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RESEARCH ARTICLE

Isolation and anti-inflammatory activity of colchicinoids from *Gloriosa superba* seeds

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Abstract

Gloriosa superba L. (Liliaceae) seeds, known as "kalihari" (Hindi), were phytochemically investigated for colchicine (well known for gout treatment) and other related alkaloid content. Colchicine, 2-demethylcolchicine, 3-demethylcolchicine, and *N*-formyl-*N*-deacetylcolchicine were alkaloids isolated from the seeds. The isolated samples have been standardized for their purity with respect to the reference standard using HPLC. The structures were confirmed by NMR spectroscopy and were analyzed by spiking them along with colchicine reference by HPLC. The purity of colchicine, 2-demethylcolchicine, 3-demethylcolchicine and *N*-formyl-*N*-deacetylcolchicine were 99.82, 96.78, 98.71, and 98.13% respectively. The compounds were subjected to an anti-inflammatory study by using the formaldehyde inflammagen-induced inflammation model. Oral administration of colchicine at 2, 4, and 6 mg/kg body weight resulted in 48.9, 68.7, and 79.1% inhibition respectively, while 30.9% inhibition was seen in the phenylbutazone 100 mg/kg treated group once daily for a period of 4 days. The results clearly indicated that the colchicine is more effective as an anti-inflammatory agent compared with phenylbutazone, the standard drug used in the study, whereas the oral administration of 6 mg/kg body weight of 2-demethylcolchicine, 3-demethylcolchicine and *N*-formyl-*N*-deacetylcolchicine showed very poor activity (41.6, 40.4, and 41.1% activity respectively).

Keywords: Gloriosa superba*; colchicinoids; anti-inflammatory activity; paw edema*

Introduction

Gloriosa superba L. (Liliaceae) grows throughout tropical regions in India and is a well known source of colchicine (Chopra et al., 1956). (-)-Colchicine is an effective drug treatment for intense pain associated with a gout attack (Singer, 1996). Clinical experience shows that colchicine may be an extremely promising adjunct to conventional treatment and may ultimately serve as the initial mode of treatment, especially in idiopathic cases (Adler et al., 1998). The anti-inflammatory effect of colchicine is induced by inhibition of the synthesis of tumor necrosis factor α (TNFα) by macrophages and down-regulation of surface expression of TNFα-receptor on macrophages and endothelial cells, thus it interferes with the priming effect of TNFα on neutrophils before their activation by monosodium urate crystals (Li et al., 1996; Ding et al., 1990), inhibition of leukotriene B4 synthesis, a powerful chemotactic agent (Serhen et al., 1984; Reibman et al., 1986). Colchicine reduces adhesion of neutrophils to endothelium inhibiting polymorphonuclear leucocyte (PMN) function (Firdham et al., 1981), E-selectin-mediated endothelial (Asako et al., 1992) and L-selectin-mediated neutrophilic adhesiveness (Cronstein & Weissman, 1993; Cronstein et al., 1995). It has been reported that colchicine blocks the cyclooxygenase-2 (COX-2) activity, prostaglandin E_2 and thromboxane A_2 synthesis of mononuclear phagocytes with subsequent reduction of swelling and pain in gout and Familial Mediterranean Fever (FMF) (Pouliot et al., 1998). In addition, this colchicine is known for tyrosine phosphorylation and superoxide anion production inhibitor (Roberge et al., 1996), arachidonate release and

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5-lipoxygenase inhibition (Peters-Golden et al., 1996; Zurier et al., 1973), histamine inhibition (Mekori et al., 1989), insulin and parathomone release (Gillespie et al., 1968; Malaisse et al., 1975). Colchicine at high dose causes bone marrow failure, skin eruption, nettle rash, stomatitis and intestinal bleeding. Long-term administration can result in ovarian and testicular dysfunction, steatorrhea and Lyell syndrome (Ben Chetrit & Levy, 1998). In addition, it is used for familial Mediterranean fever (FMF), primary biliary cirrhosis (Kershenobich et al., 1988; Ikeda et al., 1996), psoriasis (McKendry et al., 1993), scleroderma (Torres & Furst, 1990), and sarcoidosis (Kaplan, 1960). Colchicine therapy improved BMI slightly, but significantly (Tukmen et al., 2008). Pressure activation of malignant cells promotes tumor development and impairs tumor-free survival and perioperative colchicine administration may inhibit this effect (Craig et al., 2008). The usage of colchicine is restricted because of its toxicity, possibly because of its poor purity and because the toxicity studies on colchicine derivatives such as 2-demethyl colchicine, 3-demethyl colchicine and *N*-formyl-*N*-deacetylcolchicine have not been confirmed till now. In the present investigation the purity of the colchicine was found to be 99.82%, which can be used as safely by replacing colchicine with 96% purity.

Formaldehyde-induced inflammation model was used for investigation of anti-inflammatory effect of the drug (Obukowicz et al., 1998).

Materials and methods

Plant materials

Gloriosa superba seeds were purchased during February 2005 from a vendor of Chennai, Tamil Nadu, India. The seed sample was identified by Y.P.S. Panguty, Botany Department, DSB Campus, Nainital. Solvents were procured LR grade, from Qualigens Fine Chemicals, Mumbai, India. Activated charcoal (CAS No. 7440-44-0) used in this study was untreated, particle size of not more than 75 microns and obtained from Sigma (St. Louis, MO). Alumina neutral activity I-II (neutral aluminum oxide active) (CAS No. 1344-28-1) was obtained from Merck, Mumbai, India. All the analytical solvents used were HPLC grade and were procured from Qualigens Fine Chemicals. Colchicine reference standard was procured from Chromadex.

Animals

One hundred and fourty male albino Wistar rats weighing 180-200 g were used for the experiment. All animals were fed with standard laboratory chow and tap water before the experiment. The animal laboratory was windowless with automatic temperature (22° ± 1°C) and lighting 14 h light/10 h dark controls. The rats were fasted 24 h before the experiment and allowed access to water ad libitum. There were 10 animals in all groups, which were housed in separate cages. The study has been approved from the institutional animal ethical committee (IAEC) of the Committee for the Purpose of Control Supervision of Experimental Animals (CPC-SEA).

Isolation

Gloriosa superba seeds (3 kg) were extracted using 9 L of 90% methanol at 70°-75°C, three times (each wash 3 h). The methanol extract was allowed to cool to room temperature (25-30°C) and filtered using a Hyflo Super Cel filter bed, then concentrated to 2 L under vacuum at 50°C. Water (1 L) was added to the concentrated methanol extract and was partitioned with 6 L of hexane twice. The aqueous layer was partitioned using methylene dichloride (11 L twice). The combined methylene dichloride layer was washed subsequently using 3% sodium hydroxide solution (15 L twice), 1% acetic acid solution (10 L once) and finally with demineralized water (10 L twice) to remove the phenolic impurities. The methylene dichloride layer was then concentrated under vacuum. The dried mass (54 g) was dissolved in ethanol and the solution was passed through an activated charcoal column (containing 50 g activated charcoal) and was eluted using ethanol (2 L). The ethanol elute was concentrated to dryness under vacuum at 50°C. Assay of finally dried mass (30 g) was found to be 96.7% and further purification was achieved by passing it through a neutral alumina column. The dried mass was dissolved in methylene dichloride and was passed through a column (covered using black cloth, as colchicine is light and heat sensitive) containing neutral alumina (200 g, Merck, activity I-II). The column was eluted using methylene dichloride (1 L). The combined elute was concentrated to dryness under vacuum at 40°C. The dried mass (27.6 g) was dissolved in ethyl acetate (250 mL) with heating and was concentrated to 100 mL and was kept for crystallization for 1 h at room temperature. The crystallized material was then filtered using a Buchner funnel and washed using chilled ethyl acetate (30 mL). The crystalline mass was dried under vacuum (1 torr) for 24 h at initially 40°C for 5 h and finally at 60°C for 19 h. The resultant 24.9 g light yellow crystalline mass obtained gave 99.82% purity.

The standardization using high performance liquid chromatography (Agilent HPLC model 1100) equipped with 254 nm UV detector, carried out using a Phenomenex ODS-C8 column (250 x 4.6 mm, particle size 5 μ). The mobile phase used was 450 volumes of water containing 6.8 g/L potassium dihydrogen

phosphate and 530 volumes of methanol. The flow rate adjusted 1 mL/min of mobile phase.

Formaldehyde-induced paw edema in rats

Isolated constituents were administered orally by feeding tube at doses of 2, 4, and 6 mg/kg, and phenylbutazone at a dose of 100 mg/kg once daily for a period of 4 days; the rats in the control group received the same volume of distilled water for the same period (ten animals in each group). Prior formaldehyde injection, right hind paw volume was measured with a plethysmometer; 1 h after final drug administration, 0.2 mL formaldehyde (1%, w/v) was subcutaneously injected in to the plantar surface of the right hind paw. The volumes of the right hind paws were measured after oral administration at 3, 6, and 24 h until swelling disappeared (Suleyman et al., 1999; Jain & Khanna, 1981). Edema was expressed as a percentage of pre-administration right paw volumes. Finally, the anti-inflammatory effect in animals receiving colchicine was compared with those in the control and phenylbutazone-administered groups (Table 1).

Statistical analysis

Results were represented as mean ± standard deviation of mean (SEM) or as percentages. Student's *t*-test was used for determining significance. Analysis of data indicates that results with $p < 0.05$, as compared with control, were accepted as statistically significant.

Results and discussion

Colchicine, the pharmacologically active constituent used for centuries in acute gout arthritis, has been isolated on a commercial scale with high purity (99.82%). Extractions of colchicine from *G. superba* by aqueous methanol (Kannan et al., 2007) and ethanol (Ellington et al., 2003) have also been reported which gave 95-97% purity. As toxicity of the other alkaloid constituents of *G. superba* is not known, so the most pure constituent can be provided to the consumers without any further complications. The isolation methodology involves column chromatographic separation in which a charcoal column separates the 2-demethyl colchicine and 3-demethyl colchicine while an alumina column separates *N*-formyl-*N*-deacetylcolchicine impurity, an assay more than 99.5% was achieved only by passing through the alumina column. Isolated constituents i.e. colchicine gave 99.82%, 2-demethyl colchicine gave 96.78%, 3-demethyl colchicine gave 98.71% and *N*-formyl-*N*-deacetylcolchicine gave 98.13% purity by HPLC (Table 2).

Table 1 shows the effects of 2, 4 and 6 mg doses of colchicine in formaldehyde-induced paw edema in rats. Oral administration of colchicine at 2, 4, and 6 mg/kg body weight resulted in 48.9% ($p < 0.002$), 68.7% ($p < 0.001$) and 79.1% ($p < 0.001$) inhibition respectively, while 30.9% inhibition was seen in the phenylbutazone 100 mg/kg treated group. The results have clearly indicated that colchicine is more effective (79.1% inhibition at 6 mg/kg body weight) as an anti-inflammatory agent when compared with phenylbutazone the standard drug used in the study, whereas 2-demethylcolchicine, 3-demethylcolchicine and *N*-formyl-*N*-deacetylcolchicine at the dose of 6 mg/kg body weight resulted inpoor inhibition (41.6%, 40.4%, and 41.1% of inhibition respectively).

It has been reported by Ferriera et al. (1974) that bradykinin produces edema and inflammation in the rat paw edema. The role of bradykinin in the

Table 1. Effects of colchicinoids and phenylbutazone on formaldehyde-induced paw edema in rats.

Drug	Dose (mg/kg)	Normal paw volume (mL)	Paw volume after 3h inflammation	Percentage of inflamed paw volume (mL)	(%)	Anti-inflammatory effect (%)	*P* value*
Control	–	1.07	1.86	0.79 ± 0.018	73.8	–	–
Phenylbutazone	100	1	1.51	0.51 ± 0.008	51	30.9	< 0.001
Colchicine	2	1	1.38	0.38 ± 0.059	38	48.9	< 0.002
	4	1.03	1.27	0.24 ± 0.066	23.3	68.7	< 0.001
	6	1.02	1.18	0.17 ± 0.018	15.6	79.1	< 0.001
2-Demethyl colchicine	2	0.99	1.6	0.69 ± 0.031	61.6	17.1	< 0.02
	4	1	1.53	0.53 ± 0.074	53	28.7	< 0.05
	6	0.99	1.42	0.43 ± 0.032	43.4	41.6	< 0.001
3-Demethyl colchicine	2	1	1.57	0.57 ± 0.024	57	22.8	< 0.001
	4	1.08	1.63	0.55 ± 0.05	50.9	31	< 0.01
	6	1	1.44	0.44 ± 0.029	44	40.4	< 0.001
N-Formyl-*N*-deacetylcolchicine	2	0.98	1.57	0.5 ± 0.048	60.2	19	< 0.02
	4	1.03	1.53	0.5 ± 0.019	48.5	34.8	< 0.001
	6	0.98	1.41	0.43 ± 0.014	43.8	41.1	< 0.001

*Significant difference by student's *t*-test.

Table 2. Purity profile of the colchicinoids.

Constituent	Purity %	Method of analysis
Colchicine	99.82	HPLC
2-Demethyl colchicine	96.78	HPLC
3-Demethyl colchicine	98.71	HPLC
N-Formyl-*N*-deacetylcolchicine	98.13	HPLC

pathogenesis of formaldehyde-induced inflammation was reported by Kulkarni et al. (1986). Based on the obtained results, it can be summarized that anti-inflammatory action of colchicine on inflammagen-induced edema may depend on its inhibiting capacity of formation of mediators like bradykinin.

Declaration of interest

It is our own research and no financial assistance was received for this study. There is no conflict of interest.

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