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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF ROOTS OF *GENTIANA KURROO* ROYLE IN EXPERIMENTAL ANIMAL MODELS

BILAL A. WANI[1], *D. RAMAMOORTHY[1] AND BASHIR A. GANAI[2]

[1]Department of Ecology and Environmental Sciences Pondicherry University- 605014, Puducherry, India, [2]Department of Biochemistry University of Kashmir, Srinagar 190006, J&K, India. Email: hosiramin@yahoo.co.uk

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ABSTRACT

The methanolic extract of roots of *Gentiana kurroo* Royle (Gentianaceae) an important and endemic medicinal plant of Kashmir Himalaya was screened for the presence of various bioactive plant metabolites and analgesic activity (using Eddy's hot plate method and acetic acid-induced writhing test in Swiss albino mice at an oral dose of 250 and 500 mg/kg body weight). Diclofenac sodium (10 mg/kg b.w) was used as standard drug, whereas the vehicle (0.9% normal saline) was used as negative control. The phytochemical analysis revealed the Presence of tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates. The extract showed stastically significant (P<0.05) analgesic activity in a dose-dependent manner, which were comparable with standard analgesic drug. In acetic acid-induced writhing test, the doses of 250 and 500 mg/kg b.w produced 63.38% and 73.70% inhibition of writhing reflex respectively as compared with the standard drug, which showed 71.61% inhibition. In eddy's hot plate method the extract showed significant (P<0.05) increase in reaction time at different time of observation (0-120 min) in comparison with control. The study clearly indicate that the crude methanolic root extract of *Gentiana kurroo* posses potent analgesic activity, which has provided some justification for the folkloric use of the plant as stomach-ache, pain, and anti-inflammatory.

Keywords: *Gentiana kurroo* Royle, Kashmir Himalaya, Analgesic activity, Writhing, Diclofenac sodium

INTRODUCTION

Gentiana kurroo Royle (Gentianaceae) is an important and native medicinal plant of North-Western Himalayas. It is commonly known as Indian Gentian, while in Kashmir Himalaya it is called as Nilkanth. *Gentiana kurroo* is a rosette-forming perennial herb, growing along sub-alpine North-Western Himalayas at an altitude of 1500-3000 meters above mean sea level.[1-2] In Kashmir Himalaya it is usually found on south-facing steeper slopes along dry and rocky sloppy grasslands and sparsely shrubby scrubs[3]. The roots of the plant are source of iridoid glycosides- Gentiopicrine, Gentiamarin and the alkaloid Gentianin[4]. The roots of the plant are used as bitter tonic, antiperiodic, expectorant, astringent, stomachic, anthelmintic, antipsychotic, anti-inflammatory, sedative and antibacterial[5]. The herb is also used for curing the skin disease leucoderma, bronchial asthma, and urinary infections[6]. To our knowledge, no report is available on the analgesic activity of *Gentiana kurroo* of Kashmir Himalaya in particular. So, the present study has been undertaken to evaluate the phytochemical screening and analgesic activity of the methanolic extract of *G. Kurroo* root.

MATERIALS AND METHODS

Plant material

Gentiana kurroo Royle was collected from lower reaches of Pir-panchal range of Kashmir Himalaya at an altitude of 2150 m (a.s.l) by conducting field trips. The collected plant material was properly identified by Akhtar H. Malik, Curator Centre of Biodiversity and Plant Taxonomy, University of Kashmir and specimen under voucher number *780-KASH* was deposited in Kashmir University Herbaria for further reference.

Preparation of extract

Roots of the plant were cut, properly cleaned and dried under shade. After drying, the material was chopped and then grinded to powder. Dry root powder (100 g) was packed in Soxhelt apparatus and extracted with methanol at 60-65°C. The extract was filtered through Whatmann filter paper No.1 and the solvent was removed under reduced pressure at 35-45°C using Buchi rotavapor (R-215). The dried extract was labelled as (MEGK) and stored at 4°C in storage vials for experimental use.

Phytochemical screening

Phytochemical screening for major bioactive constituents like tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolic compounds and carbohydrates was undertaken using standard phytochemical methods.

Tannins: To 2 ml of alcoholic extract, 2 ml of 5% $FeCl_3$ was added. Formation of yellow brown precipitate indicates the presence of tannins[7].

Alkaloids: To the 2 ml methanolic filtrate, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagent/Wagner's reagent/ Dragendroff reagent was added. Formation of orange precipitate indicates the presence of alkaloids[8].

Saponins: Alcoholic or aqueous extract of 2 g powder was made and subjected to frothing test. Frothing persistence indicated presence of saponins. Latter the froth was mixed with few drops of olive oil. Formation of emulsion indicated presence of saponins[9].

Cardiac glycosides: To 2 ml of alcoholic filtrate, 1 ml glacial acetic acid and 1-2 drops of $FeCl_3$ was added followed by 1 ml concentrated H_2SO_4. Appearance of brown ring at the interface indicates presence of cardiac glycosides[10].

Terpenes: To 2 ml of alcoholic extract, 5 ml of chloroform, 2 ml acetic anhydride and concentrated H_2SO_4 was added carefully to form layer. Reddish brown colouration of interface indicates presence of terpenes[11].

Flavonoids: 2 g plant material was extracted in 10 ml alcohol or water. To 2 ml filtrate, few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings were added. After 3 minutes magenta red or pink colour indicated the presence of flavonoids[7].

Phenolics: To 2 ml of alcoholic or aqueous extract, 1 ml of 1% $FeCl_3$ solution was added. Blue or green colour indicates the presence of phenols[12].

Carbohydrates: Benedict's test (test for reducing sugar) and Fehling's test (standard test for reducing sugar) were performed to confirm the presence of carbohydrates.[11-13]

Experimental animals

Swiss albino mice (25-30 g) of either sex were procured from the central animal house of Pondicherry University. They were kept under controlled room temperature (24 ± 1°C, relative humidity 60-70%) in a 12 h light-dark cycle. The animals were given a standard laboratory diet and water *ad libitum*. The animals were acclimatized to the laboratory room for 2-3 days prior to pharmacological experiments. The animals were divided into four groups each consist of six animals and were fasted overnight prior to the experiment. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Analgesic study

Acetic acid-induced writhing response in mice

To evaluate the effect of the plant extract on acetic acid-induced writhing response in mice, the method described by Collier *et al*[14] was used with slight modifications. Swiss albino mice were divided into four groups of six animals each. Group-I served as control and received 10 ml/kg b.w of normal saline intraperitonially. Group-II received standard drug Diclofenac Sodium at the dose of (10 mg/kg) i.p. Group-III and IV received methanolic extract of *Gentiana kurroo* root (MEGK) at an oral dose of 250 and 500 (mg/kg body weight) respectively. The extract and reference drug were administered 30 min before the administration of 0.75% acetic acid at a dose of 0.1 ml/10g body weight. Immediately after the injection of acetic acid, each animal was isolated in an individual cage and after three minutes, the number of writhes (abdominal contractions) was recorded for each animal for a period of 30 minutes and the percentage inhibition was calculated by following formulae.

$$\% \text{ inhibition} = \frac{(W_c - W_t)}{W_c} \times 100$$

Were,
W_c = Mean no. of writhings (control)
W_t = Mean no. of writhings (test)

Eddy's hot plate method

The animals were grouped same as in above method. All animals were selected 24 hour prior to experimentation and the animals were selected on the basis of their normal reaction time. The animals were placed on Eddy's hot plate[15] maintained at a temperature of 55 ± 1°C. The cut off time for the reaction was 15 seconds in order to avoid the damage to the paws of the animals. The time taken by the animals to lick the fore or hind paw or jump from the plate was taken as the reaction time. The reaction time was recorded at 0, 30, 60 and 120 minutes after extract administration.

Statistical analysis

All The values were expressed as mean ± S.D (n=6) for each group and the statistical significance was determined by ANOVA followed by Dunnett's test. P value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The result of the phytochemical screening of the plant showed that the roots of *G. kurroo* are rich in various active ingredients (secondary plant metabolites). It supports the resourcefulness of the plant extract[9]. The result of the phytochemical screening of the plant extract (Table 1) revealed that the plant is rich in tannins, flavonoids, phenolics, cardiac glycosides, terpenes, and alkaloids. Flavonoids, alkaloids and terpenoids in the plant may be responsible for its effects as analgesic, anti-malarial properties and its use in treatment of stomach disorder[16]. In analgesic studies, the extract showed strong analgesic activity at both the dose levels. In acetic acid-induced writhing test, the methanolic extract of *G. kurroo* root at a dose of 250 and 500 mg/kg b.w showed significant ($p<0.05$) reduction in number of writhings induced by acetic acid in a dose dependent manner which were comparable with the standard drug as shown in table-2. Maximum percentage of inhibition of writhing responses exhibited by the MEGK at 500 mg/kg was 73.70%, while the same at 250 mg/kg showed 63.38% reduction in acetic acid induced writhing, which was comparable to that of standard (diclofenac sodium) which caused 71.61% inhibition. Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGE_2 and $PGF_{2\alpha}$), bradykinins and substance P, which stimulate nerve endings[17]. It is therefore possible that extract produced analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandin[18]. In case of eddy's hot plate test, the extract showed significant ($p<0.05$) increase in reaction time (increase threshold potential of pain) in a dose-dependent manner to the thermal stimulus at different time of observation (0- 120 min.) in comparison with control. The results of hot plate test were shown in table-3. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, whereas hot plate test are most sensitive to centrally acting analgesics.

Table 1: Phytochemical screening of methanolic extract of *Gentiana kurroo* root

Phytoconstituents	Test
Tannins	++
Alkaloids	+
Saponins	+
Cardiac glycosides	++
Terpenes	++
Flavonoids	++
Phenolics	++
Carbohydrates	+

++: strong presence, +: moderate presence

CONCLUSION

In conclusion, it can be inferred from the present investigation that the methanolic extract of roots of *Gentiana kurroo* Royle possesses potent analgesic effect against different stimuli. The mechanism of analgesic activity was found to be due to an inhibition of both peripherally and centrally mediated nociceptive.

Table 2: Effect of methanolic extract of *G. kurroo* root on writhing assay

Treatment	Dose (mg/kg)	Mean number of writhes	Inhibition (%)
Control	--	53.97 ± 2.13	--
Standard	10	15.32 ± 1.79*	71.61%
MEGK-250	250	19.76 ± 1.43*	63.38%
MEGK-500	500	14.19 ± 1.26*	73.70%

Values are in Mean ± S.D; (n =6), *p < 0.05 Vs control.

Table 3: Effect of methanolic extract of *G. kurroo* root on hot plate assay

Treatment	Dose (mg/kg)	Response Time in Sec. (Mean ± S.D.)			
		0 min	30 min	60 min	120 min
Control	--	2.71 ± 0.05	2.94 ± 0.04	2.89 ± 0.11	2.97 ± 0.02
Standard	10	4.63 ± 0.03	9.68 ± 0.06*	15.69 ± 0.10*	20.45 ± 0.14*
MEGK-250	250	3.86 ± 0.07	7.85 ± 0.02*	14.92 ± 0.16*	19.37 ± 0.03*
MEGK -500	500	5.38 ± 0.10	12.28 ± 0.09*	18.48 ± 0.05*	22.16 ± 0.15*

Values are in Mean ± S.D; (n= 6), *p < 0.05 Vs control.

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