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Effect of Ocimum Sanctum (Tulsi) Leaf on High-Fat-Diet Induced Hyperlipidemic Rat

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Abstract: Dyslipidemia mediated atherosclerosis is the key pathogenesis of several cardiovascular diseases. Present study aimed to evaluate the therapeutic potential of *Ocimum sanctum* (OS) leaf consumption against high-fat-diet (HFD) induced hyperlipidemia in rat model. A total of 18 *Wistar* albino rats (200-215g) were randomly divided as control, HFD (hyperlipidemic) and OS-treated groups. HFD-group were administered with high fat diet for 15 days while, OS-treated group with *Ocimum sanctum* leaf powder (200 mg / kg of bodyweight / day) together with HFD diet for 15 days. Blood samples were analyzed for changes in plasma total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein and very-low-density lipoprotein. Plasma atherogenic index was also calculated.

HFD administration significantly increased plasma lipid levels and atherogenic index in HFD-group. The supplementation of OS significantly reduced plasma total cholesterol, triglyceride, lipoproteins and atherogenic index in OS-treated rats. In conclusion, 15 days OS leaf (powder) supplementation dose of 200 mg / kg of bodyweight / day effectively attenuated dyslipidemia in hyperlipidemic rats.

Keywords: cardioprotective, high fat diet, hyperlipidemia , Ocimum sanctum.

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Introduction

Coronary heart disease (CHD) is a complex, multifactorial disease and one of the leading causes of death among adults [1]. Atherosclerosis is the principle pathophysiological mechanism of coronary artery disease influenced by genetic and environmental risk factors particularly diet, smoking, and sedentary life-style [2]. High-fat-diet associated dyslipidemia is a social predicament and the causative factor of atherosclerotic plaque. Dyslipidemia is characterized by elevated plasma levels of cholesterol, triglyceride (TG), low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) [1,3]. Atheroma (atherosclerotic plaque) develops on tunica intima characterized by subendothelial lipid accumulation, immunocyte infiltration, proliferation of smooth muscle cells, and formation of foam cells and fatty streak [4].

Dietary modifications are usually the first choice of management for controlling hyperlipidemia or primary cases of CHD. In pharmacological intervention, different drugs such statins, fibrates, resins and niacin are used to treat primary and particularly secondary cases of CHD however, these pharmacotherapies are also associated with side-effects which limits its effectiveness. Thereof, herbal medicine together with life-style and dietary modifications are also well-recognized for their effective management of cardiovascular risks and potential opportunity for the treatment of cardiovascular diseases [5]. Most of the herbal therapies' relies on antioxidant and hypolipidemic potentials of herbs and herbal material. *Ocimum sanctum* (Tulsi) leaves are popular culinary herb and traditionally used in Ayurvedic Indian medicine to cure variety of diseases owed to its antioxidant, anti-diabetic and anti-inflammatory potentials [6,7]. The present

study aimed to investigate the cardioprotective effect of *Ocimum sanctum* leaf daily consumption in controlling the HFD-induced dyslipidemia and atherosclerosis in rat model of hyperlipidemia.

Material and Method:

Plant Material:

Fresh *Ocimum sanctum* (OS) leaves were collected from local herbal store, Karachi, Pakistan. The leaves were washed with tap water, dried under shade, segregated and chopped into small pieces. Dried OS leaves were pulverized to fine powder using electronic grinder and stored in an airtight container.

Experimental Animals:

All experiments conducted according to the "Guide for the Care and Use of Laboratory Animals" [8].

Healthy, female *Wistar* albino rats weighing 200-215 g procured from the animal facility of International Center of Chemical and Biological Sciences (ICCBS), University of Karachi were housed at animal care facility, Department of Physiology, University of Karachi. Two animals per cage (at 25±5°C, 12 hrs. light/dark cycle) were allowed to acclimatize for 1 week . All animals were fed with standard laboratory pellet diet and tap water *ad libitum*.

Modified-Diet Preparation:

The OS dried leaf powder was mixed with HFD in ratio 2% of whole diet as described in previous study [7].

Experimental Groups:

In this study, a total of eighteen rats were used which were randomly divided into three groups (6 rats each group). Group 1 (control) rats were fed with standard laboratory diet during experimental period of 15-days. Group 2 (HFD-group) animals were fed with high-fat diet for 15 days [9]. Group 3 (OS-treated group) rats made hyperlipidemic (provided with HFD for 15 days) and then fed with OS leaf powder (200 mg / kg of bodyweight / day) together with high-fat diet for another 15 days.

Collection of Blood Sample:

At the end of experiment, animals were sacrificed following overnight fast. Blood samples were collected in EDTA vacutainer and centrifuged (3000 rpm for 5 min) to separate plasma and stored at -8πC for biochemical estimation.

Estimation of Plasma Lipid Profile and Atherogenic Index:

Commercially available enzymatic activity (colorimetric) assay kits (Randox, UK) were used for the estimation of plasma total cholesterol, triglyceride, and HDL concentrations. Plasma LDL concentration was determined by using Friedewald's formula [10]. Plasma very low-density lipoprotein (VLDL) concentration was calculated via formula as described previously [11].

Plasma atherogenic index was calculated as reported previously [12].

Statistical Analysis:

All results are presented as mean ± standard error of mean (SEM). Experimental groups (control, HFD, OS treated) were tested for statistically significant differences using one-way ANOVA. The p-value of < 0.05 considered as statistically significant.

Results:

Body weight:

The average body weight of control rats was 121.5 g while body weight of HFD fed group was 212.17 g that was significantly higher from control rats ($p < 0.05$). 15 days of OS leaf consumption showed significant ($p < 0.05$) decrease in body weight compared to control and HFD groups (Table 1).

Effect of High-fat Diet

15 days of HFD administration increased the plasma total cholesterol ($p < 0.05$) and plasma triglyceride level ($p > 0.05$). Similarly, plasma lipoproteins LDL, VLD were also significantly increased in HFD group ($p < 0.05$) while, plasma HDL was significantly decreased in HFD group. Significant ($p < 0.05$) increase in the atherogenic index of plasma (AIP) was observed in HFD-group compared to control animals (Table 2).

Effect of *Ocimum sanctum* leaf treatment on hyperlipidemic rats

Ocimum sanctum (leaf powder) supplementation for 15-days decreased plasma total cholesterol ($p > 0.05$) and triglyceride ($p < 0.05$) levels in OS-treated group compared to HFD-group. Plasma HDL significantly increased in OS-treated group when compared with HFD-group however, the HDL concentration was non-significant when compared to control group. In comparison to HFD-group, plasma LDL and VLDL levels were decreased significantly ($p < 0.05$) with the 15-days OS supplementation. Animals of OS-treated group also exhibit significant decrease in plasma atherogenic index compared to hyperlipidemic animals of HFD-group (Table 2).

Table 1: Body weight comparison among experimental groups

Experimental Groups	**Bodyweight (g)**
Control	121.5 ± 3.03
HFD	212.17 ± 5.51***
OS Treated	190.5 ± 5.524$^{***/*}$

Note: Results are expressed as mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$

Table 2: Plasma lipid profile and atherogenic index comparison among experimental groups

Groups	**TC (mg/dl)**	**TG (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL**	**AIP**
Control	100.13 ± 3.5	88.62 ± 3.4	33.2 ± 0.94	49.2 ± 2.3	17.7 ± 0.68	0.42 ± 0.01
HFD	144.8 ± 11.7*	116 ± 13.3^{NS}	24.4 ± 1.16***	97.15 ± 12.5***	23.2 ± 2.6***	0.663 ± 0.06***
OS Treated	121.13 ± 5.7$^{*/NS}$	79.55 ±5.4$^{NS/*}$	30.7 ± 2.2$^{NS/*}$	74.5 ± 6.3$^{***/***}$	15.91 ± 1.08$^{***/***}$	0.413 ± 0.04$^{***/***}$

Note: Results are expressed as mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$

Discussion:

Globally, cardiovascular disease (CVD) is leading cause of morbidity and mortality [1]. Atherosclerosis is one of the key pathological mechanisms for cardiovascular diseases. Among several risk factors for atheroma development, dyslipidemia / hyperlipidemia is the principal contributing factor. Large number of research and cohort studies have shown that dietary and lifestyle modifications are the promising strategies for the prevention of CVD incidences [13]. In the present study, high-fat diet was administered for 15-days for the induction of diet-induced hyperlipidemia.

Our results indicated that after 15 days of OS leaf administration a 16% reduction in plasma total cholesterol, 31% reduction in plasma triglyceride, 23% decrease in LDL and 31% decrease in VLDL cholesterol levels while plasma HDL level was increased by 26% when compared with hyperlipidemic animals of HFD-group. High atherogenic index of plasma strongly correlated with the high CVD risk [12]. Ocimum administration showed significantly decreased AIP in OS-treated group in comparison to hyperlipidemic animals indicating the overall improvement in blood lipid regulation.

Similar to present study, several other research studies also reported the hypolipidemic effects of *Ocimum sanctum* leaf attributable to decreased liver lipoprotein production [14], decreased intestinal cholesterol absorption while accelerated fecal cholesterol elimination [7,14]. A research study on HFD-induced hyperlipidemic rats also reported the strong hypolipidemic, anti-atherogenic and antioxidant effects of *Ocimum sanctum* administration as exhibited by significant decrease in plasma lipids and lipoprotein concentration and by decreased fatty streak development and growth [15].

Conclusion:

The results of present study concluded that daily consumption of *Ocimum sanctum* may be beneficial in controlling CVD risk factors as *Ocimum sanctum* supplementation and effectively regulates dyslipidemia in experimental rat model HFD-induced hyperlipidemia.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

This was a experimental animal model study that was conducted in accordance with the “Guide for the care and use of laboratory animals”.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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