

Anti-dermatitis, anxiolytic and analgesic effects of *Rhazya stricta* from Balochistan

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Abstract: Current study was carried out on *Rhazya stricta*. Plant material was collected from Jhalmagsi Dist. Balochistan, Pakistan. Methanolic extract of *Rhazya stricta* was tested for anti-dermatitis, analgesic, anxiolytic effects, insecticidal activity and Brine shrimp Bioassay. Crude extract showed significant anti-dermatitis activity, as the results of intensity score showed mild Excoriation or erosion, moderate Edema or populations and absence of Erythema or hemorrhage, Scratching time was decreased to 1.45 and histological observations of mice treated with crude extract showed mild changes and few inflammatory cells in several microscopic fields. The results of analgesic activity were significant and the percentage inhibition of writhes were 73.54% and 69.38% at 300mg/kg and 500mg/kg respectively. The overall response of crude extract in anxiolytic activities were depressive and crude extract showed sedative effects. In *Brine shrimp* (*Artemia salina*) lethality bioassay crude extract showed dose depended significant activity, and showed positive lethality with LD₅₀ 3.3004µg/ml. Insecticidal activity was positive against *Callosbruchus analis*, the percent mortality was 40%.

Keywords: *Rhazya stricta*, anti-dermatitis, anxiolytic, analgesic.

INTRODUCTION

In developing countries traditional medicine are used for the primary health care. In developed countries use of herbal medicine is also gaining importance. Herbal medicines are less toxic and have fewer side effects so, people are turning towards alternative medicine. Throughout the world medicinal plants are important utilize source of life saving drugs. Plants extracts contains bioactive compounds used in food as additives, also used in perfumes, cosmetics, dyes, and insecticidal preparations. These bioactive compounds are collectively known as secondary metabolites (Saif *et al.*, 2007). To maintain their health and cure their ailment about 80 % of world population uses the medicinal plants. For herbal, homeopathic, allopathic formulations which are sold widely in world market and used either in modern or traditional system of medicine, there is need to introduce new important plants of established therapeutic values, as interest in medicinal plant is increasing world wide as a source of medicine (Ahmad *et al.*, 2004). In Pakistan about 6000 medicinal plant species are present, huge amount of foreign exchange can be saved if allopathic and herbal drug manufacturers carry research on these plants and utilize them (Shinwari *et al.*, 1989). Total area of Balochistan is 43.6 percent and is largest province of the country. Many medicinal plants are native to Balochistan.

In local market various wild species are available. Scientific data is very limited on potential plants. If scientific information will be available, these potential herbs can be cultivated and used for medicinal purpose (Syed *et al.*, 2006). Current study was carried out on *Rhazya stricta* Decne (Family Apocynaceae) is a small glabrous erect shrub with a smooth central stem and dense semi-erect branches (Western, 1989). *Rhazya stricta* is a perennial plant that grows in dry sub-tropical regions. It is found throughout South Asia i.e. Afghanistan, Pakistan, India and also found in Middle East countries i.e. United Arab Emrits, Saudi Arabia and Iraq (Abdullah, 1979). In Balochistan it is found in Jhalmagsi, Bolan, Naseerabad, Lasebala and Hub (Shareeque *et al.*, 2005).

R. stricta is utilized as herbal drug in many countries. In Balochistan Pakistan it is used for snake bite, as a remedy for infant diseases, for tooth and eye diseases and root decoction in water is used for fever (Shareeque *et al.*, 2005). Urinary tract diseases are treated with *R. stricta* in Unani medicine (Gilani *et al.*, 2007). Although it is used in traditional medicine, but plant has not been investigated for pharmacological and toxicological studies. In this regard current study was designed to investigate pharmacological anti-dermatitis, anxiolytic and analgesic activities of *R. stricta*.

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MATERIAL AND METHODS

Plant material

Plant material was collected from JhalMagsi district of Balochistan Pakistan. The plant was identified and voucher specimen no.1A-S-06 was deposited in the herbarium of Department of Pharmacognosy, University of Karachi, Karachi, Pakistan. The fresh leaves and stem were chopped into small pieces. The chopped material was macerated with methanol for 15 days (2 times) at room temperature. The methanolic extract was filtered and evaporated under reduced pressure in rotary evaporator to yield a dark green residue.

Experimental animals

Albino mice of either sex obtained from animal house of Aga Khan University Karachi were used to determine the Anti-dermatitis, anxiolytic and analgesic activities. Mice weighing 25-30 gm were used. Animals were kept in colony cages (five animals in each group) with access to food and water. They were maintained in a climate and light controlled room ($30^{\circ}\text{C}\pm 1^{\circ}\text{C}$ 12/12 hours light/dark cycle) at least 7 days before testing or administration of the drug.

Anti-dermatitis activity

Induction of dermatitis (sensitization method)

Contact dermatitis was produced in mice according to method described by Josphine *et al.*, 2005. Dorsal hair of the mice was shaved with electrical razor. 20 μml of dinitrochlorobenzene were applied on each dorsum of each ear and dorsal skin (in acetone olive oil vehicle 4:1) 25% for 3 days. The animals were grouped in two groups, a vehicle (acetone-olive oil treated group) and the other dinitrochlorobenzene treated group. After sensitization (after 7 days) animals were divided in three groups, first group was treated crude extract, applied topically, second group was treated with base, and third group was treated with standard drug (Betnat-N).

Dermatitis severity was evaluated by;

i) Intensity score and scratching time

Intensity score and scratching behavior are two parameters used for evaluation of dermatitis severity. These parameters are measured according to method described by Wen *et al.*, 2001. For calculation of intensity score three items were assessed, Excoriation or erosion, Edema or papulations and Erythema or hemorrhage. Grading on a scale of 0-3 were used. Where 0= absent, 1= mild, 2=moderate, 3=sever.

Scratching time is cumulative time of scratching per 10 minutes.

ii) Histological observation

Two skin samples were taken, one from control and one from skin lesion site. After 13days of sensitization and

after 13 days of treatment with crude extract of plant, skin samples were taken, fixed in formalin (10%) and embedded in paraffin. 4 μm thick section were taken and stained with hematoxylin, eosin and with toluidine blue for assessment of mast cells and luna for eosinophils. Sections were viewed on an olympus AH light microscope.

Assessment of analgesic activity by writhing and hot plate method

These tests were performed according to the modified method of Koster *et al.*, (1959) and Turner (1965). Mice were used as the test animals in this method. According to this method writhes were induced by intraperitoneal injection of solution of acetic acid 10ml/kg. 30 minutes before to the administration of the acetic acid, the animals were treated orally with the test substance. Numbers of writhes was counted for 30 minutes immediately after acetic acid administration. A reduction in the number of writhing as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhing. For hot plate analgesic activity mice were placed in an empty beaker (2 liter) on a hot plate ($55\pm 1^{\circ}\text{C}$) and their reaction to heat was observed. When the animal raised and licked the front paws they were quickly removed from the hot plate. Mice were divided into 4 groups (i.e. Group-A for control, Group-B and Group-C for 300mg/kg and 500mg/kg oral doses of crude extract respectively, and Group-D for standard). Each group comprised of 5 animals, weighing 25-30 gm. Acetyl salicylic acid (Aspirin) as 300mg/kg orally was used as the reference compound. The crude drug and the acetyl salicylic acid were diluted in distilled water and administered orally. The control animals were treated orally with the same volume of saline as the crude extract.

Evaluation of anxiolytic effects

For the evaluation of anxiolytic effect different neuropharmacological activities which includes open field test, cage crossing test, head dip test, rearing test, traction test and forced induced swimming test. All the neuropharmacological tests were performed in a calm and peaceful environment.

In each test, animals were divided into 4 groups (i.e. Group-A for control, Group-B and Group-C for 300mg/kg and 500mg/kg oral doses of crude extract respectively, and Group-D for standard). Each group comprised of 5 animals. Diazepam as 2mg/kg orally was used as standard. The crude extract and the diazepam were diluted in distilled water and administered orally. The control animals were treated orally with the same volume of saline as the crude extract. In all the tests observations were made after 30 to 40 minutes of oral dose of the test substance.

Table 1: Anti-dermatitis activity Intensity Score of mice treated with *R. stricta*

Intensity score	Control	Dermatitis induced mice	Treated with base	Treated with crude extract	Treated with Standard drug
Excoriation or erosion	0	3	3	1	1
Edema or populations	0	3	3	2	1
Erythema or hemorrhage	0	1	1	0	0

0= absent, 1=mild, 2=moderate, 3=severe

Table 2: Anti-dermatitis activity scratching time of mice treated with *R. stricta*

Intensity score	Control	Dermatitis induced mice	Treated with base	Treated with crude extract	Treated with Standard drug
Cumulative scratching Time per 10 minutes	0.10	3	3	1.45	0.45

Few microscopic fields:

- ++ = Mild changes, few inflammatory cells in several microscopic fields.
 +++ = Moderate and focal changes, few to numerous inflammatory cells in nearly all microscopic fields.
 ++++ = Severe and extended changes, numerous inflammatory cells in all microscopic fields.
 +++++ = Very severe and extended changes, inflammatory cells in all microscopic fields.

Table 4: Effect of crude extract of *R. stricta* on acetic acid induced writhing in mice.

Treatment	Dose mg/kg orally	Mean No. of writhes $\pm$ S.E.M	Inhibition %
Control	0.5ml Saline	130 $\pm$ 3.48	00
Crude extract of <i>R. stricta</i>	300 mg/kg	34.4 $\pm$ 4.81	73.54*
	500mg/kg	39.8 $\pm$ 5.51	69.38*
Aspirin	300mg/kg	36.4 $\pm$ 2.27	72*

Mean $\pm$ S.E.M; N = 5; Significance with respect to control (*=Significant results)

Table 5: Effect of crude extract of *R. stricta* on hot plate analgesiometer in mice

Group	Variation flicking time with $\pm$ SEM (Time in sec at 55 $\pm$ 1°C)						210 min	270 min
	0	30 min	60 min	90 min	120 min	150 min		
Control	0.17 $\pm$ 0.0141	0.14 $\pm$ 0.0142	0.13 $\pm$ 0.021	0.15 $\pm$ 0.0143	0.17 $\pm$ 0.024	0.15 $\pm$ 0.15	0.13 $\pm$ 0.014	0.16 $\pm$ 0.014
Treated 300mg/kg	0.118 $\pm$ 0.013	0.13 $\pm$ 0.020	0.13 $\pm$ 0.035	0.166 $\pm$ 0.033	0.178 $\pm$ 0.020	0.142 $\pm$ 0.015	0.154 $\pm$ 0.012	0.158 $\pm$ 0.011
500mg/kg	0.114 $\pm$ 0.008	0.124 $\pm$ 0.020	0.096 $\pm$ 0.012	0.16 $\pm$ 0.023	0.14 $\pm$ 0.021	0.158 $\pm$ 0.024	0.128 $\pm$ 0.012	0.158 $\pm$ 0.015
Aspirin 300mg/kg	0.15 $\pm$ 0.0143	0.39 $\pm$ 0.0036	0.38 $\pm$ 0.0082	0.39 $\pm$ 0.0012	0.44 $\pm$ 0.0051	0.45 $\pm$ 0.0086	0.33 $\pm$ 0.0081	0.216 $\pm$ 0.0091

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

Table 6: Anxiolytic effects of crude extract of *R. stricta*

Dose mg/kg (body weight)	Open field Test	Head dip test	Cage crossing test	Rearing test	Traction Test	Mean mobility time
Control 0.5ml	126 $\pm$ 3.97	31.6 $\pm$ 4.75	36.67 $\pm$ 1.604	42.67 $\pm$ 0.93	2.29 $\pm$ 0.073	5.33 $\pm$ 0.049
Diazepam 2 mg/kg	85 $\pm$ 2.1213*	15.2 $\pm$ 2.17*	09 $\pm$ 0.317*	14.66 $\pm$ 0.982*	3.94 $\pm$ 0.050*fall	2.82 $\pm$ 0.133*
CE <i>R. stricta</i> 300 mg/kg	91.4 $\pm$ 5.035*	14.4 $\pm$ 2.226*	17 $\pm$ 3.040*	10.2 $\pm$ 3.352*	2.04 $\pm$ 0.285*	2.76 $\pm$ 0.474*
CE <i>R. stricta</i> 500 mg/kg	89.2 $\pm$ 5.228*	10.4 $\pm$ 1.326*	14.8 $\pm$ 1.085*	8.8 $\pm$ 1.157*	2.89 $\pm$ 0.252	1.78 $\pm$ 0.281*

Values are the mean number of head dips, cage crossing and rearing in 3 min, Traction test= mean time taken by mice to travel iron rod; forced induced swimming test = Mean mobility time in 6 min, (mean $\pm$ S.E.M), (n=5); *Significant difference between control group and treated group; $p < 0.05$.

Open field activity

The apparatus for open field activity consists of 76 X 76 cm square area with clear walls 42 cm high. The floor of the apparatus is divided by lines into 25 equal squares. Test was performed according to method described by Kennett *et al.*, 1985 and Turner 1965. Animals were placed in the center square of the open field and number

of Squares crossed with all four paws was counted for 30 minutes.

Head dip test

It is an exploratory test. A specially designed square shaped Head dip box having three holes in each side was used in this study. The observation was to count the

Table 7: Brine shrimp lethality bioassay of *R. stricta*

DOSE (µg/ml)	No. of shrimps	No. of survivors	LD ₅₀ (µg/ml)	Std. Drug	LD ₅₀ (µg/ml)
1000	30	0	3.3004	Etoposide	7.4625
100	30	12			
10	30	20			

number of head dips by the mice through these holes in specified time (Sanchez *et al.*, 2002; Kasture *et al.*, 2002 and Debprasad *et al.*, 2003). Mice weighing 25 to 30 gm were used in this test. The control and drug treated animals were placed individually in the head dip box and the observations were made for 30 minutes.

Table 8: Insecticidal activity by contact toxicity method of *R. stricta*

Name of insect	% mortality		Sample
	+ve control	-ve control	
<i>Tribolium castaneum</i>	100	0	0
<i>Sitophilus oryzae</i>	100	0	0
<i>Rhyzopertha dominica</i>	100	0	0
<i>Callosobruchus analis</i>	100	0	40

Cage crossing test

Mice in a specifically designed having rectangular shape. Both control and treated mice were placed in to the cage and their cage crossing movements were noted in 30 minutes. The test is important for the motor activity of animal. This test was performed according to the method described by Florence *et al.*, 2000.

Rearing test

Rearing is also an exploratory behavior test. Mice weighing 25 to 30 gm were used as the test animals. A 1000 ml glass beaker lined with white paper on bottom was used in this study. The observation was count the upward movements of the animal locating the body in an erect position in the beaker (Sanchez *et al.*, 2002; Kasture *et al.*, 2002 and Sakina *et al.*, 1990). The observations were made for 30 minutes.

Traction test

This test was used to determine the sedative or stimulant activity of the extract (Sanchez *et al.*, 2002; Kasture *et al.*, 2002 and Debprasad *et al.*, 2003). Mice weighing 25 to 30 gm were used for this test. The observation was to determine the time taken by the animal to travel an iron rod of one-meter length. Firstly mice were trained to make them able to walk on iron rod. Any increase or decrease in time taken by the crude extract treated animals from control animals to travel the rod were noted.

Forced induced swimming test

Forced induced swimming test was performed according to Sanchez *et al.*, 2002 and Turner 1965. This test determines the muscle and CNS activity of the crude extract. Mice weighing 25 to 30 gm were placed

individually for six minutes in the glass tub filled with water at room temperature up to the marked level. Mouse when placed in water suddenly starts to move its front and hind paws. The activity time of animal is determined with the help of stopwatch out of total observation time of six minutes.

Brine shrimp bioassay

The Crude plant extract was prepared for the estimation of LD 50 activity in brine shrimp. Test was performed according to method described by Mayer *et al.*, 1982. The crude extract was prepared at 10, 100 and 1000µg/ml concentrations in methanol. Incubation period of samples was 24 h from 22°C to 25°C. Triplicate of assay for each concentration was performed. The numbers of the active nauplii were counted, death percentage was calculated at each does.

Insecticidal activity

Exposure method of insecticidal activity of Crude Extract of *R. stricta* was performed (Rahman *et al.*, 2001). *Callosobruchus analis*, *Tribolium castaneum*, *Sitophilus oryzae* and *Rhyzopertha dominica* were used as test organisms. Standard drug for activity was Permethrin. The dose of the crude extract was adjusted to 1019.10.69µg/cm². Incubation temperature for activity was 30°C. After the incubation, the mean mortality percentage was calculated by using formula.

$$\% \text{ Mortality} = \frac{\text{Number of insects (sample)} \times 100}{100 / \text{Number of insects (control)}}$$

STATISTICAL ANALYSIS

All the calculations were expressed as mean with $\pm$ SEM. The significance of difference between means was determined by Dunnett's t-test and values of $P < 0.05$ were considered significant and $P < 0.01$ as highly significant (Alcaraz and Jimenez, 1989).

RESULTS

R. stricta crude extract showed significant result in dermatitis induced mice (Table 01). In dermatitis induced mice treated with crude extract intensity score was Excoriation or erosion 1, Edema or populations 2, Erythema or hemorrhage 0. Results revealed that intensity score were significant and comparable with standard drug. In anti-dermatitis activity scratching time (table 02) the scratching time of mice treated with crude was 1.45 mins. Results reveals that scratching time per 10 min in

dermatitis induced mice treated with *R. stricta* was decreased from 3 minutes to 1.45 min, which is comparable with standard.

The histological observations of mice treated with crude extract showed mild changes and few inflammatory cells in several microscopic fields (table 03). Results of dermatitis induced mice treated with *R. stricta* were comparable with standard drug.

In acetic acid induced writhing test crude extract of *R. stricta* showed significant dose related inhibition of number of writhes (table 04). In control (vehicle treated) animal, mean number of writhes induced by intraperitoneal injection of acetic acid was 130, which was reduced to 34.4 and 39.8 in animal treated with 300mg/kg and 500mg/kg oral doses of the test substance respectively. Results were significant and comparable with aspirin, which produced 36.4 writhes.

Effect of crude extract of *R. stricta* on hot plate analgesiometer in mice (table 05) shows that the crude extract slightly increases the pain threshold by hot plate method.

Anxiolytic effects were studied by determining the neuropharmacological activities (table 06). The open field activity was diminished. In head dip test exploratory activity was significantly affected and was dose related. The result of cage crossing test indicates that Cage crossing test was more depressant at 300mg/kg oral dose of *R. stricta*. The rearing activity was also decreased. The rearing activity was more depressant at 500mg/kg oral dose of *R. stricta*. The effects were comparable with standard drug. The result of traction test shows that the time taken to travel iron rod was decreased at the dose 300mg/kg orally. In forced induced swimming test the mean mobility time of animals treated with crude extract of *R. stricta* for 300mg/kg oral was 2.22 minutes and for 500mg/kg oral was 2.00 and diazepam 2mg/kg 2.82 minutes. The mobility time was decreased and immobility time was increased. The crude extract shows significant results as compare to diazepam. Findings indicates that the crude of *R. stricta* possesses both sedative and muscle relaxant activities.

Brine shrimp (*Artemia salina*) lethality bioassay were performed with the crude extract of *R. stricta* (Table 07). This cytotoxicity test was performed at the doses of 1000, 100 and 10 µg/ml. The sample shows dose depended significant activity. The number of survived shrimps at the doses of 10µg/ml were 20 out of 30, at 100µg/ml were 12 out of 30 and at 1000µg/ml were 0 out of 30. Crude extract showed positive lethality with LD₅₀ 3.3004µg/ml. The insecticidal activity was performed on *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosbruchus analis* (table 08). Results showed that the crude extract of *R. stricta* have insecticidal activity

against *Callosbruchus analis*. The percentage mortality was 40%.

DISCUSSION

In various cultures drugs from natural sources have been used for thousands of years for the treatment of several diseases due to their less side effects (Sharafkhaneh *et al.*, 2007). Balochistan has great potential for medicinal plants, and these plants have been used from hundreds of years to cure the disease. *R. stricta* is used in various countries as a herbal drug (Shareeque *et al.*, 2005). In Balochistan it is used for the treatment of various medical conditions. Analysis of results of anti-dermatitis activity obtained revealed that significant anti-dermatitis activities have been obtained with crude extract of *R. stricta* and comparable with that of standard drug. Major constituents of *R. stricta* are alkaloids (Gilani *et al.*, 2007) which may contribute to decreasing the symptoms of dermatitis. Further studies may lead to isolation of active compound responsible for that activity. In acetic acid induced writhing test crude extract of *R. stricta* showed significant dose related inhibition of number of writhes. Results were highly significant and comparable with aspirin. The percentage inhibition of writhes with two doses of crude extract was 73.5% (300mg/kg) and 69.38% (500mg/kg) whereas with aspirin it was 72%. On hot plate analgesiometer crude extract of *R. stricta* slightly increases the pain threshold by hot plate method. *R. stricta* is reported to have alkaloids and this activity may be due to these alkaloids. Further studies are needed to find out the single active constituent having analgesic activity.

In anxiolytic studies the effect of methanolic extract of *R. stricta* on CNS has been studied. The results indicate that the extract significantly decreased the locomotor activity as shown by the results of the open field and cage cross, rearing, traction, forced swimming test. The pharmacological profiles of the present investigation of the methanol extract of *R. stricta* was similar to that of diazepam; it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, the use of *R. stricta* in traditional medicine may be due to its CNS action and ability to relieve pain validated by present studies. However, further investigation is needed to determine the exact phytoconstituents that are responsible for the CNS depressant activities of the methanol extract of *R. stricta*. The insecticidal activity of crude extract of *R. stricta* shows activity against *Callosbruchus analis*. The percentage mortality was 40%. The crude extract of *R. stricta* has been shown to possess insecticidal components. It would be interesting to investigate whether the insecticidal compounds in *R. stricta* are alkaloids, triterpenes, flavonoids or other classes of compounds. *Brine shrimp* lethality assay is a rapid, less

expensive and commonly used bioassay for exploring plant extracts bioactivity which is recognized in many cases to explore cytotoxic and anti-tumor activities. This significant lethality of *R. stricta* crude extract to *brine shrimp* shows that it contains active constituents which may possess cytotoxic effects and need further studies.

CONCLUSION

In traditional medicine successful use of herbal drugs makes a clear indicative to search the clue for the therapeutic and pharmacological superiority of many of them when compared with synthetic drug. Phytopharmaceuticals a new generation of treatment is capable of treating diseases more effectively with less side of as compared with synthetic drugs. In this regard current study was carried *R. stricta*. This plant contains many types of active compounds having different chemical structure. Medical application and biological and pharmacological activity of bio- active constituents of this plant has not studied extensively. So current study was done to investigate the biological and pharmacological activities of this plant. A drug-development program may lead to development of new drugs with the bioactive compounds from this plant. Although crude extracts from various parts of *R. stricta* have medicinal applications, new drugs can be prepared after detail study of its bioactivity, mode of action, pharmacokinetics, toxic effects and after standardization and conducting clinical trials. Herbal drugs having less toxic effects are becoming more popular and the scenario of world is now changing towards the use of plant preparation, development of new drugs from *R. stricta* should be considered for the control of many diseases.

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