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In Vitro Anti-Diabetic and Anti-Arthritic Activity of Hydro Ethanolic Extract of *Cynodon Dactylon*

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A B S T R A C T

Objective: The present study is aimed to evaluate the *in-vitro* anti-diabetic and anti-arthritic activity of hydro ethanolic extract of whole plant of *Cynodon dactylon*. **Methods:** Non-enzymatic glycosylation of hemoglobin method, Glucose uptake in yeast cells method, and Alpha-amylase inhibition assay method were performed for *in-vitro* anti-diabetic activity using metformin as standard drugs. Inhibition of protein denaturation method using diclofenac as a standard for *in-vitro* anti-arthritic activity. **Results:** The concentration of 10mg/ml of hydro ethanolic extract of plant material showed the maximum results as compared to standard drugs used. **Conclusion:** As per the results obtained in the present investigation indicates that the hydro ethanolic extract of whole plant of *Cynodon dactylon* is having potential anti-diabetic and anti-arthritic activity.

Keywords: *Cynodon dactylon*, Anti-diabetic, Anti-arthritic, Diclofenac sodium, Metformin.

A R T I C L E I N F O

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1. Introduction

Diabetes is a medical syndrome described by hyperglycemia due to absolute or relative deficiency of insulin with interruptions of carbohydrate, fat and protein metabolism resulting from deficiency in insulin secretion or

insulin action, or both (1).Wide-reachingly, the diabetic population is rapidly increasing particularly in the developing countries (2). The current worldwide diabetic population is about 200 million, and this will be doubled after few years (3). It is caused by shortage or ineffective production of insulin by pancreas which outcomes in increase in concentrations of glucose in the blood. It is found to damage many of the body systems, particularly the blood vessels of eye (retinopathy), kidneys (nephropathy) along with affecting the nerves (neuropathy) (4).

Arthritis is one of the most familiar disorders related to musculoskeletal system that may affect many tissues and organs but primely targeting synovial joints.Amongvarious types of arthritis osteoarthritis and rheumatoid arthritis are commonly occurring ones(5). Osteoarthritis is degenerative disorder of joints. Rheumatoid arthritis is an autoimmune disorder affecting joints on both sides of the body like both hands, both wrists, and both knees.It is a manifold condition that leads to pain, swelling, stiffness, loss of utility in joints(6). It is a familiar disease having maximum frequency in 3rd to 4th decades of life with 3-4 times higher predominance in female(7).The assorted pro inflammatory molecules which include reactive oxygen species, leukotrines, prostaglandins and cytokines released by macrophages are engaged in the occurrences of this disorder. The regulation of these mediators produced by macrophages and other immune cells and modulation of metabolism arachidonic acid by inhibiting enzymes like COX and LOX are the prospective target for conditions of chronic inflammations(8).

The commonly used drugs for treating diabetes are Insulin analogues, sulfonyl urea's and biguanides etc., which have several adverse effects and in case of arthritis treatment even though various categories like immune suppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, they proffer only short-term relief and produce relentless side effects including bleeding of gastrointestinal system , renal diseases and cardiovascular toxicity which limit their helpfulness in the treatment of the disease(8).

To overcome these problems the world has started looking back into our ancient medical history which mostly includes the herbal medicines. Herbal drugs comprise a major part in all traditional system of medicine and are an achievement of popular therapeutic assortment (9). The bioactive compounds of herbal plants are used as anti diabetic, chemotherapeutic, anti inflammatory, anti arthritic agents where no suitable cure is present in modern medicines (10). The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easily available, lucrative, less toxic effects (11). A number of investigations confirmed that agents derived from plants can be used in traditional medicine and many of the plants were found with good antidiabetic and anti arthritic activity (12). Our work is based on the study of one of the indigenous plant which shows inhibitory effect on glucose utilization, and is in use as a hypoglycemic agent and is claimed to be effective in rheumatism (7) in traditional system of medicine. Hence, the present study was undertaken to evaluate *in vitro* antidiabetic and antiarthritic activity of whole plant extract of *Cynodon dactylon* (CD).

Cynodon dactylon of family Poaceae is a hardly perennial grass, is one of the most common occurring weeds in India. The plant is folk remedy for cancer, carbuncles, cough, and snakebites, anti inflamatory,gout and rheumatic affections(8). Compounds include flavanoids, phenols, saponins and cyanogenic glycosides (13). Cynodon dactylon also claimed to shows various pharmacological activities like anti-inflammatory, antidiabetic, antibacterial, antidiarrheal, hepatoprotective, CNS depressant, antifungal, antiulcer, antioxidant, anticancer(14).

2. Materials and Methods

Collection of plant material

The whole parts of *Cynodon dactylon* was collected from medicinal garden and was dried for seven days and powdered, stored in air tight containers for further studies**.**

Preparation of plant extract

The whole plant of *Cynodon dactylon* was coarsely powdered material and 50 gms powder was soaked in 500 ml of hydro ethanol (50:50) and kept under maceration for 72 hrs at room temperature. The extract was concentrated to ¼ of its original volume by distillation as it was adapted to recover low temperature. The extract was then weighed and the percentage of extractive value was calculated in terms of air dried weight of plant material.

Drugs and chemicals

Glucose, Hemoglobin, Gentamycin, Phosphate Buffer, Ascorbic acid, Yeast, Alpha-amylase, Acetate buffer, Metformin, Diclofenac sodium, Potato starch, Iodine-iodide indicator.

Phytochemical Screening: For preliminary phytochemical analysis the freshly prepared crude ethanolic extracts of leaves were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids and alkaloids by using standard phytochemical screening procedures(15).

Methodology

Assessment of *in-vitro* Anti-diabetic activity

i) Non –enzymatic glycosylation of haemoglobin assay method: To 1 ml of haemoglobin solution, 1 ml of gentamycin solution and 1 ml of different concentrations of plant extracts (2,4,6,8,10µg/ml) were added. The reaction was started by the addition of 1 ml of 2% glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature. The concentrations of glycosylated haemoglobin at the incubation period of 0, 24 and 72 hrs were estimated in colorimeter at 520 nm. Here metformin is taken as a standard drug. % inhibition was calculated as previously published protocol (16).

ii) Glucose uptake in Yeast cells method

The commercial baker's yeast (10 gms) is dissolved in 100 ml of distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts ((2,4,6,8,10µg/ml) were added to 1ml of glucose

solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension followed by vortex and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 rpm for 5 min) and amount of glucose was estimated in the supernatant and is estimated by glucometer. Metformin was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using previously published protocol (17)

iii) Inhibition of alpha amylase enzyme assay method

1ml of substrate-potato starch (1% w/v), 1ml of drug solution of 5 different concentrations such as 2, 4, 6,8,10µg/ml, 1ml of α- amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2pH) was added. The mixture was incubated for 1hr.then 0.1 ml iodine-iodide indicator (635mg iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565nm in colorimeter. The percentage increase of α-amylase inhibition were calculated using previously published protocol (18).

Assessment of in-vitro Anti-arthritic activity

i) Protein Denaturation Method

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin (from hens egg), 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of 2, 4, 6, 8,10µg/mlof extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 370 C in BOD incubator for about 15 mins and then heated at 700 C for 5 mins. 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 as mentioned in previously published protocol (19).

3. Results and Discussion

3.1. Chemistry

The preliminary Phytochemical screening tests for the ethanol extract of *Cynodon dactylon* leaves (Table 1) revealed the presence of carbohydrates, alkaloids, flavones, tannins, steroidal glycosides, phenols,saponins, proteins, etc. Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic and anti-arthritic activity of the plant. Anti-diabetic and anti-arthritic effect of *Cynodon dactylon* was studied significantly by testing various in-vitro parameters. At different concentrations of plant extract (2, 4, 6, 8, 10 mg/ml) provided the significant action against non-enzymatic of haemoglobin method, glucose uptake method, alpha-amylase method and denaturation of protein.

DISCUSSION

Diabetes is a today a foremost degenerative disease in the world affecting at least 20 million people and is having complications like hypertension, atherosclerosis, microcirculatory disorders, etc. Plant materials remain an important resource to challenge serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries [(20)].

In earlier studies,the aqueous extract of *Cynodon dactylon* had significant antidiabetic potential along with significant hypoglycemic and Hypolipidemic effects. Different doses of aqueous extract of *C. dactylon* significantly decreased the blood glucose, urea, serum cholesterol, serum triglycerides, creatinine, total heamoglobin, glycosyalated heamoglobin.

In-vitro non-enzymatic glycosylation of haemoglobin method is not significant to detect diabetes but is more essential to evaluate the control of diabetes. The haemoglobin present in the RBC (red blood corpuscles) has an affinity to get bound to glucose and form glucose-haemoglobin complex. The higher the blood-glucose concentration, the higher is the amount of glucose-bound (called glycosylated) haemoglobin. Thus the glycosylated haemoglobin amount is a definite guide to the concentration of glucose in the blood. Amount of Glycated haemoglobin should not cross 12%. Our study reveals promising activity of C.N in avoiding the binding of glucose to surface proteins of erythrocytes mainly due to their antioxidant properties (21). The percentage inhibition of glycosylation is dose dependent, as dose increases, inhibition increases. Because as the drug concentration increases formation of complex of glucose-haemoglobin decreases and free haemoglobin increases, which show the inhibition of glycosylated haemoglobin The activity of test compound was found to be better as that of standard drug metformin.

In the Glucose uptake in Yeast cells method the process of transport of glucose across the membrane of the yeast cell has been being paid attention as invitro screening process for hypoglycaemic effect of various drugs and medicinal plants. Current studies on the non metabolizable sugars transport and certain metabolizable glycosides put forward that transport of sugar across the membrane of yeast cell is mediated by stereospecific membrane carriers. It is reported that in yeast cells (Saccharomyces cerevisiae) transport of glucose is intensely complex and it is generally accepted that glucose is transported in yeast is by process of facilitated diffusion. Facilitated carriers are particular carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose. The amount of remaining glucose in the medium after a particular time offers as an indicator of the uptake of glucose by the yeast cells(21). Results indicated that test drug *Cynodon dactylon* had greater efficiency in increasing the glucose uptake by yeast cells as much as that of standard drug metformin.In α-Amylase inhibition process, the Alpha amylase is an enzyme that hydrolyses alpha bonds of large alpha linked polysaccharide like glycogen and starch to give up glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of these polysaccharides and avoid break down of polysaccharide in mono and disaccharide and the formation of starch – iodine complex due to inhibition of α- amylase. The *Cynodon dactylon* showed a good result in α-amylase inhibition assay, implying that *Cynodon dactylon* might be effective in breaking down starch to minimized availability of glucose(21). The results infer that the *Cynodon dactylon*

may have noteworthy effect in maintaining postprandial glucose concentration. Some literature have reported that protein denaturation is one of the source of rheumatoid arthritis (22). Auto-antigens production in rheumatic diseases may be due to in vivo denaturation of proteins. Mechanism of denaturation possibly involves modification in electrostatic, hydrophobic, hydrogen and disulphide bonding. Numerous anti-inflammatory drugs have shown dose dependent capability to inhibit thermally provoked protein denaturation (23). In our present study, ethanolic extract of *Cynodon dactylon* inhibited heat induced protein denaturation and might be one of the reason of acquiring anti-inflammatory and anti-arthritic activity. The ethanolic extract of *Cynodon dactylon* is significant antiinflammatory activity at the concentration of 10 mg which is comparable to the standard drug diclofenac sodium. The anti-inflammatory activity of the extracts with increase in concentration the activity is also increased.

Table1: Antimitotic activity of the compounds (7a-e) by onion root tip method

Compound	**Concentration in ml/mg**	**Total number of dividing cells**	**Total number of cells**	**% dividing cells**	**average**	**% of dividing cells compare to control**	**% of inhibition compare to control**	**ID50 mmol/L x10^{-6}**
Control solution (1.2% alcohol)		55 36 48	210 216 222	26.19 16.66 21.62	21.493	100	0	0.0
1	1.44	18 46 52	202 230 245	8.911 20.00 21.22	16.71	77.75	22.25	3.24
7a	1.19 2.34 3.50	6 9 8	158 169 122	3.792 5.325 6.557	5.226	25.979	74.021	1.522
7b	1.25 2.50 3.60	18 25 48	281 343 514	6.405 7.288 9.338	7.677	38.163	61.837	1.986
7c	1.30 2.52 3.93	34 40 45	510 341 489	6.666 8.797 9.202	8.221	38.253	61.74	2.091
7d	1.17 2.35 3.52	6 9 4	158 169 122	5.555 5.194 7.633	4.133	19.232	80.767	1.452
7e	1.19 2.38 3.57	7 8 10	126 154 131	6.382 8.928 6.159	6.127	30.408	69.592	1.709

Table 1: Phytochemical Screening

S.No	**Phytochemical constituents**	**Test**	**Results for ethanolic solvent**
1.	Alkaloid	Dragendroffs test Wagners test Mayers test	+ + + + ++
2	Cardiac glycoside	Keller kiliani test	+ +
3	Phenolic compounds and flavonoids	Magnesium-HCL Gelatine test	+ + + +
5	Terpenoid and Steroid	Salkowski test	+ +
6	Saponins	Frothing test	+ +
7	Carbohydrates	Benedict test	+ +
8	Proteins	Biuretic test	_ _

Note: (+)= Present ; (-) = Absent

In-Vitro Antidiabetic Activity

Table 2: Non-Enzymatic Method of *Cynodon Dactylon*

control	**concentration of extracts mg/ml**	**absorbance of extract**	**absorbance of standard**	**% inhibition of extract**	**%inhibition standard**
0.041	2mg/ml	0.041	0.031	0 %	24.39%
	4mg/ml	0.027	0.031	34.14%	24.39%
	6mg/ml	0.023	0.023	43.90 %	43.90%

	8mg/ml	0.014	0.014	65.85%	65.85%
	10mg/ml	0.014	0.005	65.85%	87.80%

Table 3: Glucose uptake in yeast cells of *Cynodon dactylon*

Control	concentration of extracts mg/ml	Glucose conc of standard	Glucose conc of extract
430mg/dl	2mg/ml	269mg/dl	289mg/dl
	4mg/ml	267mg/dl	280mg/dl
	6mg/ml	256mg/dl	275mg/dl
	8mg/ml	242mg/dl	261mg/dl
	10mg/ml	230mg/dl	250mg/dl

Table 4: Alpha-amylase inhibition activity of *Cynodon dactylon*

Control	concentration of extracts mg/ml	absorbance of extract	absorbance of standard	% inhibition of extract	%inhibition standard
0.061	2mg/ml	0.037	0.031	39.34%	49.18%
	4mg/ml	0.027	0.023	55.73%	62.29%
	6mg/ml	0.023	0.017	62.29%	72.13%
	8mg/ml	0.023	0.014	62.29%	77.04%
	10mg/ml	0.009	0.009	85.24%	85.24%

***In-vitro* Anti- Arthiritic Activity**

Table 5: Protein Denaturation method

control	concentration of extracts mg/ml	absorbance of extract	absorbance of standard	% inhibition of extract	%inhibition standard
0.0706	2mg/ml	0.041	0.037	41.42%	47.14%
	4mg/ml	0.031	0.031	55.71%	55.71%
	6mg/ml	0.027	0.0177	61.42%	75.71%
	8mg/ml	0.0178	0.008	75.71%	88.57%
	10mg/ml	0.0088	0.005	88.57%	92.85%

4. Conclusion

Finally it can be concluded that the hydroethanolic extract of *Cynodon dactylon* showed considerable antidiabetic and antiarthritic activity as compared to the respective standard drugs. The underlying reason behind this marked activity may be related to its significant antioxidant, anti-inflamatory, hypoglycemic and in vitro alpha amylase inhibiting activities. However more comprehensive in vitro and in-vivo studies are needed to elucidate the exact mechanism of its antidiabetic and anti arthritic activity. Further, the active principle responsible for such activities is required to be isolated and studied.

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