Antidiarrheal, Analgesic and Pediculicidal Activities of *Gymnosporia royleana*

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ABSTRACT

Objective: In order to scientifically validate the folk uses, extracts of aerial parts of Gymnosporia royleana were screened for in-vivo antidiarrheal and analgesic activity and in-vitro pediculicidal activity.

Methods: Methanol extract of aerial parts of Gymnosporia royleana (GR) was screened for in-vivo antidiarrheal activity in wistar rats using castor oil induced diarrhea model, and for analgesic activity using acetic acid induced writhing and hot plat induced pain models in mice. Methanol extract and its various solvent fractions were also screened for in-vitro pediculicidal activity using human head lice.

Results: In antidiarrheal assay, GR extract showed a considerable reduction in the number of wet feces as well as total number of feces in a dose dependent fashion. GR extract produced 26.55%, 77.60% and 84.06% inhibition of diarrhea at doses of 100 mg/Kg, 200 mg/Kg and 400 mg/Kg body weight. In acetic acid induced writhings model, the extract demonstrated the dose dependent analgesic effect which was highest for 600 mg/Kg body weight dose, however, it was less than that of Diclofenac sodium. In thermally induced pain model, GR extract exhibited significant analgesic effect in a dose dependent fashion and at 600 mg/Kg dose, analgesic effect was comparable to that of morphine (20 mg/Kg body weight). The pediculicidal activity of the plant extracts were found to be insignificant when compared to control.

Conclusion: The results of in-vivo studies strongly support the antidiarrheal and analgesic use of the plant in folk medicine, however, in-vitro pediculicidal assay provided contradictive evidence regarding the pediculicidal use of the plant.

Keywords: Antidiarrheal analgesic, celastraceae gymnosporia royleana, pediculicidal

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INTRODUCTION

Gymnosporia royleana is a native plant of northern areas of Pakistan and belong to the genus *Gymnosporia* (*Celastraceae*). The plant is a thorny shrub with stiff branches and flowers in the month of March and April. Flowers are white in color and after maturation forms 3-gonous capsule bearing black seeds (1, 2). Northern areas like the valleys of Kaghan, Kashmir, Swat and Buner are its natural habitats (1, 2), where it is used in local traditional medicines as analgesic, antidiarrheal, anti-dysentric, antispasmodic, antimicrobial, gastroprotective, anticancer and as pediculicidal (3, 4). Despite of the widespread use of the plant in folk medicine as well as potential of the genus to display diverse pharmacological activities, a very limited number of scientific studies have been carried out on *G. royleana* for the validation of its folkloric uses. The only reported studies include; the evaluation of the root extracts of *G. royleana* for potential antimicrobial, phytotoxic and anticancer properties, screening of the leaf extracts of the plant for anti-hemolytic, anti-lipid peroxidation and antioxidant properties and screening of leaf extracts for antiproliferative effect in prostate cancer (2,3,5). In the current investigation, we have made an attempt to scientifically validate the traditional analgesic, antidiarrheal and pediculicidal use of the plant in various models.

MATERIALS AND METHODS

Plant collection, identification and grinding

The aerial parts of GR were collected from Mata, in district Swat, of KPK province and identified by taxonomist, Dr. Ghulam Jeelani, department of Botany, University of Peshawar. A voucher specimen (Bot. 20044/pup) was submitted in the said institution.

The aerial parts of the plant were garbled and washed with distil water to remove any foreign material and dirt. Then the material was placed for several weeks, under shade at room temperature, until sufficiently dried. After sufficient drying, the material was chopped and finely powdered by using mechanical grinder.

Extraction and fractionation

Extraction of chemical constituents from *G. royleana* was accomplished using cold maceration technique. Powdered plant material (9.7 Kg) was macerated repeatedly in methanol at room temperature and intermittent shaking. The combined filtrate was evaporated using rotary evaporator (Bucchi, Rotavapor R 200), that finally provided crude extract (580 g). This methanolic extract was suspended in distilled water and was shaken in a separating funnel with n-hexane (3 x 1.5 L), dichloromethane (3 x 1.5 L), ethyl acetate (3 x 1.5 L) which resulted in respective fractions and the remnant aqueous part.

Experimental animals

BALB/c mice and Wistar rats were utilized for conducting bioassays. These animals were procured from National institute of health (NIH) Islamabad, Pakistan. The animals were acclimatized prior to experimentation, in the laboratory conditions. During acclimatization period standard diet and tap water *ad libitum* were provided to the animals.

Ethics

In-vivo pharmacological studies were carried out according to the standard experimental guidelines and procedures, as approved by the ethical Committee (05/EC-15/pharm), Department of Pharmacy, University of Peshawar, Pakistan.

Anti-diarrheal effect

Anti-diarrheal activity of *GR* extract was tested in rats using the reported protocol with slight modification. Thirty albino wistar rats (150-200g) fasted for 18 hrs were distributed into 5 groups, i.e. negative control, positive control and three test groups, with each group consisting of six animals. After an initial 4 h observatory period, each rat was orally given 2 ml castor oil to induce diarrhoea. The negative control group received N/saline (10 ml/Kg, *p.o*); the positive control group received loperamide (3 mg/ Kg, *p.o*); whereas, the test groups were given methanolic extract of *GR* at 100 mg/Kg, 200 mg/Kg, 400 mg/Kg doses orally, half an hour before castor oil administration. Parameters like onset of diarrhoea, number of wet faeces, total number of faecal output, were observed for a further period of 4 h (6, 7).

Antinociceptive activities

Acetic acid-induced writhing

Thirty BALB/c mice weighing about 18-22 gm and of either sex were selected. Five groups (n = 6) were made from these mice and were starved for 18 hrs before test. Group 1, which served as negative control, was treated with N/ saline 10 ml/Kg, through intraperitoneal route; groups 2, 3 and 4 were given methanol extract at 150, 300 and 600 mg/Kg doses, respectively, through intraperitoneal route; group 5 was served with 10 mg/Kg diclofenac sodium, orally and assigned

as positive control. After half an hour, 1% aqueous solution of acetic acid (10 mg/Kg) was administered to all the mice through *i.p*. route. After a lag time of 5 min post acetic acid administration, the abdominal writhings were determined for further 10 min (8). The percent analgesic effect was calculated according to the following formula.

$$\% \text{ analgesic effect} = 100 - \frac{\text{No. of writhings in test animals}}{\text{No. of writhings in control animals}} \times 100$$

Hot plate test

Mice were grouped into five classes (n = 6), deprived of food 2 hrs prior to the start of experiment and were then pre-tested at 55± 0.1 °C on hot plate (Havard apparatus). Mice showing greater latency time than 15 seconds, during pre-testing were rejected. Groups 1 and 2 were assigned as negative and positive control and were treated with N/saline (10 ml/Kg) and Tramadol (30 mg/Kg *i.p*.) respectively. Groups 3, 4 and 5 were given 150, 300 and 600 mg/Kg (body weight) of crude methanolic extract, respectively. 30 minutes later, the mice were positioned one by one on hot plate and the latency of nociceptive responses (jumping, paw licking or flicking) was recorded (in sec). A cut-off time of 30 seconds was selected for each animal so as to avoid any tissue damage. Latency times were recorded for each group at 0, 30, 60, 90 and 120 minutes (9).

% analgesic effect was then calculated by using the following equation:

$$\% \text{ analgesic effect} = 100 - \frac{\text{Latency time of test} - \text{Latency time of control}}{\text{Cut-off time} - \text{Latency time of control}} \times 100$$

Insecticidal activity

Insecticidal activity of extract and subsequent fractions of aerial parts of *GR* against human head lice (*Pediculus humanus capitis*) was determined by using filter paper diffusion bioassay, according to the reported protocol (10, 11).

Head lice were collected by dry combining from the heads of infested school children (aged 5-10 yrs.) at a local primary school, with the consent of their guardians. After combing, the lice were carefully collected and placed in clean plastic bottles. The adults, nymphs and nits of *Pediculus humanus capitis* were identified and separated. The lice were protected from sunlight and heat. All the extracts were dissolved in distilled water so as to obtain 5%, 10% and 20% concentrations.

After collection, the head lice were immediately subjected to *in-vitro* tests. The lice were carefully sorted using a dissecting microscope for any damaged or killed lice. Next a disc of Whatman No. 1 filter paper (9-cm in diameter) was placed in separate petri dishes. Ten lice were carefully placed on the filter paper disk and 0.50 ml of (i) each of the test solution; (ii) distilled water (negative control); and (iii) varying concentrations of commercial benzyl benzoate lotion (positive control) was added to separate petri dishes. After this, each petri dish was placed in a dark chamber at 70 ± 1% relative humidity and 26 ± 0.5 °C for 1 hr. After this initial exposure, the lice of each group were transferred to another petri dish, containing a clean filter disk soaked with 0.5 ml distilled water and placed again in dark place under the aforementioned conditions and observed 18 hrs later again under a dissecting microscope. Lice were regarded as dead when no peristalsis or movements of appendages were seen after several minutes. The test was performed in triplicate and % inhibition was determined by the following formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{Number of living insects in test}}{\text{Number of living insects in control}} \times 100$$

Statistical analysis

The data was expressed as mean ± S.E.M of 6 animals. For statistical analysis, student t-test or ANOVA followed by Dunnett's test was applied for multiple comparisons. Effects were considered to be significant at the $P < 0.05$ level. Graph-pad prism 5 software was employed for statistical analysis.

RESULTS

Antidiarrheal activity

Castor oil induced diarrhea test

During this test, administration of castor oil to rats produced watery diarrhea that was evident in the control group as increased frequency of wet feces. Rats that were pretreated with GR extract showed a considerable reduction in the number of wet feces as well as total number of feces in a dose dependent fashion. GR extract produced 26.55%, 77.60% and 84.06% inhibition of diarrhea at doses of 100 mg/Kg, 200 mg/Kg and 400 mg/Kg body weight as shown in table 4. It is fascinating to note that GR extract at 400 mg/Kg dose caused stronger inhibition of diarrhea as compared to Loperamide (standard) which achieved 80.06% inhibition of diarrhea at 2 mg/Kg body weight dose. Furthermore, GR extract had also significant effect on the overall number of feces (both normal and wet). The test results showed that *GR* extract reduced total number of feces by 20.35%, 43.83%, and 55.38%, which in the case of standard drug Loperamide was 58.12% as shown in table 1 and figure 1.

Analgesic activity

Acetic acid-induced writhings

The crude methanolic extract of *GR* produced significant analgesic effect as represented by table 2. The data demonstrated the dose dependent analgesic effect which was highest for 600 mg/Kg body weight, however, it was less than that of Diclofenac sodium (standard drug). Individually, the % analgesic effect of crude methanolic extract was 31.57%, 55.90%, and 68.88% at 150 mg/Kg, 300 mg/Kg and 600 mg/Kg body weight respectively, where as in the case of diclofenac sodium, it was 83.79 % at *i.p.* dose of 10 mg/Kg. This test revealed that the methanolic extract of GR possessed signifi-cant analgesic properties.

Hot plate model

In thermally induced pain model, GR extract exhibited significant analgesic effect at 150 mg/Kg, 300 mg/Kg and 600 mg/Kg, which in the latter case was compare-able to that of morphine (20 mg/Kg body weight). These results also revealed that the analgesic effect was in a dose dependent manner. The analgesic effect was observed as an increase in latency time (seconds) that was recorded at 0 min, 30 min, 60 min, 90 min and 120 min post vehicle, GR extract and morphine administration, respectively. The most significant analgesic effect for extract as well as standard drug was observed after 90 minutes of administration which was 29.90% and 36.00% respectively.

Pediculicidal activity

The pediculicidal activity of various extracts of GR was tested against human head lice (*Pediculus capitis* humanis), and results have been presented in table 4 and figure 4. The data

suggested the pediculicidal activity of the plant extracts to be insignificant when compared to control. The maximum pediculi-cidal effect produced by any of the test extract was only 20%, and produced by hexane fraction at the highest tested concentration. Moreover, crude methanolic extract, hexane fraction and ethyl acetate fraction produced very mild pediculicidal activity at the highest concentration tested, whereas the activity of dichloromethane and aqueous fraction was insignificant when compared to control.

DISCUSSION

Antidiarrheal activity

Diarrhoea basically occurs whenever the secretory process surpasses the absorption capacity of the intestines via any of the following four mechanisms: (a) increased secretion of fluids from the mucosal surfaces; (b) diminished absorption of water and electrolytes; (c) Hyper motility; and (d) increased permeability. Although the frequent discharge of intestinal contents during diarrhoea is beneficial in the sense that it facilitates the removal of injurious intestinal contents, yet, the resulting dehydration can be life threatening (12).

In castor oil induced diarrhea model, ricinoleic acid, the principle component of castor oil is believed to be responsible for the induction of diarrhea (13, 14). As a result of the irritant effect of ricinoleate, the peristaltic activity of small intestine increases along with altered permeability of mucosa to electrolytes. Furthermore the ricinoleate also stimulate the production of PG E_2 by mucosal cells which also increase the secretory and motility function of intestines (15). Recent studies have shown that the ricinoleic acid also directly stimulates the EP3 receptors of intestinal smooth-muscles thus causing hyper-motility of the intestines (16, 17). From the

above discussion it is evident that any agent which can modify any of the above mentioned factors, will have some effect on diarrhea.

The data of our study suggest that *GR* extract has strong inhibitory effect against castor oil induced diarrhea and at 400 mg/kg dose its inhibitory effects were even stronger than the standard drug loperamide. From this we hypothesize that the antidiarrheal effect of GR extract might partly be due to antihypermotility effects of the extract as well as its inhibitory effect on prostaglandins may also be involved to some extent.

Based on our findings, there is a strong need to conduct a mechanism based study of antidiarrheal activity of GR extract and also to isolate the compounds responsible for antidiarrheal activity of the plant, in order to develop newer and safer antidiarrheal drugs.

Analgesic activity

The underlying algogenic mechanism of acetic acid is considered to be provoked by the injurious effect of this chemical on cell membranes leading to the liberation of arachidonic acid, which undergo a stepwise conversion into prostaglandins and other inflammatory mediators, depending upon the enzymes, cycloxygenases (COXs) and lipoxygenases (LOXs). The prostaglandins (PG E_2) thus formed, subsequently act on the local peritoneal nociceptors, causing the perception of pain in mice, which is observed by pharmacologists as writhings (constriction of abdominal muscles). This argument has been supported by the presence of enhanced levels of prostaglandins in peritoneal fluids post acetic acid administration, especially the levels of PG E_2 and PG $F_2\alpha$ (18,19) along with products of lipoxygenase pathway.

The crude methanolic extract of GR demonstrated significant analgesic effect in this model. These results suggested that the antinociceptive action of the crude methanolic extract of

GR might be linked to restricted formation of PG's and bradykinin or via antagonizing the effects of these metabolites of arachidonic acid.

Furthermore the results of hot plate test suggest the involvement of centrally mediated analgesic mechanism as well and according to the data the central mechanism predominates.

Pediculicidal activity

Hence, this study provided contradictive evidence regarding the pediculicidal use of the plant in folk medicine.

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AUTHORS' NOTE

HK and IK designed the experiments, HK, AA, and FG performed the experiments, NK and AS executed statistical analysis of data whereas, ZU, GA and IR wrote the paper.

Authors declare that there is no conflict of interest.

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Table 1: Effects of methanolic extract of GR on castor oil-induced diarrhea in rats.

Sample	Onset time (min.)	Total number of feces	% inhibition of defecation	Number of wet faeces.	% inhibition of Diarrhea
Control	62.39 ± 1.81	5.11 ± 0.74	-	4.33 ± 0.47	-
Loperamide 2	119.53 ± 2.98*	2.14 ± 0.51*	58.12	0.83 ± 0.11*	80.83
GR 100	71.63 ± 2.24	4.07 ± 0.78	20.35	3.18 ± 0.34	26.55
GR 200	103.64 ± 2.82*	2.87 ± 0.29*	43.83	0.97 ± 0.11*	77.60
GR 400	139.14 ± 3.71*	2.28 ± 0.73*	55.38	0.69 ± 0.07*	84.06

*Values are reported as mean ± S.E.M. for group of 6 animals. *P < 0.05, **P < 0.01 in comparison to control.*

Table 2: Analgesic activity of crude methanolic extract of GR in acetic acid induced writhings model.

Sample	**Dose (mg/Kg i.p.)**	No. of writhings (10 min.)	% inhibition
Saline	10 ml/Kg	62.38 ± 2.16	-
Extract	150	42.69 ± 2.46*	31.57
	300	27.51 ± 1.91*	55.90
	600	19.41 ± 1.79**	68.88
Diclofenac	10	10.11 ± 1.37**	83.79

*Values are reported as mean ± S.E.M. for group of 6 animals. The data was analyzed by ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01 in comparison to control.*

Table 3: Antinociceptive effect of methanolic extract of GR and Tramadol on hot plate induced pain in mice.

Treatment	Dose. (mg/Kg)	Latency of nociceptive response in min. (mean ± S.E.M.)				
		0	30	60	90	120
Vehicle	-	8.22 ± 0.23	8.48±0.18	8.61±0.43	8.68±0.29	8.74±0.51
Extract	150	8.36 ± 0.36	9.52±0.27	9.81±0.22	10.63±0.54*	9.94±0.37
	300	8.32 ± 0.53	10.14±0.39*	10.63±0.43*	12.05±0.62**	11.87±0.68**
	600	8.31±0.27	11.91±0.51*	13.84±042**	14.79±0.78**	14.31±0.29**
Tramadol	20	8.51±0.37	12.59±0.74**	15.89±0.52**	16.24±0.23**	15.62±0.23**

*Values are reported as mean ± S.E.M. for group of 6 animals. The data was analyzed by ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01 in comparison to control.*

Table 4 Pediculicidal activity of extract and fractions of aerial parts of GR:

Test sample	% concentration	Average % mortality
Distilled water	-	3.3
Hexane extract	10	6.7
	20	13.3
	30	20.0
Dichloromethane	10	3.3
	20	10.0
	30	10.0
Ethyl acetate extract	10	0
	20	6.7
	30	16.7
Aqueous extract	10	6.7
	20	3.3
	30	10.0
Methanolic extract	10	13.3
	20	13.3
	30	16.7
Benzyl benzoate 25% w/v	10	83.3
	20	96.7

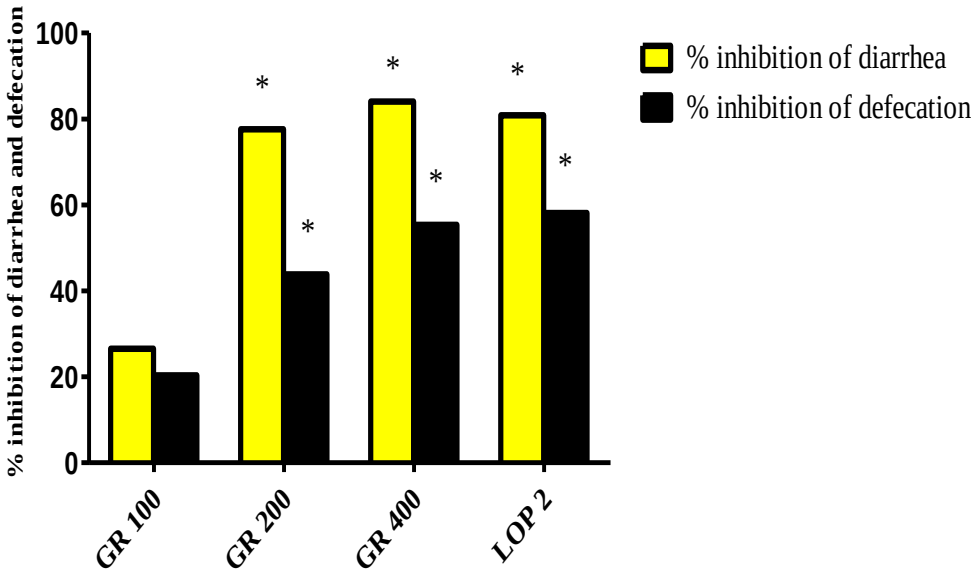

Fig 1: Effects of methanolic extract of GR on castor oil-induced diarrhea in rats.

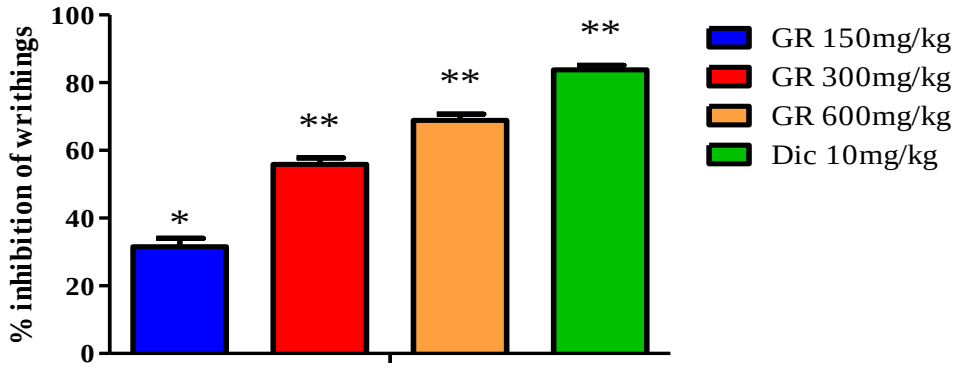

Fig 2: Antinociceptive activity of crude methanolic extract of *GR* in acetic acid induced writhings model.

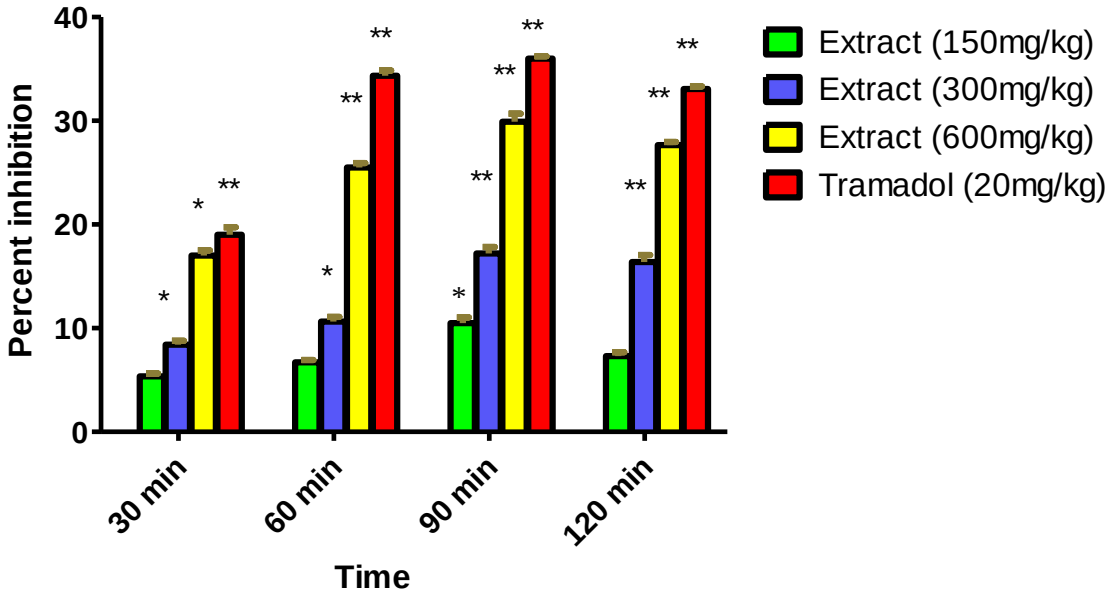

Fig 3: Antinociceptive effect of methanolic extract of *GR* and Tramadol on hot plate induced pain in mice

Bars represent mean ± S.E.M. (n=6). ANOVA followed by Dunnett's test was applied to the data to determine the level of significance in comparison with negative control. *P < 0.05, **P < 0.01