

Anti-Inflammatory and Antioxidant Activity of the Hydroalcoholic Extract of *Cynodon Dactylon* L. Pres.

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Abstract: The present work was aimed to study the anti-inflammatory activity of *Cynodon dactylon* L. Pres.; using hydroalcoholic extract of entire plant on rodent models. The experimental paradigm included pulmonary edema in mice and granuloma pouch technique in rats. In pulmonary edema model; mice received hydroalcoholic extract of *Cynodon dactylon* L. Pres. (HECD) 600 mg/kg, p.o. Mice were challenged with intrapleural injection of 0.1 ml of 1 % suspension of carrageenan in saline, one hour after administration of drugs or vehicle, on the right side of the thorax. In Granuloma pouch technique rats received HECD 600 mg/kg, p.o. and after 24 hour were challenged with carrageenan (4 ml of 2% w/v of carrageenan solution in saline, s.c.). Indomethacin (10 mg/kg) was used as reference standard. The effect of the HECD against pulmonary edema in mice; was found to be significantly (**P<0.01) effective as well as the effect of HECD on the increased protein infiltration induced by carrageenan was found to be significant (**P<0.01).

Key words: *Cynodon dactylon*, anti-inflammatory activity, Indomethacin, Carrageenan.

I. INTRODUCTION

In ancient Greek and Roman era, inflammation was regarded as a single disease entity and the result of a disturbed state of body fluids. The interesting concept of inflammation was then outlined that inflammation itself should not be considered as a disease, but as a salutary operation consequent either to process of inflammation as a succession of changes occurring in a living tissue when it is injured providing that the injury is not of such a degree of severity so as to at once destroy its structure and vitality. It is defined as a series of responses due to release of endogenous substances following injury by the vascularized tissues of the body. Pain that accompanies inflammation and tissue injury may cause local stimulation of pain fibers and enhanced pain sensitivity. Most currently available and commonly used drugs to treat symptoms of inflammation and most types of arthritis include the group of non steroidal anti-inflammatory drugs (NSAIDs). A major aspect of mechanism of action of the NSAIDs is of both cyclooxygenase isoenzymes, namely cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activities, and thereby synthesis of prostaglandins and thromboxane. In addition to sharing many therapeutic activities NSAIDs share many unwanted side effects like gastric ulceration, intolerance, inhibition of platelet function, renal damage etc [1].

India is the birth place of renewed system of 'Indigenous medicine' such as Siddha, Ayurveda and Unani. India is

enriched with flora and therefore plants have been used since ancient times for the simple remedies and became popular owing to the expertise of the physicians and the genuine and higher efficacy of the drugs^[2]. They are not yet accepted by modern system of medicine since they lack, the so called 'scientific validity for their therapeutic value'. Hence it was aimed to bring the treasure of these traditional systems of medicine to the modern system of medicine by providing the scientific validity. Furthermore, the fact that the discovery of a new drug is time consuming, expensive affair, then perhaps the logical choice would be in exploring plant products as potential medicines to the maximum possible usage.

A lab scale study was carried out to evaluate the anti-inflammatory and antioxidant activity of the hydroalcoholic extract of *Cynodon dactylon* L. Pres.

II. MATERIAL AND METHODS

The entire plant was collected in the month of August 2005 from the garden of 'Bharati Vidyapeeth's college of pharmacy, CBD-Belapur, Navi Mumbai, Maharashtra and was authenticated by Dr. Pathak and Dr. Naik (*Cynodon dactylon* Pers. (Graminae)^[4], accessory number: 4490, dated on 29 February 2003; Nicholas piramal research centre, herbarium museum). The shade dried plant material was reduced to coarse powder manually weighing 500g. The dried powder was extracted with hydro-alcoholic solution (50% Ethanol) by simple hot extraction method under reflux at $60 \pm 5^\circ\text{C}$ for 10 to 12 hrs. The extraction was carried out thrice, each time with fresh hydro-alcoholic solvent. The resultant extract was combined and then concentrated under reduced pressure at 50°C using Rotavac evaporator. The concentrated extract was treated with petroleum ether to remove chlorophyll pigments. The extract was vacuum dried and stored in an air tight container in the desiccator. The percent yield obtained was 3.6% w/w.

III. EXPERIMENTAL DESIGN

Male, Wistar albino rats weighing between 80-120 grams were procured from Bharat Serum and Vaccines Ltd., Thane. Male, Swiss albino mice weighing between 20-25 grams were procured from Haffkine's institute, Parel, Mumbai. The animals were housed in standard polypropylene cages and kept under controlled room temperature ($27 \pm 1^\circ\text{C}$) and relative humidity (60-70 %) in 12 hours light and 12 hours dark cycle. The animals were given a standard laboratory diet (Amrut

pharmaceuticals, Thane) and water *ad libitum*. The experimental protocol was approved by the institutional animal ethics committee (IAEC).

A) Pulmonary edema in mice

Male albino mice weighing between 25 ±2 grams were selected. Mice were fasted for 12 hours prior to dosing. Mice were divided into 3 groups of 6 animals each. Group-I (carrageenan control) received 0.2 ml of distilled water orally, group-II received 10 mg/kg of indomethacin (reference standard), and group-III received aqueous alcoholic extract 600 mg/kg, *p.o.* Mice were challenged with intrapleural injection of 0.1 ml of 1 % suspension of carrageenan in saline, one hour after administration of drugs or vehicle, on the right side of the thorax. The mice were sacrificed by cervical dislocation, 2 hours after carrageenan challenge. The lungs were quickly dissected free from the trachea and weighed.

Calculation: Significant changes in the test lung weight to the carrageenan control, was considered to reflect pulmonary edema.

$$\text{Pulmonary edema} = \frac{\text{Lungs weight}}{\text{Body weight}} \times 10,000$$

B) Granuloma pouch technique

Male wistar rats weighing between 160-200 grams were selected. Rats were fasted for 16 hours prior to dosing. Rats were divided into 3 groups of 6 animals each. Group-I (carrageenan control) received 0.2 ml of distilled water orally, group-II received 10 mg/kg of indomethacin (reference standard), and group-III received aqueous alcoholic extract 600 mg/kg, *p.o.* 24 hours prior to carrageenan challenge the back of the animals were shaved and disinfected. With a very thin needle a pneumoderma was made in the middle of the dorsal skin by injection of 8 ml of air under light ether anesthesia. 4 ml of 2% w/ v of carrageenan solution in saline was injected subcutaneously in the resulting air pouch. After 6 hours the rats were sacrificed by dropping them into the euthanasia chamber, the exudate was collected and the protein estimation of the exudate was carried out.

C) Erythrocyte membrane stabilization.

The blood was collected from male rats by decapitation in a vial containing heparin (anticoagulant). Twenty microlitres of fresh blood was added to a vial containing 1 ml of phosphate buffer saline (PBS, pH 7.4). The drug extract or indomethacin in PBS was added to the vials (in triplicate) so as to achieve final concentrations of 100, 250, 500, 750 and 1000 microgram /ml and PBS (15 microlitres) was added to control vials and mixed properly. Prior to addition of the drug solution to the vials, the drug solution was subjected to the centrifugation at 3000 x g for 10 minutes to give a clear solution. The vials containing the reaction mixture were incubated in a water bath at 37° C for 15 minutes, the mixture

were heated at 54° C for 25 minutes. The mixture was centrifuged to give a clear supernatant. The absorbance of the supernatant was measured at 540 nm using UV Visible spectrophotometer.

Calculation: The percent inhibition of haemolysis with respect to the control was calculated as follows:

$$\text{Percent inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where, A_{control} : Absorbance of control group, A_{sample} : Absorbance of test/ standard group.

D) Determination of DPPH radical Scavenging activity (in vitro)

The free radical scavenging activity of *Cynodon dactylon* L. Pres. was measured using DPPH. 1.0 ml of (0.1 mM) DPPH solution in ethanol was added to 1.0 ml of extract solution in ethanol at different concentration (10-3000 µg/ml). Twenty minutes later, the absorbance was measured at 517 nm. Ascorbic acid (Vitamin C) was used as a reference standard^[16].

Calculation: The percent inhibition of DPPH radical scavenging activity was calculated using the following equation.

$$\text{Percent inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where, A_{control} is absorbance of the control reaction and A_{test} is absorbance in presence of sample of extract. The antioxidant activity of extract was expressed as IC50. IC50 value was defined as concentration in (µg/ml) of extracts that inhibits the formation of DPPH radicals by 50 %.

IV. RESULT DISCUSSION CONCLUSION

a) Pulmonary edema in mice

Table 1: Effect of 50% hydroalcoholic extract of *Cynodon dactylon* L. Pers. on pulmonary edema in mice.

Treatment	Pulmonary edema (mg)
Positive Control	83.28 ± 2.84
Indomethacin (10 mg/kg, <i>p.o.</i>)	63.19 ± 3.71
<i>Cynodon dactylon</i> (600mg/kg, <i>p.o.</i>)	69.72 ± 2.34

Pulmonary edema^[5] is the name given to the condition in which there is leakage of blood elements from the pulmonary capillaries. The effect of the hydroalcoholic extract of *Cynodon dactylon* L. Pres. against pulmonary edema in mice (table 1); represents that, the drug was found to be significantly ($P < 0.01$) effective in the dose employed. The pulmonary edema developed after the intrapleural injection of carrageenan was significantly ($p < 0.01$) inhibited by the hydroalcoholic extract of *Cynodon dactylon* L. Pres. at a dose of 600mg/kg. Indomethacin^[7] 10 mg/kg was serve as a

reference standard also inhibit pulmonary edema significantly ($p < 0.01$). (Table 1)

b) Granuloma Pouch Technique:

Table 2: Effect of hydroalcoholic extract of *Cynodon dactylon L. Pres.* on exudate formation and protein leakage in granuloma pouch technique.

Treatment	Protein (mg/ ml)
Positive Control	2.48 ± 0.10
Indomethacin (10 mg/kg, p.o.)	1.28 ± 0.05
<i>Cynodon dactylon</i> (600 mg/kg, p.o.)	1.83 ± 0.10

An important characteristic of the inflammatory reaction is a sustained elevation in vascular permeability causing massive infiltration of cells and proteins^[8]. This increased permeability may be because of endothelial destruction, intracellular contraction or by direct injury. The mediators responsible for the increased vascular permeability are histamine, serotonin, bradykinin and prostaglandins^[9]. The prostaglandins like, PGE₁, PGE₂, PGF_{1 α} and PGF_{2 α} evoke increased vascular permeability in the skin of rat and man^[7]. The result indicates that the hydro-alcoholic extract of *Cynodon dactylon L. Pres.* is able to inhibit the prostaglandins which are mainly responsible for protein infiltration in inflammation. The hydroalcoholic extract of *Cynodon dactylon L. Pres.* significantly ($P < 0.01$) inhibited protein exudation into the carrageenan pouch (1.28 milligram/ dl), but the inhibition was less prominent than indomethacin (1.83 milligram/ dl) as indicated in table 2.

c) Erythrocyte Membrane Stabilisation:

Table 3: Effect of hydroalcoholic extract of *Cynodon dactylon L. Pres.* on heat-induced haemolysis of rat erythrocytes.

Concentration (Microgm/ml)	Percent inhibition	
	Indomethacin	<i>Cynodon dactylon</i>
100.00	58.49 ± 1.03	28.99 ± 4.39
250.00	63.74 ± 0.61	40.29 ± 5.55
500.00	65.65 ± 0.71	53.91 ± 4.79
750.00	68.55 ± 0.82	57.10 ± 2.09
1000.00	75.13 ± 0.43	72.46 ± 2.85

A protective effect on heat induced erythrocyte lysis is considered to be a biochemical index of anti-inflammatory drug action^[10]. The membrane of the erythrocyte may be considered a model of the lysosomal membrane^[11]. The agents which can thwart the rupture of the latter, and thereby prevent the damage to the tissue caused by the release of the hydrolytic enzymes contained within the lysosomes may be expected to alleviate some manifestations of inflammation. The hydro-alcoholic extract of *Cynodon dactylon L. Pres.* may act as a membrane stabilizer and thus may inhibit the release of enzymes which can cause damage to the tissue. Percent inhibition of haemolysis of erythrocytes is presented

as mean \pm SEM. Results in table 3; demonstrate that the hydro-alcoholic extract is able to protect erythrocytes against heat-induced lysis in a dose-dependent manner. However, at higher concentration hydro-alcoholic extract has an inhibitory activity i.e. 72.46 ± 2.85 against heat induced lysis of erythrocytes almost similar to that of indomethacin (reference drug) shows 72.46 ± 2.85 of percent inhibition.

d) In Vitro Anti-Oxidant Activity:

Table 4: DPPH radical scavenging activity

Concentration (microgram/ml)	Percentage inhibition	
	Ascorbic acid	<i>Cynodon dactylon L. Pres.</i>
10	22.62 ± 0.61	2.33 ± 0.66
50	51.70 ± 0.96	12.74 ± 1.03
100	76.44 ± 1.73	28.38 ± 1.43
500	84.23 ± 1.0	36.03 ± 1.22
1000	89.99 ± 0.17	48.43 ± 1.16
1500	92.01 ± 0.71	60.47 ± 1.50
2000	92.69 ± 0.52	70.34 ± 0.81
3000	94.67 ± 0.58	85.61 ± 0.76

These appear to be the main endogenous sources of most of the oxidants produced by the cells. It is suggested that free radical damage the cells which leads to pathological changes such as hepatotoxicity, nephrotoxicity, rheumatic arthritis etc^[12]. Anti-oxidants are beneficial components that fight with free radicals and neutralize them before they can attack the cells and hence prevent damage to cell proteins, lipids and carbohydrates^[13]. Therefore, anti-oxidant^[15] activity is important in view of the free radical theory of aging and associated diseases. DPPH radical scavenging is a common and reliable method used for screening of anti-oxidant activity *in vitro*^[14]. The ability of a compound to reduce DPPH provides a basis for the anti-oxidant activity. The results indicate an anti-oxidant activity of the extract which is of less potency than the ascorbic acid. This anti-oxidant activity may be responsible, atleast partly, for the anti-inflammatory activity of the extract. Free radical scavenging effect of hydro-alcoholic extract of *Cynodon dactylon L. Pres.* on DPPH was tested and results are indicated in table 14. Ascorbic acid was used as a reference drug with an IC₅₀ value of 132.80 microgram/ml. The IC₅₀ value of the hydroalcoholic extract of *Cynodon dactylon L. Pres.* was 1341.11 microgram/ml.

V. CONCLUSION

As the plant contain the flavonoid, these compounds are known to target the prostaglandins, which are involved both in the later phase of inflammation as well as in pain perception. Since flavonoids possess radical scavenging activity these flavonoids^[3] may also be responsible for the anti-oxidant activity of *Cynodon dactylon L. Pres.* Hence the anti-inflammatory activity and antioxidant activity of the

hydroalcoholic extract of *Cynodon dactylon* L. Pres. may be attributed to the presence of flavonoids. The results are in accordance with the study previously conducted anti-inflammatory and analgesic studies on hydro-alcoholic extract of *Cynodon dactylon* L. Pres.^[17].

REFERENCES

- [1]. Crofford L.J. (2013). Use of NSAIDs in treating patients with arthritis, *Arthritis Res Ther*, 15 (3).
- [2]. Pandey M. M. , Rastogi., and Rawat A. K.S.(2013). Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation, *J Evid Based Complementary Altern Med*, 1-12.
- [3]. Kaliyaperumal, Ashokkumar, Kumarakurubaran S., Muthukrishnan S.D., (2013). *Cynodon dactylon* (L.) Pers.: An updated review of its phytochemistry and pharmacology., *J. Med. Plants Res*. 7(48), 3477-3483.
- [4]. Paul R., Mandal A. and Datta K.A., (2012). An updated overview on *Cynodon dactylon* (L.) Pers., *Asian j plant sci res*. 3(1), 11-14.
- [5]. Oram S., Pulmonary oedema, (1958). *Respr Med*, 52(2) 121-125.
- [6]. Georgewill O.A., Georgewill U.A. and Nwankwoala R., (2010). Anti-inflammatory effect of *Moringa oliefera* lam extract in rats, *Asian Pac. J. Trop.*, 133-235.
- [7]. Ricciotti E. and FitzGerland G.A., (2011). Prostaglandins and Inflammation, *Arterioscler Thromb Vasc Biol*, 31(5) 986–1000.
- [8]. Davidson R.M. and Seneff S., (2012). The initial common pathways of inflammation, disease, and sudden death, *Entropy*, 14, 1399-1442.
- [9]. Riberio A. N. et.al., (2016). Analgesic and inflammatory actions on bradykinin route of a polysulfated fractions from alga *Ulva lactuca*, *Int J Bio Macromol*, 92, 820-830.
- [10]. Sen S., (2013). Anti inflammatory, analgesic, and antioxidants activities of *Pisonia aculeate*: Folk medicinal use to scientific approach. *Pharm. Bio.*, 426-431.
- [11]. Labu Z.K., Laboni F.R., Howaldar M.S.I., Rashid M. H., (2015). Membrane stabilization as a mechanism of anti inflammatory and thrombolytic activities of ethanolic extract of arial parts of *Spondiasis pinanata* (Family: anacardiaceae) *PhOL.*, 2, 44-51.
- [12]. Li S., et.al., (2015). The role of oxidative stress and antioxidants in liver disease, *Int J Mol Sci.*, 16, 26087-26124.
- [13]. Huy L. P. and Huy C.P., (2008). Free radicles, antioxidants in disease and health, *Int J Biomed Sci* 4(2) 89-96.
- [14]. Kedare S.B. and Singh R.P.J., (2011). Genesis and development of DPPH method of antioxidant assay, *J Food Sci Technol* 48(4) , 412-422.
- [15]. Mansouri et al., (2014). Antioxidant effect of hydero-alcoholic extract of pomegranate (*Punica gsrantum* L.) on some of tissues, *Biotech. Res. Asia*, 11(2), 441-447.
- [16]. Shekhar C.T. and Goyal A., (2014). Antioxidant activity of DDPH radical scavenging method of *Agerataum conyzoides* Linn. leaves, *Am J Med.*, 1(4), 244-249.
- [17]. Dhande, S*, (2013) Anti-Inflammatory and Analgesic Properties of The 50 % Ethanolic Extract of *Cynadon Dactylon* Pers, *Int. J Pharm Sci Rev Res*, (2), 8-16.