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Assessment of *In-vitro* Anti-Inflammatory Activity of *Cynodon Dactylon* and Acyclovir Showing Synergistic Effect by Albumin Denaturation and Membrane Stabilization Assay

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Abstract

The present research was carried out the evaluation of the anti-inflammatory property of methanolic extract of *Cynodon dactylon* and Acyclovir as individual and in combination by two different methods viz., Albumin denaturation assay and Membrane stabilization assay. Ten different concentration of *Cynodon dactylon* methanolic extract and Acyclovir solution (100, 200, 300, 400,500, 600, 700, 800, 900, 1000mg) were using in these studies. Anti-inflammatory action was observed in dose dependent manner. In protein denaturation method at concentration of 1000ppm extract showed maximum protection (82.46%) and standard drug provided (73.23%) protection where as concentration of 500ppm acyclovir showed maximum protection (28.94%). The combination of both i.eat 1000ppm of extract and 500ppm of acyclovir showed maximum protection at the ratio of 80:20 (84.76%). Similarly, in membrane stabilization test, the selected extract at concentration of 1000ppm showed maximum membrane stabilization (89.1%) and standard drug provided (78.44%) protection, acyclovir showed maximum protection at concentration of 500ppm (28.62%).The combination of both i.e. at 1000ppm of extract and 500ppm of acyclovir showed maximum protection at the ratio of 80:20 (91.49%).Hence, from these results, we concluded that methanolic extract of *Cynodon dactylon* methanolic extract and acyclovir shows synergistic effect at different concentration when compared to standard NSAID drug (Diclofenac sodium). In addition, phytochemical analysis of *Cynodon dactylon* methanolic extract showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, phytosterol etc. It reveals that these phytochemical constituents are responsible to maximum protection of protein denaturation and albumin denaturation and membrane stabilization assay.

Keywords: *Cynodon dactylon* extract; Acyclovir; Albumin denaturation; Membrane stabilization; Phytochemical analysis

Introduction

Denaturation of tissue proteins and lysis of RBC membrane are well documented causes of inflammatory diseases. Production of auto antigens in certain inflammatory diseases may be due to denaturation of proteins or lysis of RBC membrane *in-vivo*. The *in-vitro* anti-inflammatory effect of individual extracts and in combination was evaluated against denaturation of egg albumin and membrane stabilization of RBC. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the extracts throughout the selected concentration ranges. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by extracts and reference drug diclofenac sodium. From the IC 50 values it becomes evident that the extracts were more active than diclofenac sodium, being effective in lower concentrations. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation. In the present study, the relatively high viscosity of control dispersion substantiated this fact [1].

During inflammation, there are lyses of lysosomes which release their component enzymes that produce a variety of disorders. Non-steroidal anti-inflammatory drugs (NSAIDs) exert their beneficial effects by either inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes. Exposure of red blood cells (RBCs) to injurious substances such as hypotonic medium, heat, methyl salicylate or phenyl hydrazine results in the lysis of the membranes, accompanied by haemolysis and oxidation of haemoglobin [2]. Since human red blood cell (RBC) membranes are similar to lysosomal membrane components, the inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of *Cynodon dactylon* extract with diclofenac sodium as a standard.

The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators. The results showed that *Cynodon dactylon* extract, which perhaps stabilized the red blood cell membrane by preventing the release of lytic enzymes and active mediators of inflammation [3].

Experimental

Materials and Methods

Plant material: The plant material (leaves) was collected from local market and was authenticated by Dr H. M Pandit, Khalsa College, Matunga. The fresh leaves were washed under running tap water to remove adhere dirt, followed by rinsing with distilled water, shade dried and pulverised in a mechanical grinder to obtain coarse powder.

Extraction of Bioactive Components: 10 gram of powdered drug (*Cynodon dactylon*) was weighed and extracted with methanol as solvent using soxhlet apparatus for 24hrs. The process was repeated three times using the same solvent. The extracts were dried under desiccators and the percentage yield was calculated. The methanolic extract was used for the preliminary phytochemical investigation, anti-inflammatory activity [4].

In Vitro Anti-Inflammatory Activity

Inhibition of albumin denaturation

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37 °C in incubator for about 15mins and then heated at 70 °C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Membrane Stabilization Property

Preparation of Red Blood Cells (RBCs) suspension

Fresh whole human blood (10ml) was collected and transferred to the heparinzed centrifuged tubes. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10%v/v suspension with normal saline [5].

Heat Induced haemolysis

The 2ml reaction mixture is consisted of 1ml of test extract at various concentrations and 1ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 560C for 30min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5min and the absorbance of the supernatants was taken at 560nm [6]. The experiment was performed in triplicate. % of membrane stabilization activity was calculated by the formula mentioned below:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Phytochemical analysis

The dried powdered samples were subjected to qualitative tests for the identification of phytochemical constituents according to standard procedures.

Results and Discussion

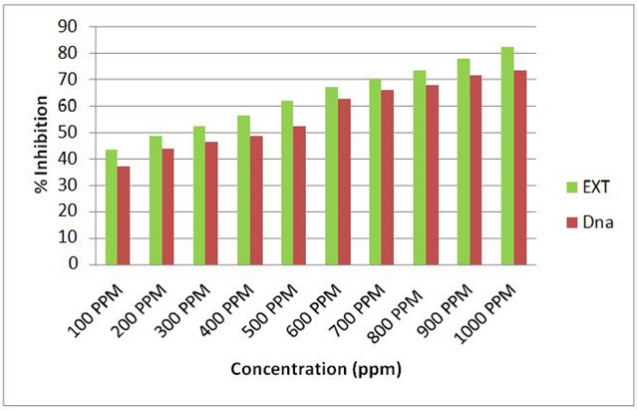

Figure 1: Graph showing % inhibition of protein denaturation activity of *Cynodon dactylon* extract.

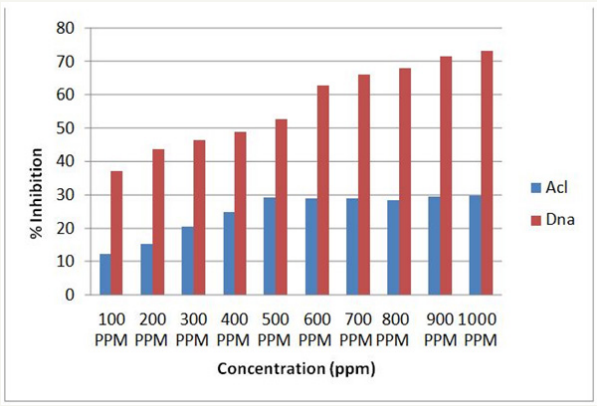

Figure 2: Graph showing % inhibition of protein denaturation activity of acyclovir.

In the present investigation, the *in vitro* anti-inflammatory effect of *Cynodon dactylon* and Acyclovir as individual and *Cynodon dactylon* extract and acyclovir in combination was evaluated against denaturation of egg albumin and Membrane stabilization method [7]. The results are summarized below. The present findings revealed a concentration dependent inhibition of protein

(albumin) denaturation and Membrane stabilization method by *Cynodon dactylon*, Acyclovir as individual and *Cynodon dactylon* extract and acyclovir in combination throughout the concentration range of 100 to 1000µg/ml. Diclofenac sodium (at the concentration range of 100 to 1000µg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation; nonetheless, the effect of diclofenac sodium was found to be less compared to *Cynodon dactylon* extract as well as in combination of *Cynodon dactylon* extract and Acyclovir (Table 1-6) (Figure 1-4).

Table 1: Effect of *Cynodon dactylon* extract on protein Denaturation.

Concentration		% Inhibition of Protein Denaturation		Viscosity	
Cynodon Dactylon Extract	Diclofenac Sodium	*Cynodon Dactylon* Extract	Diclofenac Sodium	*Cynodon Dactylon* Extract	Diclofenac Sodium
100	100	43.53	37.07	1400	1700
200	200	48.46	43.69	1300	1500
300	300	52.61	46.3	1300	1500
400	400	56.3	48.76	1300	1400
500	500	62	52.61	1200	1300
600	600	67.23	62.76	1200	1300
700	700	70.15	66.15	1100	1200
800	800	73.23	68	1000	1200
900	900	77.84	71.53	1000	1200
1000	1000	82.46	73.23	900	1100
Control				2000	

All values are mean ± S.D (NMT 2%).

Table 2: Effect of acyclovir on protein denaturation.

Concentration		% Inhibition of Protein Denaturation		Viscosity	
Acyclovir	Diclofenac Sodium	Acyclovir	Diclofenac Sodium	Acyclovir	Diclofenac Sodium
100	100	12.3	37.07	2400	1700
200	200	15.23	43.69	2300	1500
300	300	20.3	46.3	2200	1500
400	400	24.76	48.76	2100	1400
500	500	29.23	52.61	1900	1300
600	600	28.76	62.76	2000	1300
700	700	29.07	66.15	1900	1200
800	800	28.46	68	2000	1200
900	900	29.53	71.53	1900	1200
1000	1000	29.84	73.23	1900	1100
Control				2000	

All values are mean ± S.D (NMT 2%).

Table 3: % inhibition of protein denaturation activity of combination of extract and acyclovir.

Concentration Ratio (*Cynodon Dactylon* :Acyclovir)	% Inhibition of Protein Denaturation	Viscosity (Cp)
0.479166667	39.38	1500
0.888888889	51.53	1400
30:70	56.3	1300
40:60	61.53	1300
50:50:00	66.76	1200
60:40:00	70.76	1200
70:30:00	83.53	1100
80:20:00	84.76	900
90:10:00	83.84	900
Control		2000

All values are mean ± S.D. (NMT 2%).

Table 4: Effect of *Cynodon dactylon* extract on membrane stabilization.

Concentration		% Inhibition of Haemolysis		Viscosity	
Cynodon Dactylon Extract	Diclofenac Sodium	*Cynodon Dactylon* Extract	Diclofenac Sodium	*Cynodon Dactylon* Extract	Diclofenac Sodium
100	100	25.74	18.56	1500	1700
200	200	34.25	24.79	1400	1600
300	300	41.07	31.73	1300	1500
400	400	50.89	37.7	1300	1400
500	500	57.36	44.91	1200	1300
600	600	64.9	50.05	1100	1300
700	700	70.41	56.52	1000	1200
800	800	76.28	62.75	900	1200
900	900	83.83	69.1	800	1100
1000	1000	89.1	78.44	700	1000
Control 2100					

All values are mean ± S.D (NMT 2%).

Table 5: Effect of acyclovir on membrane stabilization.

Concentration		% Inhibition of Haemolysis		Viscosity	
Acyclovir	Diclofenac Sodium	Acyclovir	Diclofenac Sodium	Acyclovir	Diclofenac Sodium
100	100	9.94	18.56	2500	1800
200	200	13.53	24.79	2400	1700
300	300	15.44	31.73	2400	1600
400	400	17.36	37.7	2300	1500
500	500	27.66	44.91	2300	1400
600	600	24.19	50.05	2200	1300
700	700	25.5	56.52	2200	1200
800	800	26.52	62.75	2100	1200
900	900	28.14	69.1	2100	1100
1000	1000	28.62	78.44	2000	1000
Control		2100			

All values are mean ± S.D (NMT 2%).

Table 6: Effect of combination of extract and acyclovir on membrane stabilization.

Concentration Ratio (Cynodon Dactylon :Acyclovir)	% Inhibition of Haemolysis	Viscosity (Cp)
0.479166667	45.5	1400
0.888888889	62.63	1300
30:70	69.9	1200
40:60	81.79	1100
50:50:00	87.3	1000
60:40:00	88.74	900
70:30:00	89.72	800
80:20:00	91.49	700
90:10:00	90.17	600
Control		2100

All values are mean ± S.D. (NMT 2%).

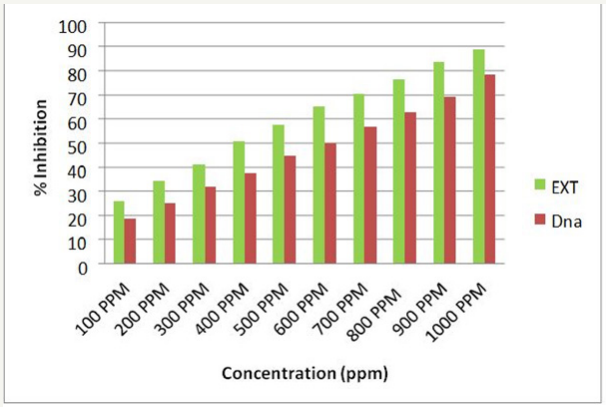

Figure 3: Graph showing % inhibition of haemolysis activity of *Cynodon dactylon* extract

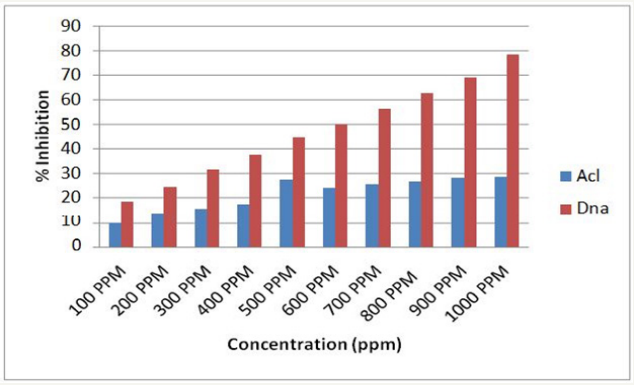

Figure 4: Graph showing % inhibition of haemolysis activity of *Cynodon dactylon* extract

Protein denaturation test showed highest % inhibition found at concentration ratio of extract (1000ppm): acyclovir (500ppm) [80:20v/v] and membrane stabilization test highest % inhibition of haemolysis found at concentration ratio of extract (1000ppm): acyclovir (500ppm) [80:20v/v] [8].

Table 7: Results of qualitative phytochemical tests.

Sr. No	Test	Observations
1	Test for Alkaloids	(+)
2	Test for carbohydrate	(+)
3	Test for Flavonoids	(+)
4	Test for Glycosides	(+)
5	Test for Proteins	(+)
6	Test for Phytosterol	(+)
7	Test for Tannins	(-)
8	Test for Saponins	(-)
9	Test for Fixed oil and Fats	(-)

(+): Presence; (-): Absence.

In addition that, dried powdered samples were subjected to qualitative tests for the identification of phytochemicals constituents according to standard procedures. The preliminary phytochemical investigation showed the presence of phyto constituents such as alkaloids, carbohydrates, flavonoids, glycosides, proteins, phytosterol were observed in *Cynodon dactylon* methanolic extract (Table 7).

So from the results of our study reveals that extract of *Cynodon dactylon* and Acyclovir in combination at the ratio 80:20 are capable of control the inhibition of denaturation of albumin and membrane lysis in rheumatic disease. Our present studies indicate that extract and acyclovir in combination exhibits strong anti-inflammatory property could be potential source of anti-inflammatory property. The inhibition of albumin denaturation and membrane stabilization was studied to establish the mechanism of anti-inflammatory activity of *Cynodon dactylon* and Acyclovir in combination showing synergistic effect. Therefore, our *in vitro* studies on extract of *Cynodon dactylon* demonstrate the significant anti-inflammatory activity. The results show that the extracts of Cynodo dactylon exhibited anti-inflammatory activities might be due to the presence of active principles such as polyphenolic content, alkaloids and flavonoids [9]. From the results of the study, it can be concluded extract of *Cynodon dactylon* and Acyclovir in combination possessed anti-inflammatory property.

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References

1. Vadivu R, Lakshmi KS (2008) *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) Moore ssp laurina. Bangladesh Journal of Pharmacology 3(2): 121-124.
2. Lee SJ, Chinen J, Kavanaugh A (2010) Immunomodulator therapy: monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins. J Allergy Clin Immunol 125(2 Suppl 2): S314-S323.
3. Mythilypriya R, Shanthi P, Sachdanandam P (2008) Efficacy of Siddha formulation Kalpaamruthaa in ameliorating joint destruction in rheumatoid arthritis in rats. Chem Biol Interact 176(2-3): 243-251.
4. Berbert AA, Kondo CRM, Almendra CL, Matsuo T, Dichi I (2005) Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. Nutrition 21(2): 131-136.
5. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Chungag BNN, et al. (2010) An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. Journal of Medicinal Plants Research 4(9): 789-795.
6. Chaudhary J, Garland WA (2012) Chemical inhibitors of inhibitors of differentiation. Google Patents.
7. Sostres C, Gargallo CJ, Lanas A (2013) Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. Arthritis Res Ther (15 Suppl 3): S3.
8. Dhikav V, Singh S, Pande S, Chawla A, Anand KS (2003) Non-steroidal drug-induced gastrointestinal toxicity: mechanisms and management. J Indian Acad Clin Med 4(4): 315-322.
9. Kokate CK, Purohit AP, Gokhale SB (2010) Pharmacognosy. Niraliprakashan, Pune, India, 3: 3.70-3.71.