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INVESTIGATION OF ANTI-INFLAMMATORY PROPERTIES OF *SWERTIA CHIRAYTA* AND *GLORIOSA SUPERBA*

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Abstract

Gloriosa superba (Liliaceae) is one of the oldest ingredients of species from ancient time. Tubers roots and seeds are two most important part of glory lily used for variety of purpose. *Swertia chirata* (Gentianaceae) is widely used in India to treat fever and malaria. It is also used to treat liver diseases. The whole plant methanol and aqueous extracts of *Swertia chirayta* possessed maximum anti-inflammatory activity in a dose dependent manner i.e 50 mg/kg and 100 mg/kg in carrageenan induced animal models. Further screening of the potent extracts of the plant confirmed the presence of xanthone (1, 5-dihydroxy-3, 8-dimethoxy xanthone, m.p.185^{0}C, yellowish crystalline needles from methanol) was obtained. The structure of the fraction was confirmed by spectral analysis (UV, IR, NMR). The methanol and aqueous extracts of tubers of *Gloriosa superba* also possessed good anti-inflammatory in a dose dependent manner i.e 100 mg/kg and 200 mg/kg in carrageenan induced animal models. Further screening of the extracts confirmed the presence of colchicines in the extracts. The present study thus revealed the presence of potent anti-inflammatory drugs in these plants.

Keywords: *Gloriosa superba*, *Swertia chirayta*, Methanol extracts, Xanthones, Colchicines, Anti-inflammatory activity

Introduction

Traditional system of medicine is found to have utilities as many accounts. Due to population rise adequate supply of drug and high cost of treatment in side effect along with drug resistance has been encountered in synthetic drugs, which has lead to an elevated emphasis for the use of plants to treat human diseases. The affordability of herbals has also drawn the attraction towards their use. India is one of the oldest civilizations which is known for rich repository of medicinal plants. *Gloriosa superba* is one of the oldest species from ancient time. Being native form Indian specially Southern India it is known as glory lily and climbing lily- in English; Karihari- in Hindi; Langli- in Sanskrit. Antimicrobial and *in vitro* antioxidant activities of the plants were reported [1- 3]. Analgesic and anti-inflammatory properties of *Gloriosa superba* were determined [4]. Larvicidal and antipox viral potential of the plants were reported [5, 6]. *Swertia chirayta* is considered the most important for its medicinal properties. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin (most bitter compound isolated till date), swerchirin, swertiamarin and other active principles of the herb. Herbal medicines such as Ayush-64, Diabecon, Menstrual syrup and Melicon V ointment contain chirayta extract in different amounts for its antipyretic, hypoglycemic, antifungal and antibacterial properties. Anti-inflammatory activities of *Swertia chirayta* were determined [7, 8]. In the present study different solvent extracts of tubers of *Gloriosa superba* and whole plant extracts of *Swertia chirayta* were screened for their anti-inflammatory activity in carrageenan induced animal models.

Materials and Methods

Plant material

The authenticated sample was collected from Forest Research Institute, Dehradun (U.K), India and was further confirmed in Botanical Survey of India (BSI), Dehradun. Voucher specimens have been deposited in BSI, Dehradun, India.

Preparation of plant extracts

The method [9] was adopted for preparation of plant extracts with little modifications. Briefly four 20 g portions of the powdered plant material were soaked separately in 100 ml of water, hexane, methanol and petroleum ether for 72 h. Each mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatmann filter paper no1 (Whatmann, England). The filtrate obtained were concentrated in vacuo using rotary evaporator at 30^{o}C.

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Determination of *in vivo* anti-inflammatory activity

Animals

Male albino rats (180–200 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals [10]. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1ºC with a 12-h light/dark cycle. The institutional animal ethical committee has approved the protocol of the study.

Carrageenan-induced edema in rats

6 Groups of five animals each were used for each of the plants. Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The solvent extracts of *Gloriosa superba* and *Swertia chirayta* at dose of 50, 100 and 200 mg/kg were administered orally 60 minutes before carrageenan injection. Aspirin (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 1, 2, 3, and 4 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Statistical analysis

The results were expressed as mean ± S.D. Statistical significance was determined by analysis of variance and subsequently followed by Turkey's tests. P values less than 0.05 were considered as indicative of significance. The analysis was performed using INSTAT statistical software.

Results and Discussion

The anti-inflammatory effects of the solvent extracts of *Swertia chirayta* and *Gloriosa superba* in carrageenan-induced edema in rat's hind paws are presented in Table 1 ;Figure 1 and Table 2; Figure 2. The anti-inflammatory activities of both the plant extracts were found to have effect in dose-dependent manner. There was a gradual increase in edema paw volume of rats in the control groups. However, in the test groups, methanol extracts of *Swertia chirayta* possessed maximum anti-inflammatory activity in a dose dependent manner i.e 50 mg/kg and 100 mg/kg in carrageenan induced animal models in comparison to that of aqueous extracts. The same results were found in case of *Gloriosa superba*, methanol extracts possessed maximum anti-inflammatory activity in a dose dependent manner i.e 100 mg/kg and 200 mg/kg in carrageenan induced animal models in comparison to aqueous extracts. The results showed that methanol fractions of the whole plant of *Swertia chirayta* causes significant reduction in inflammation i.e 92 % (100 mg/kg) followed by crude aqueous extract i.e 85 % (100 mg/kg) compared to standard anti-inflammatory drug aspirin i.e 68.62% (25 mg/kg). The dose of 200 mg/kg of solvent extracts of the plant, *Swertia chirayta* was found to be lethal dose for carrageenan induced mice and most of rats lead to death. In case of *Swertia chirayta* extracts, the values of reduction in paw volume, 0.10 ± 0.002, 0.12 ± 0.002 and 0.14 ± 0.002 were found significantly of methanol extract, aqueous extract and aspirin, respectively at 4 h after carrageenan administration. The methanol fractions of the tubers of *Gloriosa superba* causes significant reduction in inflammation i.e 85 % (200 mg/kg) followed by crude aqueous extract i.e 76 % (200 mg/kg) compared to standard anti-inflammatory drug aspirin i.e 68.62% (25 mg/kg). In case of *Gloriosa superba* extracts, the values of reduction in paw volume, 0.11 ± 0.002, 0.13 ± 0.002 and 0.14 ± 0.002 were found significantly of methanol extract, aqueous extract and aspirin, respectively at 4 h after carrageenan administration. There was no reduction in inflammation found in case of rats treated with petroleum ether and hexane extracts of both the plants. The present study provides evidence that the methanol fraction and aqueous extract of *Swertia chirayta* and *Gloriosa superba* acts as potent anti-inflammatory agent in dose- dependent manner in rats in acute inflammation model. Our results are found to be correlated with the previous studies [4, 7, 8]. Further studies on the isolation and identification of the potent extracts confirmed the presence of Xanthones (in *Swertia chirayta*) and Colchicines (in *Gloriosa superba*) in the plant extracts responsible for anti-inflammatory activity. The active principles of the extracts were re-again screened for their anti-inflammatory activity.

Table 1: Anti-inflammatory activities of different extracts of *Swertia chirayta*

Paw volume (ml) ± SD

Experiment	Control	Aspirin (25mg/kg orally)	Methanol extract (100 mg/kg)	Aqueous extract (100mg/kg)	Petroleum ether (100mg/kg)	Hexane (100mg/kg)
1h after treatment	0.25±0.003	0.21± 0.003	0.23± 0.003	0.28±0.003	0.20± 0.003	0.34± 0.003
2h after treatment	0.25±0.003	0.18± 0.003	0.20± 0.003	0.24±0.003	0.15± 0.003	0.34± 0.003
4h after treatment	0.25±0.003	0.14± 0.002	0.10± 0.002	0.12±0.002	0.30± 0.002	0.34± 0.002

±, S.D, Standard Deviation

Figure 1: Anti-inflammatory activities of different extracts of *Swertia chirayta*

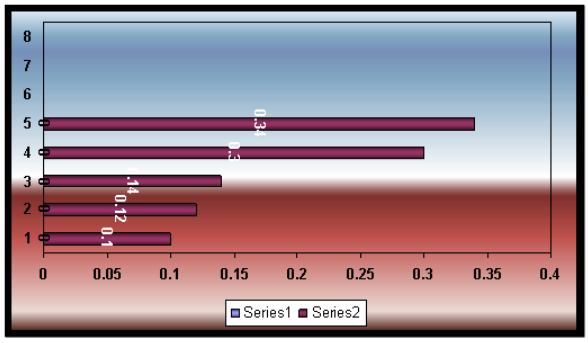


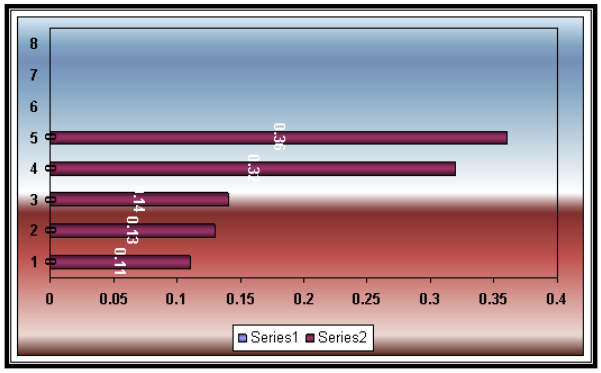
Table 2: Anti-inflammatory activities of different extracts of *Gloriosa superba*

Paw volume (ml) ± SD

Experiment	Control	Aspirin (25mg/kg orally)	Methanol extract (200 mg/kg)	Aqueous extract (200mg/kg)	Petroleum ether (200mg/kg)	Hexane (200mg/kg)
1h after treatment	0.25±0.003	0.21± 0.003	0.26± 0.003	0.28±0.003	0.29± 0.003	0.34± 0.003
2h after treatment	0.25±0.003	0.18± 0.003	0.22± 0.003	0.25±0.003	0.30± 0.003	0.33± 0.003
4h after treatment	0.25±0.003	0.14± 0.002	0.11± 0.002	0.13±0.002	0.32± 0.002	0.36± 0.002

±, S.D, Standard Deviation

Figure 2: Anti-inflammatory activities of different extracts of *Gloriosa superba*



Conclusion

The present study thus confirmed that *Swertia chirayta* and *Gloriosa superba* can be used as potent anti-inflammatory agents. The active principles of these plant(s) extracts must be further optimized in a specific amount of dose using different inflammation induced animal models. These potent compounds can be further utilized to formulate a new potent anti-inflammatory drug.

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