HYPOLIPEDEMIC EFFECT OF CYNODON DACTYLON ON HISTOPATHOLOGICAL STUDY AND DNA FRAGMENTATION ANALYSIS IN EXPERIMENTALLY INDUCED HYPERCHOLESTEREMIC RATS

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ABSTRACT

Hypercholesteremia is one of the risk factors for coronary artery disease. The present study highlights the efficacy of Ayurvedic herbal formulation Cynodon dactylon (Bermuda grass) on histopathological study and DNA fragmentation analysis in experimentally induced hypercholesteremic rats. Four groups of rats were employed namely control, hypercholesterolemia rats (4% Cholesterol + 1% cholic acid), Cynodon dactylon treatment in hypercholesteremic rats and Cynodon dactylon alone treated rats. Results of DNA fragmentation was found to be greater in High cholesterol diet fed groups. Lesser fragmentation was found in high cholesterol diet + Cynodon dactylon treatment group when compared to the high cholesterol diet group. Cynodon dactylon alone treated group is comparably similar to that of normal group (lesser fragmentation). Histopathological study of thoracic aorta of Cynodon dactylon treated group shows decrease in atherogenicity compared to untreated high cholesterol diet fed rats. The data demonstrated that Cynodon dactylon formulation was associated with hypolipidemic effects on the experimentally induced hypercholesteremic rats.

INTRODUCTION: Coronary heart disease resulting from progressive atherosclerosis remains the most common cause of morbidity and mortality all over the world 1. In developing countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the verge of cardiovascular epidemics 2, 3. The circulatory system disorders are going to be the greatest killer in India by the end of the year 2015 4.

Hyperlipidemia (mainly increased level of cholesterol or low density lipoprotein (LDL)-cholesterol) is an important risk factor in the initiation and progression of atherosclerotic lesions 5, 6. The beneficial effect of lowering elevated serum cholesterol level in the prevention of coronary heart disease is well established 7, 8. Generally the therapeutic purpose of using hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol established 9. Some of the major limitations in the effective pharmacological treatment of hyperlipidemia are the constraints imposed on healthcare resources, particularly in the low-and middle income countries 10. There is a need to tackle this physiological problem as it is attaining grave proportions globally. In this scenario, the problem may be tackled by the use of natural agents due to their cost effectiveness and minimal side-effects 11. In recent times, much research interest has been focused on various herbs that possess hypolipidemic properties that may be useful in reducing the risk of cardiovascular diseases 12.

Cynodon dactylon Pers. (CD), (Family: Graminae, Dhub in Hindi, Bermuda grass in English) is a creeping grass found in warm climates all over the world between 45° south and north attitude 13. The juice of the plant is astringent and is applied externally to fresh cuts and...
wounds. It is also used in treatment of catarrhal ophthalmia, dropsy, hysteria, epilepsy, insanity, chronic diarrhea and dysentery.

The plant is folk remedy for calculus, cancer, carbuncles, cough, hypertension, snakebites, stones, gout and rheumatic affections. Ethanol extract of aerial parts of *C. dactylon* has also marked CNS depressant and antioxidant activities. Roots and rhizomes of this plant are used in different diseases like chronic diarrhea, inflammation, skin rashes and excess bleeding. It has also antiestrogenic, antimicrobial, anathematic, antihistaminic, antiemetic, antipyretic, antidiabetic and antioxidant activities.

In some provinces of Iran (such as Azerbaijan and Kurdistan), *Cynodon dactylon* which is locally named as Chayer, has been traditionally used for cardiovascular diseases. Many people of these regions believe that the extract of the rhizome has curative effects in coronary artery diseases and in heart failure. In addition, uncontrolled studies by some Iranian cardiologists have shown cardio protective effects in the patients who used the plant traditionally.

Hence, the present study was undertaken to evaluate the hypolipidemic effect of *Cynodon dactylon* in experimentally induced hypercholesteremic rats.

**MATERIALS AND METHODS:**

**Drug and dosage:** The plant material was collected at Chennai, Tamil Nadu and was authenticated by, Captain Srinivasa Murthi Drug Research Institute for Ayurveda, Arumbakkam, and Chennai. The whole plant up to 500g was extracted with boiling water for 48hrs. The resulting extract were filtered and concentrated in Rota vapor under reduced pressure. The concentrated extract was lyophilized to get a powder (yield 15.8% w/v). The lyophilized powder was administered at the dose of 750mg/kg body weight for 45 days.

**Animals:** Healthy male albino rats (200-250g) of Wistar strain were used for the study with the approval of Institute’s animal ethics committee. The animals were purchased from Central Animal House Block, Sri Manakula Vinayagar Medical College & Hospital (SMVMCH). The animals were housed in a large spacious cage, bedded with husk and were given food and water. The animal house was ventilated with a 12hr light/dark cycle, throughout the experimental period. Animal experimentation was conducted according to the current institutional regulations. The animals were maintained on a commercial rat feed manufactured by M/s. Pranav Agro Industries Ltd., India, under the trade name ‘Amrut rat feed’. The feed contains 5% fat, 21% protein, 55% nitrogen free extract, 4% fiber (wt/wt) with adequate vitamin and mineral content.

At the end of the experimental period (45 days), the animals were sacrificed by cervical decapitation. The aorta was excised immediately, washed with cold saline. The liver tissues were stored at -20°C. A 10% homogenate of liver tissue was prepared using 0.01M Tris HCl buffer pH 7.4. The aorta was fixed by 10% formalin for histopathological studies.

**Experimental design:** The animals were divided into four groups of six rats as follows:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
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<tbody>
<tr>
<td>Group I</td>
<td>Control rats fed with normal diet for 45 days</td>
</tr>
<tr>
<td>Group II</td>
<td>Rats fed with hypercholesteremic diet (HCD) for 45 days</td>
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<tr>
<td>Group III</td>
<td>+ administered with CD (750mg/kg body weight) orally for last 30 days</td>
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<tr>
<td>Group IV</td>
<td>Rats administered with normal diet for 45 days + administered with CD alone (750mg/kg body weight) orally for last 30 days</td>
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The high cholesterol diet (HCD) consists of normal rat chow supplemented with 4% cholesterol (w/w) and 1% cholic acid (w/w).

**Histological studies:** For light microscopic studies, the aorta samples of control and test animals which were fixed in 10% formalin, embedded in paraffin, sectioned at 4μm thickness and stained with hematoxylin and eosin. Hematoxylin and eosin stain for aorta has been used in this study was according to culling.

**Electrophoretic detection of DNA fragmentation:** The isolation of DNA from liver was done according to the method of Lwasa et al.

**Spectrophotometric analysis of DNA sample:** The concentration and purity of the DNA samples were checked as follows. To 990μl of sterile distilled water, 10μl of DNA samples was added and mixed uniformly. Using appropriate blank as reference, the DNA was
scanned in 1 cm path length quartz cuvette at 260nm and 280nm in a double beam model Shimadzu, UV-VIS spectrophotometer.

Purity assessment formula = \( \frac{OD_{260}}{OD_{280}} \)

Thus the concentration of the DNA/RNA was calculated by using the above formula.

DNA/RNA concentration (µg/ml)  
= \( \frac{OD_{260} \times 50 \text{ (or } 40) \times \text{ dilution factor}}{1} \)

Where, 50 = Standard quantity of dsDNA/1(OD\(_{260}\)) and 40 = Standard quantity of RNA/1(OD\(_{260}\)).

Dilution factor = 100(10µl made up to 1.0 ml)

DNA samples corresponds to 1 µg of DNA were electrophoresed on 1.0% agarose gel with a constant voltage of 50v for 2 hrs. Gel were stained with ethidium bromide and viewed under UV-transilluminator and photographs were taken using Nikon cool pix 4500, digital camera.

RESULTS:

**FIG. 1:** EFFECT OF CYNODON DACTYLON ON THE HYPERCHOLESTEROLEMIC INDUCED DNA-FRAGMENTATION IN CONTROL AND EXPERIMENTAL RATS

*Fig. 1* shows the agarose gel electrophoretic pattern of DNA fragmentation in control and experimental rats. It was found that that there was a marked increase in the levels of DNA fragmentation in HCD induced rats (lane 2) when compared to control rats. *Fig. 2d* extract treatment restores the HCD induced DNA fragmentation to near control (lane 3). Cynodon dactylon itself had no change in the level of DNA fragmentation when compared with control rats.

**FIG. 2A:** HISTOPATHOLOGICAL STUDY OF AORTA OF CONTROL RATS

*Fig. 2A* showed no pathological changes in rats of control group (100X).

**FIG. 2B:** HISTOPATHOLOGICAL STUDY OF AORTA OF HYPERCHOLESTEREMIC RATS

*Fig. 2B* shows rats fed with an atherogenic diet developed typical plaques characterized by vaccine of thickening of the intima, migration of smooth muscle cells to the intima (shown by arrow mark), adhesion and infiltration of macrophages and mononuclear cells to the intima, appearance of foam cells and punctuate and lamellar calcification under the endothelium are seen (100X).

**FIG. 2C:** HISTOPATHOLOGICAL STUDY OF AORTA OF HYPERCHOLESTEREMIC RATS FED WITH CYNODON DACTYLON

*Fig. 2C* shows the histopathological study of aorta of hypercholesteremic rats fed with Cynodon dactylon.
Fig. 2C shows pathological changes of thoracic aorta in HCD + CD groups were less visible than that in atherosclerotic group, but shown less thickening of the intima and foam cells count (100X).

Oxygen free radicals are reported to be generated during hypercholesterolemic atherogenesis. Oxidative modification of lipid, i.e. oxidized low-density lipoprotein (Ox-LDL) has been suggested to play a critical role in the pathogenesis of atherosclerosis. LDL oxidation plays an important role in plaque growth and degenerative changes leading to cell disruption and death.

Agarose gel electrophoresis of cellular DNA showed ladder pattern (streak) of DNA fragmentation in HCD administered group, this might be due to increased endogenous DNAs activity by ROS which cut the internucleosomal region of liver cell (hepatocytes) into many fragments. This internucleosomal DNA fragmentation will activate caspase, which in turn activates apoptosis from many studies it has been proposed that ROS produced by HCD are known to be potent inducers of cardiac apoptosis.

DNA fragmentation was found to be greater in HCD fed groups, indicating extensive oxidative damage and DNA strand breaks, DNA fragmentation could be observed as discrete bands. Lesser fragmentation was found in HCD + CD treated group when compared to the HCD alone group. Cynodon dactylon alone treated group is comparably similar to that of normal group (lesser fragmentation).

The results of the present study indicate that Cynodon dactylon can reduce the oxidative damage caused by oxidized LDL on arterial walls of HCD rats due to its antioxidant property. Endothelial – intimal thickening and DNA fragmentations are increased in HCD group, which have been reversed to normal by treating with Cynodon dactylon.

Hence, these data suggest that Cynodon dactylon possesses anti-atherogenic properties.

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