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Anti-inflammatory Activity of *Crinum defixum* Ker-Gawl

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ABSTRACT

Crinum defixum Ker-Gawl is a bulbous herb which has a wide geographical distribution in India. It is commonly called Bon-naharu (meaning wild garlic) in Assam. Traditionally the bulb has been reported to have nauseant, emetic, emollient, diaphoretic properties and it is also used in various inflammatory conditions. The anti-inflammatory activity of the bulbs of the plants has been investigated in the present study in order to establish its traditional claims. The ethyl acetate, chloroform and ethanol extracts of bulbs of *Crinum defixum* were screened for anti-inflammatory activity by using carrageenan induced rat paw edema method. The study revealed that the ethyl acetate extract of the plant had significant anti-inflammatory activity than the chloroform and ethanol extracts. The study supports the ethanomedicinal use of this plant for inflammatory conditions.

Keywords: Nonsteroidal anti-inflammatory drugs, *Crinum defixum* Ker- Gawl, Carrageenan, Extracts of bulbs.

INTRODUCTION

C. defixum Ker-Gawl (Amaryllidaceae) is a bulbous herb which has a wide geographical distribution in India and is common on riverbanks and swampy places in Deccan and Bengal. It is commonly called Bon-naharu (meaning wild garlic) in Assam and it is found abundantly growing wild on riverbanks of Dhansiri River of Assam. [1] The bulb of this plant is fusiform, stoloniferous base and it has cylindrical neck. Flowers are sessile, fragrant at night and tinged with red. [2] Traditionally the leaves were used to treat pimples, body swelling, dropsy, carbuncles, paronychia, leprosy, fever, diarrhea and leucorrhea. [3-5] The juice prepared from the leaves of this plant is instilled into the ear to treat otitis. The bulb has nauseant, emetic, emollient and diaphoretic properties. It is also used in the treatment of burns, whitlow and carbuncle. [5] *C. defixum* is reported to contain the active constituents such as caranine, crinamine, crinine, galanthamine, galanthine, haemanthamine and hippestrine. [6] Recently a new alkaloid 5α-hydroxyhomolycorine has also been reported. [7] Ethanolic extract of dried bulbs of *Crinum defixum* has been reported to have the free radical scavenging and analgesic properties. It is also reported to have anti-genotoxic property. [8]

In view of the traditional claims and due to variety of active constituents in the plant, the present study was carried out to evaluate the anti-inflammatory activity of various extracts obtained from the bulbs of this plant.

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MATERIALS AND METHODS

Plant material

The bulbs of *Crinum defixum* Ker-Gawl were collected in Pakal forest area of Warangal district, Andhra Pradesh in the month of January. The collected plant material was identified and authenticated by V. S. Raju, Botanist and Professor of Department of Botany, Kakatiya University, Warangal. A voucher specimen (No. VCPVSP-CD02) has been kept in the museum for future reference. The bulbs were thoroughly checked for the presence of any foreign organic matter and then the bulbs were coarsely size reduced.

Preparation of the extract

The coarse powder (100 g) prepared from the bulbs of *Crinum defixum* was extracted successively with various solvents in increasing order of polarity from petroleum ether, chloroform, ethyl acetate and ethanol. The filtrates obtained were concentrated under reduced pressure and the extractive values were calculated. The dried extracts were used for preliminary phytochemical screening by performing various chemical tests. [10]

Animals

Male Albino mice (25 ± 5g) and rats (180-200g) were used. They were procured from Mahaveera enterprises, Medipalli, Hyderabad, India. Animals were housed in poly acrylic cages under standard conditions with an ambient temperature of 18 ± 2°C and 12-h-light/12-h-dark cycle. Animals had free access to standard chow diet and water *ad libitum*. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional Animal

Ethical Committee (IAEC No. VCP/2011/10/3/6), Vaagdevi College of Pharmacy, Kakatiya University, Warangal.

Acute toxicity test

The acute toxicity tests were performed according to the OECD (Organization of Economic Cooperation and Development) 423 guidelines. [11]

Rat paw edema test

Carrageenan-induced paw oedema test of Winter et al., (1962) was used for screening of the anti-inflammatory activity of ethyl acetate and chloroform extracts of bulbs of *Crinum defixum.* [12] The rats were divided into eight groups of six animals each. All the drugs/extracts were suspended in 1% Carboxy Methyl Cellulose (CMC) and were administered orally. Group 1 served as control and received only vehicle [1% Carboxy Methyl Cellulose (CMC) suspension]. Group 2 was standard group which received Diclofenac sodium (100 mg/kg). Group 3 and 4 were treated with 200 mg/kg and 400 mg/kg chloroform extracts. Group 5 and 6 were treated with 200 and 400 mg/kg ethyl acetate extracts. Group 7 and 8 were treated with 200 and 400 mg/kg of ethanolic extracts. Thirty minutes after the administration of test drug and the extracts, 0.1ml of 1% carrageenan in normal saline was injected into the sub plantar region of right hind paws of all groups. A mark was kept at the malleolus to facilitate uniform dipping at subsequent reading. The hind paw volume of all the groups was measured with plethysmometer at zero hour. Later on the paw volumes of all groups were measured at 1, 2, 3 and 4 hours respectively. The percentage inhibition of paw edema in the various treated groups was then calculated by using the formula

$$\% \text{ Inhibition of edema} = (1 - Vt/Vc) \times 100$$

Where Vc = edema volume in control group

Vt = edema volume in test group.

RESULTS AND DISCUSSION

The percentage yield of various extracts is shown in Table 1. In the present study ethyl acetate, chloroform and ethanol extract were used. The petroleum ether extract was not used in the study because of its resinous nature and due to the very low yield obtained. All the extracts were subjected to phytochemical screening which revealed the presence of alkaloids, glycosides, steroids, saponins, flavonoids, phenols and carbohydrates as shown in Table 2. Alkaloids and glycosides were present in chloroform, ethyl acetate and ethanol extracts. Flavonoids and tannins were found in ethyl acetate and ethanol extracts. Steroids were found in petroleum ether and chloroform extract. Saponins were found in all the extracts.

The acute toxicity studies were performed by increasing dose of extracts from 50mg/kg to 2000mg/kg. The results revealed that lethality was not observed up to 2000 mg/kg. Hence it was considered as safe dose and $1/10^{th}$ the above dose was used for the evaluation of the anti-inflammatory activity.

Table 1: The percentage yield of chloroform, ethyl acetate and ethanol extracts

S. No	Extract	% Yield (w/v)	Colour	Consistency
1	Petroleum ether	0.1	Yellowish	Resinous
2	Ethanol extract	1.2	Blackish brown	Semisolid
3	Ethyl acetate extract	1.8	Reddish brown	Semisolid (hard)
4	Chloroform extract	0.9	Reddish brown	Semisolid/ Resinous

Table 2: Preliminary Phytochemical Screening of *Crinum defixum* bulb extracts

Name of Test	Petroleum Ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids				
Dragendroff's test	-	+	+	+
Mayer's test	-	+	+	+
Wagner's test	-	+	+	+
Hager's test	-	+	+	+
Flavanoids				
Schinoda test	-	-	+	+
NaOH test	-	-	+	+
Glycosides				
Borntrager's test	-	-	-	-
Baljet's test	-	+	+	+
Keller-Kiliani test	-	+	+	+
Saponins				
Foam test	+	+	+	+
Steroids				
Salkowski test	+	+	-	-
Liebermann-Burchard's test	+	+	-	-
Liebermann's test	+	+	-	-
Carbohydrates				
Molisch's test	-	-	-	+
Fehling's test	-	-	-	+
Benedict's test	-	-	-	+
Barfoed's test	-	-	-	+
Proteins				
Millon's test	-	-	-	-
Biuret test	-	-	-	-
Amino acids				
Ninhydrin test	-	-	-	-
Phenolics/tannins				
$FeCl_3$ test	-	-	+	+
Lead acetate test	-	-	+	+

The results of anti-inflammatory activity revealed that, all the extracts of *Crinum defixum* have shown dose dependent anti-inflammatory activity as shown in Table 3. At a dose of 400mg/kg the chloroform, ethyl acetate and ethanol extracts have shown maximum inhibition of edema (44%, 66% and 56% respectively). Out of the all extracts tested the ethyl acetate extract at a dose of 400mg/kg body weight showed maximum activity (66%) which was comparable to standard. The Carrageenan induced rat paw oedema is commonly used inflammatory model to investigate the anti - inflammatory activity. The inflammation produced by carrageenan has a biphasic effect i.e., the first phase is due to release of histamine and serotonin (5HT) which occurs within 0-2 hours, and second accelerating phase of swelling is attributed to prostaglandin release (>4h). In this study ethanol and ethyl acetate extract bulbs of *Crinum defixum* showed dose dependent activity in both the phases of inflammation. Whereas the chloroform extract showed less activity in both the phases. Out of all the extracts the ethyl acetate extract (400 mg/kg, *p.o.*) showed maximum inhibition in comparison with the control. The extent of inhibition by the ethyl acetate extract was found to be more in the second phase inflammation which may be attributed to flavonoids and phenolic compounds present in the ethyl acetate extract. The observed activity might be due to inhibition of prostaglandin synthesis. Ashih *et al*., 2010 have reported that the phenolic compounds present in the ethanolic extract of bulbs have analgesic and antioxidant activity. [13] Our reports also reveal that the anti-inflammatory activity can be attributed the flavonoids and phenolic compounds present in the plant.

It is evident from the results that the chloroform extract has little anti-inflammatory activity which can be attributed to

Table 3: Effect of *Crinum defixum* extracts on carrageenan-induced paw edema in rats

Drug/Extract	Dose(mg/kg)	Edema Volume (ml) (Mean ± SEM)			
		1 h	2h	3h	4 h
Control	-	0.32 ± 0.06	0.40 ± 0.08	0.49 ± 0.08	0.43 ± 0.08
Standard	100	0.13 ± 0.05 (59)***	0.14 ± 0.05 (65)***	0.15 ± 0.05 (69)***	0.17 ± 0.04 (60)***
Ethanol	200	0.20 ± 0.07 (34)**	0.23 ± 0.04 (43)***	0.26 ± 0.05 (46)***	0.25 ± 0.04 (42)***
	400	0.15 ± 0.054 (54)***	0.16 ± 0.06 (59)***	0.18 ± 0.025 (63)***	0.19 ± 0.02 (56)***
Ethyl acetate	200	0.2 ± 0.03 (38)***	0.22 ± 0.07 (45)***	0.25 ± 0.03 (49)***	0.24 ± 0.04 (44)***
	400	0.14 ± 0.04 (56)***	0.15 ± 0.04 (61)***	0.16 ± 0.051 (66)***	0.175 ± 0.04 (59)***
Chloroform	200	0.24 ± 0.04 (26)*	0.28 ± 0.04 (31)**	0.31 ± 0.04 (35)***	0.30 ± 0.049 (29)**
	400	0.2 ± 0.02 (36)**	0.24 ± 0.04 (41)***	0.27 ± 0.04 (44)***	0.25 ± 0.04 (40)***

Values are given as Mean ± SEM at different doses and time intervals, n = 6; *P<0.05, **P<0.01, ***P<0.001 compared to control (One-way ANOVA; post hoc Dunnet test)

lack of flavonoids and phenolic compounds in it. Even though the alkaloids are present in the chloroform extract the anti-inflammatory was comparatively low when compared to control. Basing on these evidences it can be concluded that the ethyl acetate extract has anti-inflammatory activity, which may be due to the presence of flavonoid and phenolic compounds. This study can be an evidence for the traditional claims of the herb for treating various inflammatory conditions. However, there is need for further research work to isolate and identify the active constituents responsible for the anti-inflammatory activity of the ethyl acetate extract of *Crinum defixum.*

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