Evaluation of anti-inflammatory activity of *Cynodon dactylon* Pers. on carrageenan induced paw edema in rats

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In the present study, anti-inflammatory property of chloroform-methanolic extract isolated from *Cynodon dactylon* Pers. (Dhub Grass) was investigated in carrageenan induced rat paw edema. The extract showed significant inhibition of carrageenan induced paw edema at three doses of 125, 250 and 500 mg/kg used for both acute and chronic models in the study and is comparable with standard anti-inflammatory drug, Indomethacin. It can therefore be concluded from the present study that chloroform-methanolic extract of *C. dactylon* possesses anti-inflammatory property.

**Keywords:** *Cynodon dactylon*, Dhub Grass, Rat paw edema, Carrageenan, Anti-inflammatory.

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**Introduction**

Inflammation is a local response of living mammalian tissue to injury due to any agent. Inflammation manifests usually in the form of painful swelling associated with some changes in skin covering the site¹. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Chronic inflammation leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process². Carrageenan-induced paw oedema is widely used for determining the acute phase of the inflammation. Histamine, 5-HT and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation³, whereas prostaglandins are detectable in the late phase of inflammation⁴. Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses⁵. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases⁶. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary. *Cynodon dactylon* is a common grass weed which has been regarded to possess various medicinal properties⁷. The plant possesses antimicrobial, antiviral activity and has also been used to treat urinary tract infection, calculi and prostatitis. It also has significant application in treating dysentery, dropsy and secondary syphilis. The aqueous plant extract is used as diuretic, anti-emetic, anti-inflammatory and purifying agent⁸. In traditional system of medicine, the whole plant, or the rhizomes alone, is applied topically in gout, rheumatic affections and as a styptic to wound⁹,10. The objective of this investigation was to ascertain the scientific basis of its use in treatment of inflammation, on which there is no previous data available. Hence in the present study effort has been made to establish the scientific validity to the anti-inflammatory property of *C. dactylon* extract using carrageenan induced paw edema in experimental rats.

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Materials and Methods

Plant material

The fresh entire plants with roots of *Cynodon dactylon* Pers. were collected from the local area of Bagalkot district, Karnataka, India. Herbarium was prepared and specimen was further identified and authenticated in Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka. The whole plant material was washed in 10% KMnO$_4$ along with root, dried under shade and then pulverized to get uniform moderately coarse powder (# 44). The sieved powder was stored in airtight high density polyethylene container before extraction.

Drug and Chemicals

The drug carrageenan was purchased from S. D. Fine-Chemicals and other chemicals were purchased from commercial sources of analytical grade.

Preparation of extracts

Dried plant material was abridged to a fine powder with a mechanical grinder. The powdered plant material was extracted with petroleum ether and then defatted powder was refluxed with chloroform-methanol. The chloroform-methanolic extract of *C. dactylon* (CMCD) was concentrated to dryness and stored.

Collection of animals

Sprague-Dawley rats of either sex (225-285 g) were obtained from institute’s animal house and used for this study. They were housed under standard laboratory conditions and were fed with commercial rat feed (Amruth, Sangli, Maharashtra) and water *ad libitum*. All the experiments were carried out in accordance with direction of Institutional Animals Ethics committee (HSKCP/IAEC, Clear/2007-08, Dated 28/11/2007).

Acute toxicity study

The acute toxicity study was performed as per the method described by Litchfield and Wilcoxon (1949), and LD$_{50}$ was calculated accordingly. The CMCD in the dose range of 100-2000 mg/kg was administered orally to different groups of mice (n=10). The animals were examined at every 30 min up to a period of 3 h and then occasionally for additional 4 h period, finally 24 h mortality was recorded. All the animals found to be safe. The dosing schedule was followed as per OECD (guidelines 425). The anti-inflammatory activity was performed on experimental rats at three dose levels 125, 250 and 500 mg/kg of body weight.

Carrageenan paw edema in rats

Anti-inflammatory activity of CMCD was assessed by carrageenan paw edema. Rats were divided in to 6 groups (n = 6). Animals of all the groups injected with 0.1 mL of carrageenan in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group-I animals (normal) received vehicle (5% Tween 80), Group-II animals (carrageenan control) received vehicle (5% Tween 80), 30 min prior administration carrageenan injection. Group-III, the standard reference group was given p.o., aqueous solution of Indomethacin (5 mg/kg), 30 min prior administration carrageenan injection. Group-IV, Group-V and Group-VI received 125, 250 and 500 mg/kg of CMCD, 30 min prior administration carrageenan injection.

The paw volume was measured plethysmographically immediately after injection, again at ½, 1, 2, 3, 4 and 5th hour and eventually 24 h after challenge. The percentage inhibition of edema was calculated for each group with respect to the vehicle received control group of animals$^{11,12}$.

Statistical evaluation

The difference of average values between treated animals and control groups were calculated for each time and statistically evaluated. All the values were expressed as mean ± SEM and one-way ANOVA was applied to determine the significance of the difference between the control groups and rat treated with the test compounds. A value of *P* <0.05 was considered to be significant.

Results and Discussion

As shown in the Table 1, subplantar injection of carrageenan in rats (control) showed time dependent significant increase in paw thickness when compared to normal and group. At ½ h the significant (*P*<0.01) increase paw volume and at 1 h there is a significant (*P*<0.001) increase in paw volume and it sustained through the study when compared to the normal group. At 1st h there is no significant change in paw volume in Indomethacin, 125 mg/kg and 250 mg/kg of CMCD treated groups was observed, but 500 mg/kg of CMCD treated group showed significant (*P*<0.05) change. At 2nd h also 125 mg/kg, 250 mg/kg of CMCD groups changes were insignificant but Indomethacin and 500 mg/kg of CMCD groups were significant (*P*<0.05). At 3rd h Indomethacin and 500 mg/kg of CMCD groups showed significant (*P*<0.01) and (*P*<0.001) changes,
respectively; 125 mg/kg, 250 mg/kg of CMCD treated groups were again insignificant. At 4th h Indomethacin and 500 mg/kg of CMCD groups are significant (P<0.001), 125 mg/kg and 250 mg/kg of CMCD treated groups showed significant effect (P<0.05) and (P<0.01), respectively. At 5th h 125 mg/kg of CMCD treated group is significant (P<0.01), Indomethacin, 250 mg/kg and 500 mg/kg of CMCD treated groups shown significant effect (P<0.001) when compared to control group. The percentage of inhibition of paw volume for 125 mg/kg is 1.04, 1.88, 2.79, 6.82, 9.69, 11.78, for 250 mg/kg, 8.91, 7.62, 4.84, 7.36, 12.52, 19.46, for 500 mg/kg 7.29, 12.43, 13.69, 22.58, 25, 24 and for Indomethacin treated group is 2, 2, 11.56, 15.26, 20.12, 23.63 at 1, 2, 3, 4, and 5th h. The percentage of inhibition of paw volume for 500 mg/kg of CMCD group is more prominent than Indomethacin treated group.

Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins13-17. We observed that CMCD showed significant inhibition against carrageenan-induced rat paw edema in the dose dependent manner to possess the anti-inflammatory effect may be due to presence of flavonoid18. This response tendency of the extract in carrageenan induced rat paw edema revealed good peripheral anti-inflammatory properties of the CMCD extract. In earlier studies it has been reported that a flavonoids, cyanidin and β-carotene possess anti-inflammatory activity19-21. The presence of flavonoids, cyanidin and β-carotene might be responsible for the anti-inflammatory activity in CMCD extract also. Thus, it is concluded that the extract of C. dactylon produces significant anti-inflammatory activity in dose dependent manner.

Conclusion
The findings of the present study have demonstrated that chloroform methanolic extract of C. dactylon has potent anti-inflammatory property and it justifies the traditional use of this plant in the treatment of various types of inflammation.

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References