Antihyperlipidemic activity of *Cynodon dactylon* extract in high-cholesterol diet fed Wistar rats

S. Rashmi Kaupa, Nayanatara Arunkumarb,*, Leigelin Kavitha Bernhardt a, Rakesh Gorantla Vasavii, Sandeep Sanjeev Shettyd, Sheila Ramesh Pai b, B. Arunkumare

a Department of Physiology, International Center, Kasturba Medical College (Manipal University), Manipal, Karnataka, India
b Department of Physiology, Center for Basic Sciences, Kasturba Medical College, Bejai (Manipal University) Mangalore, Karnataka, India
c Department of Anatomy, International Center, Kasturba Medical College (Manipal University) Manipal, Karnataka, India
d Department of Pharmacology, Manipal College of Pharmaceutical Sciences (Manipal University) Manipal, Karnataka, India
e Department of Chemistry, Mahatma Ghandhi Memorial College, Udupi, Karnataka, India

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Abstract  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). Administration of cholesterol showed significant elevation (*p* < 0.001) of TC, LDL-c, VLDL-c, and TG concentrations, and of the TC:HDL-c ratio (*p* < 0.05). Concurrent administration of *C dactylon* extract caused a significant decrease (*p* < 0.001) in the concentrations of serum TC, LDL, HDL, VLDL TGs when compared with cholesterol fed control rats. The TC:HDL-c ratio was also declined significantly (*p* < 0.001). These results suggest

* Corresponding author. Department of Physiology, Center for Basic Sciences, Kasturba Medical College, Bejai, Mangalore-575004, Karnataka, India.
E-mail address: nayanaarun@hotmail.com (N. Arunkumar).

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lipid-lowering effects of *C dactylon*, which serves as a new potential natural product for preventing hyperlipidemia.

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**Introduction**

For centuries, the plant kingdom has been one of the largest areas from which novel medicines are derived. The constituents of plants often have medicinal and research value, such as for treating diseases and understanding basic natural science. Many recent studies have focused on plant extracts in order to find new chemicals that could be beneficial to mankind. *Cynodon dactylon* Pers. (Family: Gramineae, *Durba* in Bengali, *Dhub* in Hindi, Bermuda grass in English) is a creeping grass found in warm climates all over the world between 45° south and north latitudes. *C dactylon* is available throughout the year. The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is also useful in the treatment of catarrhal ophthalmia, dropsy, hysteria, epilepsy, insanity, chronic wounds, and rheumatic infections. It has also antioxidant properties and is used as an anti-inflammatory, diuretic, antiemetic, purifying agent, and also in the treatment of dysentery.

Hyperlipidemia is well known to play a major role in the development of atherosclerosis, and is widely recognized as a risk factor for cardiovascular diseases (CVD) and myocardial infarction, which is a common cause of mortality and morbidity. Chronic elevation of blood lipids may also lead to the development of fatty liver and renal damage, as indicated by the increased concentrations of liver and kidney enzymes. Accumulation of lipids impairs endothelial dysfunction, which can initiate vasoregulation, platelet and monocyte adhesion, vascular smooth muscle cell growth and oxidation of low-density lipoprotein (LDL). Although several factors such as life style, a diet rich in cholesterol, age, and hypertension, have been reported to cause heart failure, hypercholesterolemia due high levels of cholesterol, particularly LDL cholesterol (LDL-c), is mainly responsible for CVD. Hence, decreasing the prevalence of hyperlipidemic conditions is considered to be an important therapeutic approach. Accordingly, efforts have been made to identify the anticholesterol effects of various medicinal plants. Thus in the present study an attempt was made to investigate the hypolipidemic properties of *C dactylon*.

**Materials and methods**

**Plant material**

*C dactylon* was collected from the campus of Kasturba Medical College, Manipal University, Mangalore, India. It was identified and authenticated by a plant taxonomist. The whole green plant was washed with water and shade dried for 3–5 days. Five hundred grams were macerated with ethanol (80% v/v) and kept for 48 hours at room temperature (28–30°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 −20°C until use. 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.

**Acute oral toxicity study**

An acute toxicity study was carried out as prescribed by the Organization for Economic Cooperation and Development guidelines. Prior to experimentation, Wistar rats were fasted overnight (water withheld for 3–4 hours) and fixed *C dactylon* extract doses of 50, 200, 400, and 2000 mg/kg body weight were administered by gavage via canula intubation. The extract was tolerated as no death was found up to the maximum dose administered. Rats were observed individually after dosing for the first 30 minutes periodically, and daily thereafter for 14 days for any toxicity signs such as gross changes in the skin, fur, eyes, mucous membranes, or circulatory, respiratory, autonomic, and central nervous systems, or changes in behavior pattern. On the basis of earlier studies the effective dose of 400 mg/kg body weight was selected for further studies.

**Phytochemical screening**

Chemical tests were carried out on ethanolic extracts of *C dactylon* using standard procedures to identify the constituents as described by Sofowora, Trease and Evans, and Harborne. Alkaloids: About 0.2 g of the extract was warmed with 2% *H*₂*SO₄* for 2 minutes. It was filtered and a few drops of Dragencloffs 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.
procedures were carried out in strict compliance with the with or without cholesterol supplementation. All the animal water. All the rats received approximately 20 g of standard acclimatize, and were allowed free access to food and

Table 1 Phytochemical constituents of ethanolic extract of Cynodon dactylon.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Positive(+) /Negative (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

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.

Experimental animals

Twenty-four albino rats of both sexes (Wistar strain) weighing 120—130 g were obtained from the animal house of Kasturba Medical college (Manipal University), Mangalore. They were housed in the institutional experimental animal laboratory. The rats were kept in cages in a room maintained at 26—29°C with a 12-hour light—dark cycle for 4 weeks to acclimatize, and were allowed free access to food and water. All the rats received approximately 20 g of standard rat pellets (Lipton, India Ltd., Bangalore, India) per day, with or without cholesterol supplementation. All the animal procedures were carried out in strict compliance with the institutional animal ethical committee regulations.

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 hypercholesteremia.15

Experimental design

The effect of C dactylon plant extract on normal and cholesterol-fed rats were 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 (hypercholesterolemic 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 —80°C until analysis. Samples were analyzed spectrophotometrically: TG was estimated by the GPO-POD method, TC 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 = triglycerides/5

According to Fridewald low density lipoprotein cholesterol (LDL-c) can be calculated as follows:

LDL-c = Total cholesterol - (HDL-c) — (VLDL-c).

Statistical analysis

The experimental results were expressed as mean ± SEM. Data were assessed by ANOVA followed by the Student t test. A p-value <0.05 was considered as statistically significant.24 All data analyses were done using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Analysis of the phytochemical constituents of an ethanolic extract of the entire C dactylon plant (Table 1) showed the presence of glycosides, flavonoids, alkaloids, tannins, and saponins. Table 2 shows the values of the serum lipid profile in Groups I—IV. Serum total cholesterol (TC), LDL-c, VLDL-c, and TG concentrations increased significantly (p < 0.001) after 45 days of cholesterol feeding, and the TC:HDL-c ratio was also increased significantly (p < 0.05). Concurrent

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 ± 11.10NS</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 ± 8.94NS</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 ± 9.42NS</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 ± 12.62NS</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 ± 1.79NS</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 ± 0.018NS</td>
<td>1.335 ± 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 — Hypercholesterolemic 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 < 0.001; Group II versus Group IV: NS = not significant.
administration of *C dactylon* extract caused a significant decrease ($p < 0.001$) in the concentrations of serum TC, LDL-c, HDL-c, VLDL-c, and TG, and in the TC:HDL-c ratio when compared with cholesterol-fed control rats.

**Discussion**

In developing countries, the incidence of CVD is increasing at an alarming rate, and India is on the verge of a cardiovascular 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 studies.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 also in the ratio of TC:HDL-c. These results are consistent with earlier reports29,30 that established a correlation between dietary lipids and serum lipid profile. Supplementation of cholesterol in the diet rapidly results in a marked increase in the production of cholesteryl ester-rich VLDL by the liver and intestine31 and a reduced rate of cholesterol removal by the hepatic LDL receptors.32 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 in to 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.

**References**


