

**EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF
DRAKSHARISHTA PREPARED BY TRADITIONAL AND MODERN
METHODS IN HYPERLIPIDEMIC RATS**

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Summary

The objective of the present study was to evaluate the lipid peroxidation activity and related hypolipidemic activity of Draksharishta-T and Draksharishta-M prepared by traditional and modern methods and its marketed formulation in high fat diet induced hyperlipidemic rats. The antioxidant activity of Draksharishta-T and Draksharishta-M was increased in concentration dependent manner. Draksharishta-T and Draksharishta-M inhibited the ferrous sulphate induced lipid peroxidation in a dose dependent manner and showed inhibitory concentration (IC₅₀) value 230.03 and 236.11 µg/ml respectively. Oral administration of Draksharishta-T and Draksharishta-M for nine weeks at the dose of 2 ml/kg significantly reduced serum cholesterol, serum LDL and serum triglycerides while showed significant rise in serum HDL as compared to high fat diet fed control group. Marketed Draksharishta also showed significant decrease in serum cholesterol, serum LDL, serum triglycerides and showed significant rise in serum HDL. Atorvastatin (1.2 mg/kg, p.o.) was used as standard antihyperlipidemic drug. Both types of Draksharishta as Draksharishta-T and Draksharishta-M showed significant reduction in atherogenic index as compared to high fat diet fed control group which strongly supports antiatherosclerotic property of Draksharishta.

Key words: Draksharishta, Lipid peroxidation, Atherogenic index, antihyperlipidemic activity, Atorvastatin

Introduction

The association of raised serum cholesterol and triglycerides with cardiovascular disease is well known. Hypolipidemic drugs are those, which lower the level of lipids and lipoproteins in blood¹. The hypolipidemic drugs have attracted considerable attention because of their potential to prevent cardiovascular disease by retarding the accelerated atherosclerosis in hyperlipidemic individuals which causes hypertension and finally can cause heart attack. This is the second leading cause of death in the world. Heart attack can occur in any person, manifests itself in various ways- as a sudden episode of weakness of half of the body, confusion, slurring of speech, visual disturbances, headache, vertigo, altered consciousness, usually happening altogether².

Draksharishta is a polyherbal hydroalcoholic preparation and is used as blood purifier, in the treatment of anaemia and advised as a choice of remedy in respiratory problems. The chief ingredient of Draksharishta is draksha, dried fruits of *Vitis vinifera*³. The composition and properties of fruits of *Vitis vinifera*, have been extensively investigated and it was reported that they contain large amount of phenolic compounds as catechins, epicatechin, quercetin, and gallic acid, dimeric, trimeric and tetrameric procyanidins⁴. These compounds have many favourable effects on human health such as lowering of human low density lipoproteins, reduction of heart disease and cancer because of their antioxidant property⁵⁻⁸.

Therefore, we undertook the present investigation to evaluate the hypolipidemic effect of Draksharishta prepared by traditional and modern methods, Draksharishta-T and Draksharishta-M respectively in high fat diet induced hyperlipidemic albino rats.

Material and Methods

Preparation of Draksharishta-T

This was prepared by the method as given in Ayurvedic Formulary of India, Part-I³. The ingredients of Draksharishta were procured from Local market, Jamnagar. Identification of all the individual plant material was done as per Ayurvedic Pharmacopoeia of India. Authentication of all these ingredients was carried out by Dr G. D. Bagchi, Scientist, Department of Taxonomy and Pharmacognosy, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. Prepared herbarium has been deposited in the CIMAP for future reference.

According to this method, dried fruits of *Vitis vinifera* after proper crushing were placed in polished vessel of brass along with prescribed quantity of water (13L), and allowed to steep overnight. After overnight steeping, this material was warmed at medium flame until the water for decoction reduced to one fourth of the prescribed quantity (3.25L), then the heating was stopped and it was filtered through unstarched muslin cloth in cleaned and fumigated vessel and after that jaggery was added and mixed properly. Then Dhataki flowers (*Woodfordia floribunda*) and prescribed quantity of coarsely powdered prakshepa dravyas as *Cinnamomum zeylenticum* (stem bark), *Elettaria cardamomum* (seeds), *Cinnamomum tamala* (leaves), *Mesua ferrea* (stamens), *Callicarpa macrophylla* (flowers), *Piper nigrum* (fruits), *Piper longum* (fruits), *Embelia ribes* (fruits) were added and this sweet filtered fluid was placed for fermentation in incubator for fifteen days at 33°C±1°C. After fifteen days completion of fermentation was confirmed by standard tests⁹.

The fermented preparation was filtered with unstarched muslin cloth and kept in cleaned covered vessel for further next seven days. Then, it was poured in clean amber coloured glass bottles previously rinsed with ethyl alcohol, packed and labelled properly.

Preparation of Draksharishta-M

Method of preparation was same as followed with Draksharishta -T, only dhataki flowers were replaced with Yeast for inducing fermentation¹⁰.

Animals

Adult wistar albino rats, weighing between 200-220g of either sex were acclimatized to normal environmental conditions in the animal house for one week. The animals were housed in standard polypropylene cages and maintained under controlled room temperature ($22\text{ }^{\circ}\text{C}\pm 2^{\circ}\text{C}$) and humidity ($55\pm 5\%$) with 12:12 hour light and dark cycle. All the animals were given a standard chow diet (Hindustan Lever Limited), and water *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed and prior permission was granted from the Institutional Animal Ethics Committee (CPCSEA No. 07/09).

Chemicals

Thiobarbituric acid was obtained from Loba Chemie, India. Ferrous sulphate, trichloro acetic acid, potassium dihydrogen phosphate, potassium hydroxide, were of analytical grade and obtained from Ranbaxy fine chemicals.

Assay of lipid per oxidation

The extent of lipid per-oxidation in goat liver homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substances (TBARS) by using standard method¹¹ with the help of spectrophotometer.

Goat liver was purchased from local slaughter house. Its lobes were dried between blotting paper (to remove excess blood) and were cut into small pieces with a heavy-duty blade. They were then homogenized in glass-teflon homogenizing tubes in cold phosphate buffer saline (pH 7.4). It was centrifuged at 2000 rpm for 10 min, and supernatant was diluted with phosphate buffer saline up to final concentration of protein 0.8-1.5 mg/0.1ml. Protein concentration was measured by using standard method¹². To study the comparative response, the experiment was divided into five groups. Liver homogenate (5%, 3ml) was aliquoted to seven different glass petri dishes. The first two groups were treated as control and standard where buffer and Vit. E was added respectively. In the third to seventh group, different concentration (100, 150, 200, 250 and 300 $\mu\text{g/ml}$) of Draksharishta-T and Draksharishta-M were added. Lipid per oxidation was initiated by adding 100 μl of 15mM ferrous sulphate solution to 3 ml of liver homogenate. After 30 min, 100 μl of this reaction mixture was taken in a tube containing 1.5ml of 10% trichloroacetic acid. After 10 min, tubes were centrifuged and supernatant was separated and mixed with 1.5ml of 0.67% thio barbituric acid. The mixture was heated on a water bath at 85°C for 30 min, and then on boiling water bath to complete the reaction. The intensity of pink colored complex formed was measured at 535 nm.

The percentage of inhibition of lipid per oxidation was calculated by comprise the results of the test with those of controls as per the following formula i.e. Eq. (1) as-
Percentage Inhibition = (Control Absorbance- Test Absorbance) X 100/Control absorbance.

Determination of Antihyperlipidemic Activity

Experimental design

All the animals were randomly divided into the six groups with six animals in each group.

Group I (-ve Control): Normal diet (Standard chow diet)

Group II (+veControl): High Fat Diet (HFD)

Group III: HFD + Draksharishta-T (2.0 ml/kg/day p.o)

Group IV: HFD + Draksharishta-M (2.0 ml/kg/day p.o)

Group V: HFD + marketed Draksharishta (2.0 ml/kg/day p.o)

Group VI: HFD +Atorvastatin (1.2 mg/kg/day p.o)

The composition of the two diets was as follows:

Control Diet (Normal)

Wheat flour 100g

Sucrose 50g

Hydrogenated vegetable oil 5ml

Casein 20g

Cellulose 4g

Salt mixture (NaCl, KCl, CaCl₂) 1.5g

Citric acid 0.5ml

Vitamin B complex composition

High fat Diet

Wheat flour 100g

Sucrose 50 g

Hydrogenated vegetable oil 10ml

Casein 20g

Butter 10g

Cellulose 4g

Salt mixture (NaCl, KCl, CaCl₂): 1.5g

Cholesterol (dried egg yolk) 0.5g

Citric acid 0.5ml

Vitamin B complex composition.

Procedure:

Group I served as normal control and was given normal saline along with normal diet. Group II, III, IV, V and VI received high fat diet plus cholesterol for induction of hyperlipidemia. In addition to this, group III, IV and V were administered with Draksharishta-T, Draksharishta-M and marketed Draksharishta (2 ml/kg/day p.o) respectively while group VI received Atorvastatin (1.2 mg/kg/day p.o) for nine weeks

Body weight of each animal was noted at the beginning and at the end of the experiment. During the whole period, free access to food and water was provided to the animals. Twenty hours prior the end of the experiment, food was withdrawn and blood samples were taken by retro-orbital plexus. The blood samples were centrifuged for 12 min at 1600 rpm for the separation of serum. Serum total cholesterol¹⁴, serum HDL¹⁵, serum LDL¹⁵, serum VLDL¹⁶ and serum triglycerides¹⁶, were determined in each blood sample.

These parameters were estimated by using Span Diagnostic and Erba Diagnostic Kits. The LDL, VLDL and Atherogenic index were calculated by using the following Friedewald formulae¹⁵

$LDL = TC - HDL - VLDL$ (where $VLDL = TG/5$)

$Atherogenic\ index = (LDL + VLDL) / HDL$

Statistical Analysis

The results are expressed as mean \pm SEM. Statistical analysis of data among the various groups was performed by using one way analysis of variance (ANOVA) followed by the Tukey's test using Graph Pad Prism software of statistics. Significance value ($P < 0.05$) was considered statistically significant as compared to control group.

Results

Assay of lipid per oxidation

The results presented in **Fig.1**, showed that Draksharishta-T, Draksharishta-M and its marketed formulation, inhibited ferrous sulphate induced lipid per oxidation in a dose dependent manner. Draksharishta-T and Draksharishta-M at 300 μ g/ml exhibited maximum inhibition, which was nearly equal to the inhibition produced by Vit. E (5mM). The IC₅₀ value was found to be 230.03, 236.11 and 233.49 μ g/ml with Draksharishta-T, M and its marketed formulation respectively. The inhibition could be caused by the absence of ferryl-perferryl complex or by changing the ratio of ferric to ferrous or by reducing the rate of conversion of ferrous to ferric or by changing the iron itself or combination thereof¹¹.

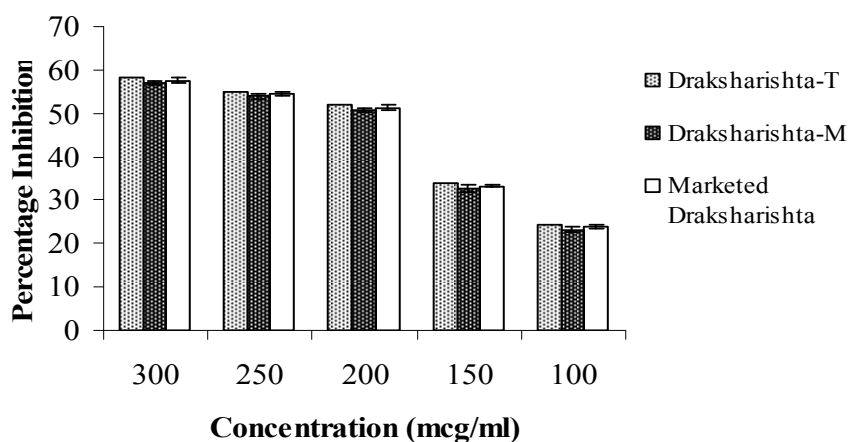


Fig.1. Effect of Draksharishta-T, M and its marketed formulation on Lipid peroxidation model

all values are shown as mean±SEM of three replicates

Hypolipidemic activity

A significant reduction in the body weight of rats was observed in Draksharishta-T, Draksharishta-M and its marketed formulation treated groups as compared to high fat diet fed control group as shown in **Fig.2**.

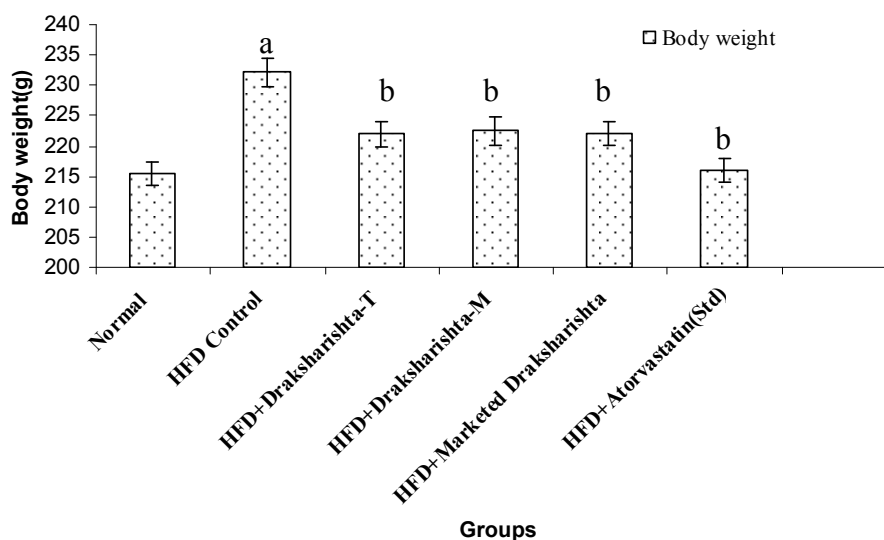


Fig.2. Effect of Draksharishta-T, M and its marketed formulation on the body weight of HFD induced hyperlipidemic rats; HFD- high fat diet

all values are expressed as mean±SEM, a ; P<0.001 significant as compared to normal group, b ; P<0.001 significant as compared to HFD control group

A one hundred seven (107%) increase in serum total cholesterol was noticed in rats fed with high fat diet as compared to rats fed with normal diet. Administration of Draksharishta-T, M and its marketed formulation showed significant reduction in serum cholesterol, serum LDL, serum triglycerides while showed significant rise in serum HDL as compared to high fat diet fed control group as shown in Fig.3.

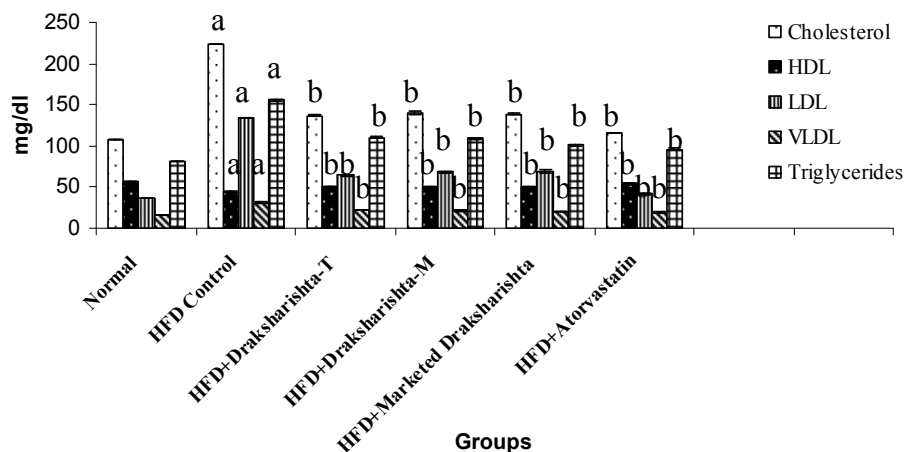


Fig. 3. Effect of Draksharishta-T, M and its marketed formulation on serum lipid profile in HFD induced hyperlipidemic rats; HFD-high fat diet

All values are expressed as mean \pm SEM, a ; $P < 0.001$ significant as compared to normal group
b ; $P < 0.001$ significant as compared to HFD control group

All the test formulations of Draksharishta as Draksharishta-T, M and its marketed formulation also showed significant decrease in atherogenic index as compared to high fat diet control group as shown in Fig.4, which strongly supports anti-atherosclerotic property of Draksharishta.

Discussion

Lipids are widely involved in oxidative reactions and these reactions, can be induced by free radicals called Reactive Oxygen Species (ROS). Oxidative stress caused by ROS in the living cell is associated with numerous diseases, like coronary heart disease, atherosclerosis, inflammation, cancer, anaemia, and age related muscular degeneration and ageing. Use of anti oxidants (substances that when present in low concentrations with those of an oxidizable substrate, significantly retard oxidation of that substance) can postpone problems caused by ROS and they retard oxidation process. Enzyme modifying actions of anti-oxidants could account for their pharmacological activities. In our present study Draksharishta-T and M were evaluated for free radical scavenging activity and showed potent anti-oxidant activity and evidenced that free radical scavenging potential helps in ameliorating disease process¹⁷.

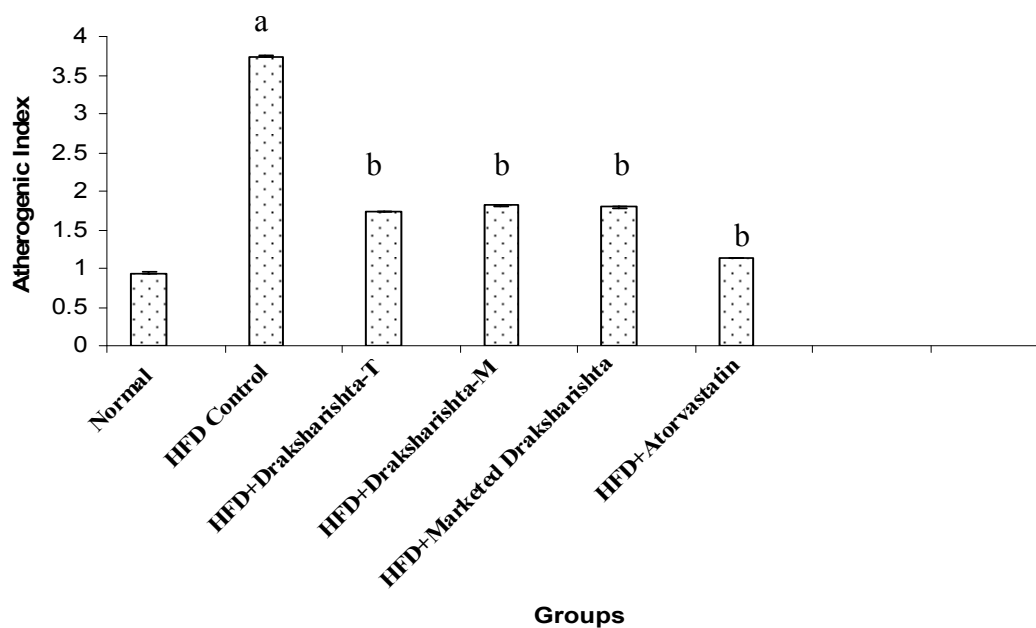


Fig.4. Effect of Draksharishta-T, M and its marketed formulation on atherogenic index in HFD induced hyperlipidemic rats; HFD- high fat diet

All values are expressed as mean \pm SEM, **a** ; $P < 0.001$ significant as compared to normal group
b ; $P < 0.001$ significant as compared to HFD control group

In the evaluation of hypolipidemic activity significant reduction in body weight was observed in Draksharishta treated groups as compared to high fat diet fed control group which suggests that certain enzymes are secreted in quantity involved in bile acid synthesis and its excretion and this may cause decrease in serum cholesterol and serum triglycerides¹⁸.

A rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence it is a direct risk factor for coronary heart disease. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries¹⁹.

HDL promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to the liver. Therefore, increased levels of HDL are desirable. On the contrary, high levels of VLDL and LDL promote arteriosclerosis. LDL, especially in its oxidized form, is taken up by macrophages via a scavenger mechanism. Therefore, anti-atherosclerotic drugs should reduce VLDL and LDL and/or elevate HDL. The search for hypolipidemic drugs follows that high level of serum cholesterol is associated with an increased incidence of coronary heart diseases. Reduction in LDL cholesterol and increase in HDL cholesterol concentration are significantly related with lipid lowering therapy²⁰.

In the present study, Draksharishta-T and M showed significant reduction in total cholesterol and LDL cholesterol level as compared to high fat diet fed control group. A significant fall in HDL cholesterol to total cholesterol ratio was observed in Group II (high fat diet treated rats). Low level of HDL cholesterol is associated with high risk of coronary artery disease. The decrease in serum triglyceride level and reduction in atherogenic index in Draksharishta treated groups is an important finding of this experiment. Most of the hypolipidemic drugs do not decrease serum triglycerides level but both types of Draksharishta as Draksharishta-T and M reduced the elevated serum triglyceride level significantly. Thus, both of these preparations maintained the serum parameters near to the normal level significantly. Reverse back of atherogenic index provides strong additional benefits in the prevention and treatment of atherosclerosis.

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