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Evaluation of Anti-inflammatory and Wound Healing Activity of *Gentiana lutea* Rhizome Extracts in Animals

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Abstract

Gentiana lutea Linn (Gentianaceae), commonly known as gentian, is widely used in the traditional system of medicine as an anti-inflammatory and wound healing agent. We examined the effectiveness of alcohol and petrol ether extracts of rhizomes of *Gentiana lutea* at 500 and 1000 mg/kg doses orally in the carrageenan-induced rat paw edema, xylol-induced mouse ear edema and cotton pellet-induced chronic inflammatory models. Both extracts showed significant dose-dependent anti-inflammatory activities in all of these models. Both extracts exhibited significant wound healing activity at 300 and 500 mg/kg, p.o., in excision, resutured incision and dead space wound models.

Keywords: Anti-inflammatory activity, *Gentiana lutea*, petrol ether extract, wound healing activity.

Introduction

Gentiana lutea Linn (Gentianaceae) is a plant native to the mountains of Europe, Turkey and Western Asia, commonly known as "gentian". Today it is cultivated in many parts of the world, including India. It is a perennial herb with erect stems and yellow flowers. The main parts used in traditional medicine to be developed are the dried rhizomes and roots. The rhizomes have a pleasant odor and sweet taste which latter becomes bitter (Trease & Evans, 1978). Gentian is reported to contain bitter glycosides, mainly gentiopicroside, alkloids, gentianine and gentiolutine, yellow coloring matter due to xanthones, pectin and fixed oils. It also contains phytosterols, phenolic acids and oligosaccarides (Bruneton, 1995).

Gentian is reported to have choleretic, anti-oxidative, hepatoprotective (Gebhardt & Wagner, 1996) and antifungal activity (Gurein & Reveilleve, 1985). It is reported to stimulate gastric acid secretion and is used in case of anorexia (Chaudhari, 1996). The roots are used to manufacture spirits, non-alcoholic beverages, frozen dairy desserts, candy and baked goods. Gentian is a main ingredient in many liver tonics and nervine formulations. Gentian is traditionally used to treat numerous gastrointestinal problems (stomachache, heart burn, gastritis, diarrhea, vomiting) as well as inflammation and wounds (Leung & Foster, 1996).

Even though *Gentiana lutea* was reported to be useful in many ailments, scientific evaluation of the plant was not reported for its anti-inflammatory and wound healing activity. Hence, in the present study, the anti-inflammatory and wound healing activity of extracts of rhizomes of *Gentiana lutea* were studied using different animal models.

Materials and methods

Plant material

Dried rhizomes of *Gentiana lutea* were purchased from Genuine Chemical Company, Bombay, India in September 2000. Further taxonomic identification was conducted by Prof. T.R. Rajan, Head of Dept. of Botany, R.L.S. College, Belgaum. The powdered material weighing 1 kg was extracted by Soxhlet using successive extraction with petrol ether (40–60 °C) 2 L/24 h and ethanol (95%) 2 L/24 h.

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Animals

Albino mice and albino rats (Wistar strain) of either sex weighing 20–25 g and 150–200 g, respectively, were used for the study. The above animals were obtained from the animal house of Shri. Venketeswara Enterprises, Bangalore. They were housed in polypropylene cages with standard pellet chow and water *ad libitum*. In all experimental sets, 6 rats and 6 mice were used for each treatment.

Toxicity study

Both alcohol and petrol ether extracts were insoluble in water, hence they were suspended in 2% Tween 80. Both the extracts were administered orally to different groups of mice in doses ranging from 1000–2500 mg/kg for the LD_{50} study using the method of Miller and Tainter (Ghosh, 1971). There was no lethality in any of the groups after 7 days of treatment.

Anti-inflammatory activity

This activity was studied using acute and chronic treatment in rats.

i) Carrageenan-induced rat paw edema

Alcohol and petrol ether extracts (500 or 1000 mg/kg) or diclofenac sodium (13.5 mg/kg), were administered orally. Thirty-minutes after drug administration, according to the technique of Turner (1965), 0.1 ml of 1% carrageenan (Sigma) in a normal saline solution was injected into the subplanter region of one of the hind paws. The paw edema volume was recorded using a plethysmometer (Basile, Italy) at different time intervals.

ii) Xylol-induced mouse ear edema

Alcohol and petrol ether extracts (500 and 1000 mg/kg) or indomethacin (25 mg/kg) was administered orally. Thirty-minutes after drug administration, inflammation was induced by a topical application of 2% xylol (20 µl) to the right ear of each mouse. The left ear was kept as control. The positive control group received only 0.5 ml of 2% Tween 80 solution. After 30 min of xylol application, the animals were killed by cervical dislocation. A 6 mm section of ear disc was obtained by punching the ear and then weighed. The inflammation induced by xylol was assessed as the increase in weight of ear punch of treated groups over controls, and this was called the edema index (Brown & Robson, 1964).

iii) Cotton Pellet-Induced granuloma in rats

Two sterilized cotton pellets, each weighing 10 mg, were implanted subcutaneously into axilla, in an anaesthetized rat (Turner, 1965). After treatment with an alcohol or petrol ether extract at 300 and 500 mg/kg for 10 days, the rats were sacrificed. The pellets were dissected out and granuloma was dried at 60 °C overnight to determine the dry weight. Results were expressed as mg/100 g.

Wound healing activity

The wound healing activity was studied in alcohol and petrol ether extracts of *Gentiana lutea* at doses of 300 and 500 mg/kg using excision wound, resutured incision wound and dead space wound models.

Excision wound method

This was produced in rats as described by Morton and Malone (1972) under light ether anesthesia. The skin of the impressed area was excised to full thickness to obtain a wound area of about 500 mm^2. The parameters studied were wound closure, epithelization time and scar area. The percentage wound closure was recorded at 0 day, 4^{th} day, 8^{th} day, 12^{th} day and 16^{th} day. The scar shape and area were traced and measured planimetrically.

Incision wound method

An incision wound was produced in rats as described by Ehrlich and Hunt (1969) under light ether anesthesia. The wound was closed with interrupted sutures, which were removed on the 8^{th} post wounding day. The breaking strength of 10-day-old wound was measured.

Dead space wound method

A subcutaneous implantation of sterilized grass piths (25 × 3 mm) was done in the rat groin (Turner, 1965). The 10-day-old granuloma were carefully dissected and cleared of adventitious tissues. The rectangular strip obtained by slitting tubular granulomous growth on grass pith was tested for tensile strength by the method of Lee (1970). Hydroxyproline estimation of granulation tissues were done as described by Woessner (1963). Histopathological studies of granuloma were done by staining with heamotoxyllin and eosin so as to enable the assessment of fibroblast population, collagen content and thickness of tissue under a light microscope.

Statistical analysis

The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) followed by Dunnet's test for individual comparison of groups with control. P-values <0.05 were considered as significant.

Results

The alcohol and petrol ether extracts of *Gentiana lutea* rhizomes were evaluated for their anti-inflammatory and wound

healing activities. Both extracts ingested up to 2500 mg/kg by the oral route failed to produce any lethality in mice. So we selected 500 mg/kg and 1000 mg/kg as the study dose of anti-inflammatory activity. For wound healing activity, a reduced dose of 300 and 500 mg/kg was used as daily doses were given. All the drugs were administered through the oral route.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

Alcohol and petrol ether extracts at doses 500 and 1000 mg/kg, as well as diclofenac sodium (13.5 mg/kg), showed significant inhibition of edema in dose-dependent manner 3 h after carrageenan-induced inflammation, when compared to control (Table 1).

Xylol-induced mouse ear edema

Alcohol and petrol ether extracts at doses 500 and 1000 mg/kg, as well as indomethacin (25 mg/kg), showed significant reduction in ear edema volume. There is also a significant increase in edema index percentage inhibition (Table 2).

Table 1. Effect of *Gentiana lutea* extracts on inflammatory models.

Treatment	dose (mg/kg)	% Inhibition of paw edema inflammation at 180 min	Weight of granuloma (mg/100 g)
Control	0.00	0.00	54.6 ± 2.22
Alcohol	500	69.78	39.4 ± 3.62*
Alcohol	1000	81.62	36.4 ± 1.16*
Petrol ether	500	34.62	38.2 ± 1.93*
Petrol ether	1000	66.72	34.2 ± 8.22*
Diclofenac sodium	13.5	84.63	31.7 ± 1.92*

Values are mean ± S.E., n = 6, P < 0.05 significant*

Cotton pellet-induced granuloma in rats

There was a statistically significant reduction in the weight of granuloma in alcohol and petrol ether extract as well as diclofenac sodium treated rats when compared to control (Table 1).

Wound healing activity

Excision method The percentage closure of excision wounds was significantly increased in both alcohol and petrol ether extract treated groups at doses of 300 and 500 mg/kg when compared to control (Table 3).

Incision method The breaking strength of the 10-day-old resutured incision wound was significantly increased in both alcohol and petrol ether extracts treated groups at 300 and 500 mg/kg doses when compared to control (Table 3).

Dead space wound In the dead space wound study, there was a significant increase in granuloma breaking strength in both alcohol and petrol ether extracts treated groups at 300 and 500 mg/kg doses when compared to control (Table 3). The histopathological study of granuloma tissue revealed that in extract treated groups, there was an increase in granulation tissue with an increase in fibroblasts and collagen content as compared to control. In hydroxyproline estimation, there was significant increase in hydroxyproline content in both alcohol and petrol ether extract treated groups at 300 and 500 mg/kg doses, as compared to control (Table 3).

Discussion

The alcohol and petrol ether extracts of *Gentiana lutea* did not show any toxicity at the various dose levels tested in albino mice. Therefore, 500 and 1000 mg/kg were selected as

Table 2. Effect of different *Gentiana lutea* extracts on mouse ear edema (orally).

Treatment	Dose mg/kg	Punch Weight (mg)	Edema Index % inhibition
Xylol	0.00	13.00 ± 0.31	100.00
Non-Treated	0.00	6.20 ± 0.2	0.00
Indomethacin	25	7.60 ± 0.24*	77.41 ± 3.20
Alcohol Extract	500	10.40 ± 0.24*	32.25 ± 4.82
Alcohol Extract	1000	8.20 ± 0.2*	76.74 ± 2.40
Petrol Ether Extract	500	11.20 ± 0.2*	19.35 ± 4.32
Petrol Ether Extract	1000	9.00 ± 0.31*	54.83 ± 2.20

Note: P < 0.05 significant*
Edema index indicates the increase in the weight of the punch biopsy of xylol treated ear over that of non-treated ear. Each value represents mean ± SE of 6 animals.

Table 3. Effect of *Gentiana lutea* extracts on wound healing models.

Drug	% Closure of excision wound area after days				Epithelization in days	Scar area in sq. mm	Incision wounds	Dead space wound	
	4	8	12	16			Wound breaking strength (g)	Granuloma breaking strength (g)	Hydroxyproline content (µg/ml)
Control	15.64	46.49	79.64	91.56	23.6 ± 0.50	46.6 ± 0.92	255.00 ± 5.60	252.00 ± 5.83	1.90 ± 0.11
Alcohol (300 mg/kg)	50.88	83.88	96.43	99.65	17 ± 0.31	32.6 ± 1.16*	418.64 ± 5.47*	304 ± 3.1*	4.96 ± 0.5*
Alcohol (500 mg/kg)	33.30	72.16	92.91	99.40	17 ± 0.31	31.6 ± 1.28*	412.64 ± 6.43*	312 ± 3.74*	5.08 ± 0.14*
Petrol ether (300 mg/kg)	50.43	85.39	96.38	99.66	17 ± 0.31	29.4 ± 1.07*	465.00 ± 7.35*	318 ± 9.02*	5.03 ± 0.09*
Petrol ether (500 mg/kg)	37.54	73.60	93.61	99.44	17.2 ± 0.37	30.4 ± 0.93*	444.00 ± 7.84*	302 ± 4.63*	5.14 ± 0.12*

Values are mean ± S.E., n = 6, $P < 0.05$ significant*

doses for the study of anti-inflammatory activity and a reduced dose of 300 and 500 mg/kg was used for wound healing activity, as a daily dose was given to the animals.

With the carrageenan-induced rat paw edema method, both the extracts of *Gentiana lutea* showed significant anti-inflammatory activity at the 500 and 1000 mg/kg doses. The anti-inflammatory activity of the alcohol extract at 1000 mg/kg was similar to that of diclofenac sodium. In the case of the xylol-induced mouse ear edema method, both the alcohol and petrol ether extracts of *Gentiana lutea* showed significant anti-inflammatory activity at 500 and 1000 mg/kg doses. The anti-inflammatory activity of the alcohol extract at 1000 mg/kg was similar to that of indomethacin. In cotton pellet-induced granuloma, the alcohol and petrol ether extracts of *Gentiana lutea* produced significant anti-inflammatory activity at 500 and 1000 mg/kg dose levels. Our studies support the earlier reported of traditional use of *Gentiana lutea* as an anti-inflammatory drug in acute and chronic inflammatory models (Leung & Foster, 1996).

Wound healing involves different phases such as contraction, epithelization, granulation and collagenation. In resutured wounds, wound breaking strength is determined, which indirectly addresses the collagenation phase of healing, and this parameter is commonly used to assess healing, perhaps because surgeons are specially interested and concerned with the strength of healed incision wounds (Patil & Kulkarni, 1985). The alcohol and petrol ether extracts of *Gentiana lutea* at doses 300 and 500 mg/kg showed significant reduction in percentage closure of excision wounds. Similarly, the breaking strength of the resutured incision wounds was increased in drug treated groups. Dead space wound provides an opportunity to study the effect on granulation and collagenation of the healing process. Such wound models have been employed for quantitative and qualitative studies on wound healing. Also, there is a significant increase in granuloma tissue breaking strength in both extract treated groups. The histopathological studies revealed an increase in the number of fibroblasts and collagen content in treated groups as compared to control. The increased amount of hydroxyproline in test groups underlines increased collagen content, which is necessary in healing of the wounds (Madden & Peacock, 1968).

This study confirms the antiinflammatory activity and wound healing activity of rhizomes of *Gentiana lutea*. The activity may be due to various glycosides, alkaloids and other compounds present in rhizomes. The antiinflammatory activity was found to be comparable to reference drugs diclofenac sodium and indomethacin, indicating that *Gentiana lutea* extracts are effective in acute models mouse ear edema and carragenan paw edema, in subacute models like the granuloma pouch method. There are many drugs which are reported to stimulate wound healing processes, like antidepressants (Muppayanavarmath & Patil, 1999), vitamin A (Ehrlich et al., 1973), vitamin E (Lee, 1953), and zinc (Diwan et al., 1979). There are many herbs which are used to treat wounds and inflammation such as *Hibiscus vitifolius* (Parmar & Ghosh, 1978), *Dalbergia volubilus* (Humayun et al., 1975), *Costus* species (Pandey et al., 1975), saponins (Bhargava et al., 1970), and toxifolin (Gupta et al., 1971),

This study confirms the anti-inflammatory and wound healing activity of the rhizomes of *Gentiana lutea*. Further studies need to be done to identify and separate the group of active constituents responsible for anti-inflammatory activity and wound healing activity from alcohol and petrol ether extracts.

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