

Antiinflammatory Activity of Flower Tops of *Gentiana Kurroo Royale* Extract

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Summary

Gentiana Kurroo Royale (GK) flower tops (Gule-Ghafis) has been traditionally used for the treatment of inflammation, pain, fever, hepatitis in Unani System of Medicine. The plant was sold in Easter countries under the name Gul-Ghafis which is *Gentiana daliurica* Fisch. But sample obtained from the major cities of India were found and identified as flower tops of *Gentiana Kurroo Royale*. However this work was aimed at the scientific validation of ethanopharmacological claim about the anti-inflammatory property of *Gentiana Kurroo Royal* flower tops extract. Air-dried flower tops of GK were blended to a fine powder extracted with 50% ethanol. The phytochemical screening, quantitative estimation (%) TLC and anti-inflammatory screening were investigated. The study was carried out on Wister rats of either sex. Animals were divided into three groups of six each. Rat paw edema induced by Carrageenan 0.1 ml of 1% carrageenan in 0.9% NaCl was administered into planter surface of right hind paw. The experimental groups-I was fed with extract in the dose of 1000 mg/ kg body weight, the animal in group-II served as control group and administered with 20 ml/ kg distilled water while standard drug Diclofenac sodium was given to group-III in the dose of 5 mg/ kg orally an hour prior to administration of carrageenan. After injection animals were observed and readings were obtained for each rat at 60, 120, 180, 240 and 300 minutes with the aid of Plethysmometer. The percentage of inhibition for each rats were calculated by the formula of New Bould (1963). The phytochemical screening were revealed the presence of alkaloids, flavonoids, glycosides, free phenols, sterols/ terpens. Percentage estimated as alkaloid 0.33 ± 0.02 , phenols 2.9 ± 0.07 , sterols/ terpens 1.35 ± 0.01 , flavonoid 0.34 ± 0.24 were found.

Thin layer chromatography of flavonoid fraction showed Robinetin-0, Luteolin, Apigenin, Kaempferol and Kaempferid. The effect of extract of flower tops of GK showed a maximum anti-inflammatory effect about 68.58% ($p < 0.01$) at 180 min. while the anti-inflammatory effect of Diclofenac sodium reached a maximum 70.58% ($p < 0.01$) at 180 min. The results indicate that the flower tops of GK posses anti-inflammatory activity against inflammation induced by carrageen an in acute phase. Thus, this study strongly indicates the non-steroidal anti-inflammatory activity. They may act by the inhibition of production of prostaglandins, other than this the presence of flavonoids may account for its anti-inflammatory pharmacological activity. This pharmacological study is validating the claims of ancient Unani Physicians regarding the anti inflammatory property of the *Gentiana Kurroo* Royale flower tops.

Keywords: *Gentiana Kurroo*, Antiinflammatory Activity

Introduction

Flower *Gentiana Kurroo* Royale (GK) commonly known as Gule-Ghafis (Gul Arabic = Flower) has been traditionally used for the treatment of inflammation, pain, fever, hepatitis, in Unani system of medicine (Ibn Sina, 1906). According Wiliam Dymock (1890) the plant is sold in Easern countries under the name of Ghafis which is actually *Gentiana dahurica* Fisch. The Gul-e-Ghafis is reported by some author as the flower tops of *Aqrmonia eupatoria* Lin., but samples obtained from market of major cities of India were found and identified as flower tops of *Gentiana Kurroo* Royale. However, this work was aimed at the scientific validation of ethnopharmacological claim about the antiinflammation property of *Gentiana Kurroo* Royale flower (Gul-Ghafis) after proper authentication of plant material were carried out.

Material and Method

Plant Material: Gul-e-Ghafis was procured from different cities of India, Aligarh, New Delhi and Mumbai, and pharmacognostically was identified as *Gentiana Kurroo* Royale. Air dried flower tops of GK were blended to a fine powder extracted with 50% ethanol, Phytochemical screening Quantitative estimation (%) and TLC were also investigated.

Chemicals: Diclofenac sodium as a standard drug and Carrageenan (Sigma) were used to induce Rat paw edema.

Animals: Wistar rats (140-190 gm) of both sexes were used for inducing edema in their paw, was studied by the method of Winter et al (1962). The animal were housed in cages under standard laboratory condition. They had free access to standard diet and water. The animal were divided into groups of six animal each and fasted for 12 hours before the experiment. The ethical guidelines for the investigation of animals used in experiments were followed in all tests.

Rat paw edema induced by carrageenan 0.1 ml of 1% of carrageenan in 0.9% NaCl was administered into planter surface of the right hind paw of the animals. The experimental group-I was fed with 50% ehtanolic extract of the test drug in the dose of 1000 mg/ kg body weight, the animal in group-II served as negative control group and administered with 20 ml/ kg distilled water while standard drug diclofenac sodium was given to group-III in the dose of 05 mg/ kg orally an hour prior to the administration or carrageenan. Before injection of carrageenan, the average volume (V_0) of right hind paw of each rat was calculated. After injection, readings (V_t) were obtained for each rat at 60, 120, 180, 240 and 300 min; with the aid of Plethysmometer. The edema was expressed as an increased in the volume of paw, and the percentage inhibition for each rat and each group was obtained with following formula described by New Bould (1963).

$$\text{Percentage of inhibition} = \left(1 - \frac{(V_t - V_0)_{\text{Control}} - (V_t - V_0)_{\text{treated}}}{(V_t - V_0)_{\text{Control}}} \times 100 \right)$$

Statistical analysis: All values are presented as mean \pm SE of six rats in each group. Difference between means were assessed by comparison with control group, followed by student 't' test. $p < 0.05$ was considered significant.

Results

Phytochemical Screening

The phytochemical analysis revealed the presence of alkaloids. Ethanolic extract showed flavonoids. Defatted ethanolic extract detected Glycosides. The free phenol, sterols/ terpense were found in petroleum ether extract. Percentage estimation of alkaloids (0.33 ± 0.02) phenols (2.91 ± 0.07) sterols/ terpenes (1.35 ± 0.01), flavonoid (0.34 ± 0.24) were noted down in Table-1. Thin layer chromatography of flavonoid fraction showed Robinetin-O, Luteolin, Apigenin, Kaempferol and Kaempferid in S/S: Toluene: ethyl formate: formic acid (50: 40: 10) (Table-2).

TABLE-1: Quantitative estimation (%) of chemical constituents of flower tops of *Gentiana Kurroo Royale*

S.No.	Phenols	Alkaloids	Sterols/ Terpenes	Flavonoids
01	2.87	0.36	1.36	0.30
02	2.88	0.34	1.35	0.30
03	2.88	0.32	1.35	0.30
04	2.98	0.31	1.35	0.33
05	2.96	0.33	1.36	0.33
Mean	2.91	0.33	1.35	0.31
SD±	0.07	0.02	0.01	0.01
SE±	0.03	0.01	0.00	0.00

TABLE-2: Thin Layer Chromatography of Fraction of Flavonoids in *Gentiana Kurroo Royale*

Solvent system	Spray treatment	Colour of spot under UV light	Rf Values	Name of std. Flavonoid
Toluene: ethyl formate: formic acid (50: 40: 10)	Polyethylene Glycol	Orange	0.31	Robinetin-0
		Yellow	0.38	Luteolin
		Green	0.46	Aigenin
		Yellow	0.48	Kaempferol
		Blue	0.59	Kaempferid

Paw Edema Induced by Carrageenan

The anti-inflammatory effect of extract of flower top of *Gentiana Kurroo* Royale are shown in Table-3. The G.K. showed (1000 mg/ kg) a maximum anti-inflammatory effect of about 68.58% in 180 minutes. While the anti-inflammatory effect by Diclofenac progressively increased and reached a maximum (70.58%) at 180 min (Table-3).

TABLE-3: Anti-inflammatory Effect of *Gentiana Kurroo* Royale and Diclofenac in Carrageenan-induced Rat Paw Edema.

Group	60 min after Carrageenan injection		120 min after Carrageenan injection		180 min after Carrageenan injection		240 min after Carrageenan injection		300 min after Carrageenan injection	
	Increase in paw volume ml (Mean \pm S.E.)	% Inh.	Increase in paw volume ml (Mean \pm S.E.)	% Inh.	Increase in paw volume ml (Mean \pm S.E.)	% Inh.	Increase in paw volume ml (Mean \pm S.E.)	% Inh.	Increase in paw volume ml (Mean \pm S.E.)	% Inh.
Control	0.06 \pm 0.01	-	0.35 \pm 0.03	-	0.51 \pm 0.08	-	0.57 \pm 0.02	-	0.59 \pm 0.01	-
Diclofenac 5 mg/kg	0.17 \pm 0.03	50.75**	0.14 \pm 0.02	61.47** *	0.16 \pm 0.04	70.58** *	0.22 \pm 0.02	62.48** *	0.24 \pm 0.02	59.45***
<i>Gentiana Kurroo</i> (GK) 1000 mg/kg	0.09 \pm 0.01	35.82*	0.22 \pm 0.02	37.68** *	1.18 \pm 0.02	68.58** *	0.23 \pm 0.02	60.04** *	0.26 \pm 0.02	55.71***

*Not significant, p value ** < 0.05, *** < 0.01

Discussion

The result of the study indicate that the flower tops of *Gentiana Kurroo* Royale posses anti-inflammatory activity against the inflammation induced by carrageenan in acute phase. The *Gentiana Kurroo* significantly inhibited paw edema as an inflammatory agent.

As a result from this study strongly indicate the non-steroidal, anti-inflammatory like activity. They may act by inhibition of the production of prostaglandins, other than this the presence of flavonoid, may account for its observed pharmacological activity. Many compounds from this class have been found to exhibit anti-inflammatory effects.

To conclude, the results showed potent anti-inflammatory agent in acute phase of inflammation. It also leads to some conclusion regarding their mechanism of action against inflammation that *Gentiana Kurroo Royale* could be acting by blocking the mediators released in later phase (i.e., prostaglandins). This pharmacological study is validating the claims of ancient Hakim (Unani Physicians) regarding the anti-inflammatory property of the *Gentiana Kurroo Royale*.

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