Assessment of Antihyperlipidemic Activity of Cynodon dactylon

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Abstract:
Cynodon dactylon (L) Pers, family-Poeace, is a perennial herb found in various regions of India. It has different names in different Indian languages such as Durva (Marathi), Durba (Bengali), Dhro (Gujarati), Garichgaddi (Telugu), Arukampillu (Tamil), Shataparva (Sanskrit) etc. Cynodon dactylon occupies a key position in ethno medicinal practices and traditional systems of medicine. It has vast medicinal value and it is used in the treatment of various diseases in the form of its powder, paste or juice. Cynodon dactylon contains many metabolites notably proteins, carbohydrates, minerals, flavonoids, carotenoids, alkaloids, and glycosides. This review attempts to encompass the available literature on Cynodon dactylon with respect to its pharmacognostic characters, traditional uses, chemical constituents, summary of its various pharmacognostic and pharmacological activities and a brief review on patents associated with it. The aim of the present study was to investigate the potential role of an ethanolic extract of the entire plant of Cynodon dactylon in lowering the plasma lipid parameters in rats fed a high cholesterol diet. Wistar albino rats were randomly divided into four groups of six and for 45 days were administered either: 0.5 ml water (negative controls); 30 mg cholesterol (hypercholesterolemic animals); C dactylon extract at 400 mg/kg body weight (positive control); or the same doses of both cholesterol and the extract (test animals). The effects of C dactylon on the lipid profile were assessed by measuring the plasma concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and very low-density lipoprotein cholesterol (VLDL-c). These results suggest lipid-lowering effects of C dactylon, which serves as a new potential natural product for preventing hyperlipidemia.

Keywords: cholesterol, Cynodon dactylon, hyperlipidemia, lipid profile.

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Introduction:
According to an estimation of the World Health Organization, about 80 percent of the world’s population uses herbs to fulfil its primary healthcare needs. More than 35,000 plant species are being used around the world as medicinal plants in traditional and ethno medicinal practices. Among numerous species...
of plants growing in India, Durva or taxonomically the Cynodon dactylon occupies a key position in ethno medicinal practices and traditional medical knowledge systems (Ayurveda, Unani, Nepalese, and Chinese) [1]. Durva consists of dried whole plant of Cynodon dactylon (Linn.) Pers. (Family: Poaceae), an elegant, tenacious, perennial, creeping grass growing throughout the country.

Cynodon dactylon possesses immense medicinal value and may be applied both externally as well as internally [2]. The plant possesses antiviral and antimicrobial activity [3]. Decoctions of root are used in secondary syphilis and irritation of urinary organs [4]. The plant is astringent, sweet, cooling, haemostatic, depurative, vulnerary, constipating, diuretic and tonic and is useful in impaired conditions of pitta and kapha, hyperdipsia, burning sensation, haemoptysis, haematuria, haemorrhages, wounds, leprosy, diarrhoea, dysentery, conjunctivitis, vomiting etc. [5]. The plant is a folk remedy for snake bites, gout, and rheumatic affections [6]. Its anthelmintic activity has been successfully investigated [7].

Apart from this, it also possesses anti-inflammatory activity [8]. Three varieties namely ‘nildurva’ with bluish or greenish stem, ‘shvetadurva’ with whitish stem and branches and ‘gandadurva’ with nodulose stem are mentioned in ‘Bhavaprakash nighantu’ [9]. C. dactylon is found in warm climates all over the world between 45° south and north latitudes. It is available throughout the year [10].

**Taxonomical classification of Cynodon dactylon:**

Kingdom-Plantae  
Division-Magnoliophyta  
Class-Liliopsida  
Order-Cyperales  
Family-Poaceae  
Genus-Cynodon  
Species-dactylon

**Phytochemistry**

C. dactylon contains 28.17% enzymes, 11.79% ash, 10.47% Proteins. Ash contains 0.77% calcium, 0.58% phosphorus, 0.34% manganese, 0.23% sodium, 2.08% potassium. Dry grass contains per 400 grams 36.16% carbohydrate, 6.04% proteins. It contains phenolic phytotoxins viz. ferulic, syringic, para coumaric, vanillic, para hydroxyl benzoic and ortho hydroxy phenyl acetic acid [11] [13]. Flavonoids and glycosides were found to be present in the aqueous extract of C. dactylon while alkaloids, glycosides and flavonoids were reported to be present in ethanol extract of the plant [8]. Other compounds like vitamin C, β carotene, fats, palmitic acid etc. have also been reported [12]. Analysis of leaves of C. dactylon by GC-MS technique revealed that C. dactylon leaves contain glycerin (38.49%), 9, 12-Octadecadienoyl chloride, (Z, Z)-(15.61%), hexadecanoic acid, ethyl ester (9.50%), ethyl -d-glucopyranoside (8.42%), linoleic acid, ethyl ester (5.32%), and phytol (4.89%) [13]. Chemical structures of few important constituents are shown in Figure 1.

![Chemical structures](image-url)
Materials and Methods:

Plant material
The plant samples of were harvested in Botanical Garden, Oriental University, Indore, M.P., India. The collected plants were identified and authentication was done by Dr. S. N. Dwivedi, HOD, Dept. of Botany, Janta PG College, A.P.S. University, Rewa, M.P., India. The whole green plant was washed with water and shade dried for 3e5 days. Five hundred grams were macerated with ethanol (80% v/v) and kept for 48 hours at room temperature (28e30◦C). The extraction was filtered and the filtrate evaporated to dryness under reduced pressure at 50◦C (yield 15.6% w/w, dry weight basis) and stored at 4◦C until use. The obtained C dactylon ethanol extract was stored at e20◦C until use.16 Extract preparation was done at the Manipal College of Pharmaceutical Sciences, Manipal University, Manipal. The extract was dissolved in distilled water to a concentration of 100 mg/ml.

Phytochemical screening
Chemical tests were carried out on ethanolic extracts of C dactylon using standard procedures to identify the constituents as described by Sofowora, [18] Trease and Evans [19] and Harborne [20].

Alkaloids: About 0.2 g of the extract was warmed with 2% H2SO4 for 2 minutes. It was filtered and a few drops of Dragendorff’s reagent added. An orange-red precipitate indicates the presence of alkaloids.

Tannins: A small quantity of extract was mixed with water and heated in a water bath. The mixture was filtered and ferric chloride added to the filtrate. A dark-green solution indicates the presence of tannins.

Glycosides: The extract was hydrolyzed with dilute HCl and neutralized with NaOH solution. A few drops of Fehling’s solution A and B were added. A red precipitate indicates the presence of glycosides.

Flavonoids: About 0.2 g of extract was dissolved in NaOH solution and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

Saponins: About 0.2 g of extract was shaken with 5 ml of distilled water and then heated to boiling. Frothing of the extracts shows the presence of saponins.

Animals and Preparation of Model
Twenty-four albino rats of both sexes (Wistar strain) weighing 120e130 g (6–7 weeks old, weighing 18-20 g) were purchased from Experimental Animal of university animal house. All animals were kept under standard environmental conditions of temperature (25±2°C), relative humidity (40–70%), and 12/12-h dark/light cycles. All animals had free access to pellet food and water ad libitum. All animals (including the mice euthanasia procedure) were approved by the animal ethics committee of the Second affiliated hospital of BR Nahata University, Mandsaur M.P., India according to the IACUC guidelines. In addition, maximum efforts were made to minimize pain and suffering of the animals.

Cholesterol supplementation in the rat basal diet
Cholesterol powder was purchased from HIMEDIA Laboratories (Mumbai, India), and supplemented to the basal diet of rats, to
induce hyper cholesteremia. [15]

**Experimental design**
The effect of *C. dactylon* plant extract on normal and cholesterol-fed rats was studied. All the rats received treatment for 45 days and were randomly distributed into four groups of six animals each. Group I served as a control rats (administered with 0.5 ml distilled water); Group II rats received dietary supplementation of cholesterol (hyper-cholesteremic rats); rats in Group III served as a positive control and were given *C. dactylon* at a dose of 400 mg/kg body weight; and Group IV rats were administered *C. dactylon* extract dose of 400 mg/kg body weight, in addition to oral administration of cholesterol. After overnight fasting the rats were sacrificed by administering sodium pentobarbitone; 40 mg/kg body weight. Blood samples were collected directly via cardiac puncture using 23G needles and 3-ml syringes and collected into EDTA tubes (Sigma Chemicals, Gillingham, Dorset, UK). The plasma was immediately separated by centrifugation at 3000 rpm (relative centrifugal force, approximately 1,500 g) for 10 minutes. They were then transferred into microcentrifuge tubes and stored under e80°C until analysis. Samples were analyzed spectrophotometrically: TG21 was estimated by the GPO-POD method, TC22 by the CHOD-PAP method, and high-density lipoprotein (HDL) was analyzed using kits (Roche Diagnostics, Mannheim, Germany). The concentration of very low-density lipoprotein cholesterol (VLDL-c) was estimated according to Fridewald’s equation: 23 $VLDL-C = \frac{\text{triglycerides}}{5}$ According to Fridewald [23] low density lipoprotein cholesterol (LDL-c) can be calculated as follows: $\text{LDL-c} = \text{Total cholesterol} - (\text{HDL-c} + \text{VLDL-c}).$

### Table 2: Effect of *C. dactylon* extract on serum lipid profile in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>85.83 ± 4.92***</td>
<td>156.50±11.10xxx</td>
<td>83.2 ± 2.23NS</td>
<td>92.2 ± 6.46</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>116.0 ± 13.4***</td>
<td>212.66±8.94xxx</td>
<td>122.0 ±11.7NS</td>
<td>95.8 ±2.23</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>57.33 ± 10.00***</td>
<td>127.33±9.42xxx</td>
<td>52.3 ± 12.3NS</td>
<td>72.2 ±7.08</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>51.20 ± 9.07***</td>
<td>72.2 ±12.62xxx</td>
<td>55.2 ±13.8NS</td>
<td>40.2 ± 2.99</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dL)</td>
<td>23.20 ± 2.69***</td>
<td>42.5 ±1.79xxx</td>
<td>24.4 ± 2.34NS</td>
<td>19.2 ±0.44</td>
</tr>
<tr>
<td>TC/HDL Ratio</td>
<td>1.512 ± 0.013**</td>
<td>1.218 ±0.018xxx</td>
<td>1.535 ±0.024NS</td>
<td>0.635 ±0.20</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
Group I- Control rats (administered with 0.5 ml distilled water); Group II- Hypercholesterolemia induced rats; Group III- Rats administered with *C. dactylon* at a dose of 400 mg/kg body weight; Group IV- Rats administered with *C. dactylon* extract dose of 400 mg/kg body weight, in addition to oral administration of cholesterol. Group I versus Group II- **p < 0.05; ***p < 0.001; Group II versus Group IV: xxxp < 0.001; Group I versus Group III- NS Z not significant.

**Statistical Analysis**
The results were presented as means ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls test or Dunnett’s T3 test. All data was performed by using the SPSS 19.0 software (IBM, Armonk, NY, USA). Differences between groups were considered statistically significant when P < 0.05.

**Results**
Analysis of the phytochemical constituents of an ethanol extract of the entire *C. dactylon* plant...
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Discussion
In developing countries, the incidence of CVD is increasing at an alarming rate, and India is on the verge of a cardio-vascular epidemic. [25,26] The presence of a high amount of cholesterol in the diet has been demonstrated to elevate total cholesterol and may increase the risk of cardiovascular complications. Agents that can lower serum cholesterol and scavenge or inhibit free radical formation have gained wide therapeutic value. Great efforts have been made to reduce the risk of CVD through the regulation of cholesterol, and the therapeutic benefits of plants have been the focus of many extensive dietary study. [27, 28]

In the present study, we investigated the lipid-lowering effect of C dactylon in rats fed a high-cholesterol diet for 45 days. Notably, the rats intubated with the dietary cholesterol showed a significant increase in the circulating total cholesterol, LDL-cholesterol, VLDL cholesterol, and in the ratio of TC:HDL-c. These results are consistent with earlier reports [29,30] that established a correlation between dietary lipids and serum lipid profile. Supplemental administration of cholesterol in the diet rapidly results in a marked increase in the production of cholesteryl ester- rich VLDL by the liver and intestine [31] and a reduced rate of cholesterol removal by the hepatic LDL receptors.[31] Consequently serum levels of LDL-c and VLDL-c are increased. A significant increase in the ratio of TC:HDL-c indicates an increased risk of CVDs. [32]

Simultaneous administration of C dactylon extract caused a significant decrease in serum TC, LDL-c, and VLDL-c, suggesting a beneficial modulatory influence on cholesterol metabolism and turnover. The reduction in the ratio of TC:HDL-c observed in the extract-treated rats might be a consequence of a higher proportion of HDL-c, which could be due to increased reverse cholesterol transport from peripheral organs to the liver.[33,34] Elevated serum TG is considered an independent risk factor for CVD [35]. TG accumulation caused by dietary cholesterol may contribute to the reduction of fatty acid beta-oxidation and the preference of cholesterol ester to afflux to LDL during the onset of biosynthesis and secretion of LDL.[36,37] A significant decline in the serum TG concentration observed in extract-treated rats supports the cardiovascular protective influence. The mechanism by which C dactylon extract lowered the serum TG concentration could be either by decreasing VLDL synthesis, by channeling VLDL through pathways other than to LDL, or an increase in lipoprotein lipase activity. Phytochemical studies of this plant have shown the presence of glycosides, flavonoids, alkaloids, tannins, and saponins. The observed hypolipidemic effect might be due to individual or synergistic action of these components, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. Alternatively, the components might exert a modulatory influence on lipogenic enzymes or by inhibition of cholesterol absorption. [38,40]

Based on the present results we suggest that C dactylon might elicit beneficial effects by lowering the plasma lipid levels of the treated rats. This is a preliminary study; it is among the earliest reported work regarding the anti-hyperlipidemic activity of C dactylon. C dactylon has been traditionally used to cure a variety of illnesses. The results of the present study add other beneficial effects. Further studies are required to gain more insight into the mechanism of hypolipidemic action.

Conclusion
Evidence from this study confirms the lipid-lowering effects of C dactylon in rats fed a high-cholesterol diet. The active components in the C dactylon might cause a decrease in
the serum lipid profile. Further studies are warranted to determine the exact mechanism leading to the observed effect; the component responsible may be a candidate for use as a prophylactic agent against hypercholesterolemia.

**Ethical Approval and Consent Participate**

The animal experimental procedures were approved by the animal ethics committee of the B.R. Nahata College of Pharmacy, Mandsaur M.P., India as per IACUC guidelines. In addition, maximum efforts were made to minimize pain and suffering of the animals.

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**References:**

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