusion method of antibacterial activity of *R. stricta* against *E.coli* is well elaborated in **Table 5**. Stock solution gives 15mm zone and dilution1 gave a zone of 11mm while as dilution 2 gave a zone of 7.33mm which is the least activity of *R. stricta* 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 *R. stricta* against *E.coli*.

Table 1: Analgesic activity of *S. strichta* dose

Control	Dose	No of writhings
Control		102 ± 1.30
R. strichta	250	100.2 ± 1.59
R. strichta	500	99 ±1.24

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
R. stricta	250 mg/kg	43.6± 3.99	82.2± 7.15	9.6±1.36	14.4± 1.50
R. stricta	500 mg/kg	42.8±0.73	84.8± 2.44	14±2.47	18.2±2.80
Asprin	300 mg/kg	15.8±0.86	32±2.12	0	0

Table 3: Activity of *R. stricta* 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 *R. stricta* 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 5: Activity of *R. stricta* 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

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. 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. The present study has shown antibacterial activity of the plant collected from Jhal Magsi District Balochistan, Pakistan. The study has shown significant antibacterial activity against *Clostridium perferingens* with 22.66mm of inhibition zone at the dose of 500mg/kg in well diffusion method. While as in disc diffusion method the zone of inhibition given was 13.66mm. The zone of inhibition given by *R. stricta* 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 *R. stricta* 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 *R. stricta* has positive activity against *C.perferingens* and *E.coli*. However it showed minimal activity against the pain induced in Albino mice.

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