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Ameliorative Effect of *Withania coagulans* on Experimentally-induced Hyperlipidemia in Rabbits

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Abstract

The present study is associated with the evaluation of phytochemical and antihyperlipidemic effect of Methanolic Fruit Extract (MFEt) of *Withania coagulans* on experimentally-induced hyperlipidemia in rabbits. Qualitative and quantitative phytochemical analysis of MFEt was performed by standard methods. The extract didn't induce any behavior change or mortality in rabbits up to 2000 mg/kg. In hyperlipidemic model, rabbits were divided into four control groups including control, hyperlipidemic control (both treated with distilled water 1 ml/kg), negative control (0.05% DiMethylSulphoxide 1 ml/kg), positive control (Simvastatin 20 mg/kg) and three test groups (MFEt200, 400 and 600 mg/kg). On completion of 14 days trial, rabbits were sacrificed to collect serum and liver tissues to estimate lipid profile, Alanine Aminotransferase (ALT), Creatine Kinase (CK), Catalase (CAT), Superoxide Dismutase (SOD), Lipid Peroxidation (LPO), HMG-CoA reductase activity and Coronary Risk Index (CRI). Quantitatively MFEt is found rich in total phenols especially flavonoids besides showing variety of constituents qualitatively. All the three doses (200, 400 & 600 mg/kg) of MFEt significantly increased serum high-density lipoprotein cholesterol and decreased total cholesterol, HMG-CoA reductase activity, triglycerides, very low-density and low-density lipoproteins with improved LPO and CRI ($p < 0.05$) in test groupsas compared to hyperlipidemic groups. In addition, normal ALT and CK levels and decreased percent inhibition of CAT and SOD were observed in test groups. The results concluded that MFEt of *W. coagulans* maintain lipid homeostasis by elevating the status of antioxidant enzymes and thereby minimizing the coronary risk in hyperlipidemic rabbits.

Keywords: Phytochemistry, antihyperlipidemic, antioxidant enzymes, *Withania coagulans*

1. Introduction

Presently, the increase prevalence of hyperlipidemia is creating an alarming situation all over the world. The relationship between hyperlipidemia and Cardiovascular Diseases (CVD) has been investigated thoroughly and well-reported [1]. The World Health Organization (WHO) reported that increase in blood cholesterol level escalates the risk of more than 50% cases of CVD in population globally [2]. Hyperlipidemia is characterized by elevated levels of Total Cholesterol (TC), Triglycerides (TG) and Low-Density Lipoprotein cholesterol (LDL-c) in blood and it initiates the hardening of coronary arteries or atherosclerosis which reduce the blood flow and induce oxygen deficiency; this silently-produced-situation eventually leads to heart failure [3, 4]. Beside genetics, many acquired factors such as age, diet (rich in saturated and *trans*-fats), sedentary life-style, alcohol consumption, hypertension and other endocrine disorders are the well-wishers of this silent killer of the heart [5]. Therefore, adopting diet modification by decreasing its fat content and bringing some exercise in daily routine to burn calories minimizes the risk of heart problems and keep the blood lipids within their normal levels. Interestingly,

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authentic lipid lowering medicines are commercially available for the treatment of this health hazards like statins, fibrates, etc, but all of these have certain side effects such as diarrhea, nausea, myositis and abnormal liver function [5]. In order to decrease these medicine-induced problems, researchers are focusing on natural remedies and evaluating medicinal plants to treat hyperlipidemia due to their effectiveness and minimal or no side effects [6].

Withania coagulans Dunal, commonly known as Paneer dodi (Hindi/Urdu), belongs to the family *Solanaceae* and is cultivated throughout Central and South Asia. The fruits of the same plant are traditionally used as cheese maker due to their enzymatic ability for coagulating milk [7]. Overall twenty four Withanolides (Steroidal Lactones) and a Dimeric Lignin Bispicropodophyl Lingucoside have been isolated from *W. coagulans* [8]. Similarly, nine compounds including Withanolides, Ergosta-5, 25-diene-3β, 24ξ-Diol and Sitosterol-β-D-glucoside are isolated from its fruits [9]. Many pharmacological activities of same *coagulans* are well-reported like anticancer, antidiabetic, antiflammatory, antimicrobial, anthelmintic, antiulcer, cardiovascular, free radical scavenging, hepatoprotective, immunosuppressive and wound healing [8, 9]. On the basis of vast medicinal value of *W. coagulans*, the present study was designed to evaluate the effect of methanolic fruit extract (MFEt) of same plant on experimentally-induced hyperlipidemia in rabbits.

2. Material and Methods

2.1 Preparation of Methanolic Fruit Extract (MFEt)

Dried fruits of *W. coagulans* were purchased from Hamdard Dawakana, Sadar, Karachi and authenticated (voucher No. KU/BCH/SAQ/06) by a Taxonomist of the Department of Botany, University of Karachi (UoK). Preparation of MFEt was done by soaking of 40 gram of ground fruit powder in 1 liter of methanol for overnight, filtered twice and concentrated by using rotary evaporator to obtain a dark brown residue [10].

2.2 Phytochemical Analysis

Detection of alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, cardiac glycosides, phlobataninns, resins, saponins, steroids, tannins and triterpenoids in MFEt was done by qualitative methods [11], whereas quantitative determination of alkaloids, flavonoids, saponins and total phenols was done by methods described by Harborne, Boham & Kocipai, Obadoni & Ochuko and Slinkard & Singleton respectively [12–15].

2.3 Dimethylsulphooxide (DMSO) and Simvastatin

DMSO (0.05%) and Simvastatin (Limitrol 20 mg) were purchased from Fisher Scientific (UK) and PharmaEvo (Pvt) Ltd, Pakistan, respectively and used as medium for administering doses of MFEt in rabbits and positive control in present study [10].

2.4 Animals

Healthy albino rabbits of both sexes (1–1.5 kg) were purchased from local supplier of UoK and kept in conventional animal house according to the internationally accepted guidelines for animal handling. These animals were given standard laboratory diet and free access to water *ad libitum*.

2.5 Determination of Acute Toxicity of MFEt

Acute toxicity of MFEt was measured by administering doses (10–2000 mg/kg) of same extract orally in separate groups (6/group) of overnight fasted rabbits while six rabbits were also ran as control treated with distilled water in a dose of 1 ml/kg. After receiving treatment, each group was kept in observation for 24 hours to monitor signs like tediousness/sedation, ruffled hair, clumping together, itching, restlessness and mortality/death [17].

2.6 Experimental Protocol for Determining Antihyperlipidemic Activity of MFEt

The experimental protocol of the present study was approved by Board of Advance Study and Research (BASR) of UoK.

2.6.1 Preparation of Hyperlipidemic Rabbits

Daily oral administration of High-Fat Inducer (HFI) in a dose of 1 ml/kg was used to induce hyperlipidemia in overnight fasted rabbits. The HFI contained cholesterol

(0.1 gm), bile salt (0.005 gm), butter (0.2 gm) in 1 ml of peanut oil [18].

2.6.2 Animal Grouping

The overnight fasted normal and hyperlipidemic rabbits were grouped (6/group) as,

Group I (control): normal rabbits treated with distilled water (1 ml/kg).

Group II (hyperlipidemic control): treated with HFI and distilled water (1 ml/kg each).

Group III (negative control): treated with HFI and 0.05% DMSO (1 ml/kg each).

Group IV (positive control): treated with HFI (1ml/kg) and simvastatin (20 mg/kg).

Group V, VI and VII (test groups): treated with HFI (1 ml/kg) and MFEt in doses of 200, 400 and 600 mg/kg respectively.

All treatments were provided orally for 14 days consecutively. After that, the rabbits were sacrificed to collect blood; serum was separated and liver was carefully dissected out for biochemical analysis.

2.7 Biochemical Analysis

Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein cholesterol (HDL-c) and liver & cardiac-specific enzymes including Alanine Aminotransferase (ALT) & Creatine Kinase (CK) respectively were estimated in serum by using commercially available kits of Randox (UK); whereas, Low-Density Lipoprotein (LDL-c), Very Low-Density Lipoprotein (VLDL-c) cholesterols and Coronary Risk Index (CRI) were calculated using the formulae:

LDL-c = TC-HDL-c-(TG/5) (given in Randox reagent kit)

VLDL-c = TG/5 [19]

CRI = TC / HDL-c [16]

Antioxidant enzymes including Catalase (CAT) and Superoxide Dismutase (SOD) in liver homogenate were estimated through methods described by Pari & Latha and Misra & Fridovich respectively [20, 21] while Lipid Peroxidation (LPO) was determined by ThioBarbituric Acid Reactive Substances (TBARS) method [22]. In addition, HMG-CoA reductase activity was determined in terms of HMG-CoA/mevalonate ratio in same homogenate via method described by Rao & Ramakrishnan [23, 24].

2.8 Statistical Analysis

Results are expressed as mean ± SD (Standard Deviation) and one way ANalysis Of VAriance (ANOVA) followed by LSD (Least Significant Difference) test was performed (SPSS, version 17.0). The differences were found significant at $p<0.05$.

3. Results

3.1 Phytochemical Analysis of MFEt

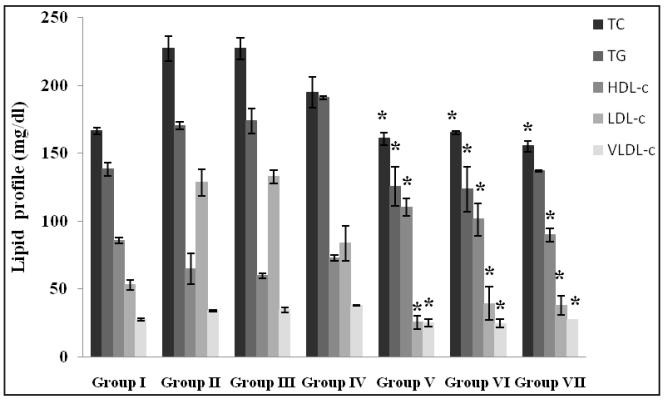
The MFEt showed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, phlobatannins, resins, saponins, steroids, tanins and triterpenoids whereas quantitatively flavonoids (200 mg), total phenols (125 mg), alkaloid (15 mg) and saponins (15 mg) per gram of starting material were found.

3.2. Acute Toxicity of MFEt

Acute toxicity in terms of behavior change and mortality was not observed in rabbits after administering MFEt from 10–2000 mg/kg.

3.3. Antihyperlipdemic Activity of MFEt

All the three doses (200, 400 & 600 mg/kg) of MFEt produced a significant ($p<0.05$) decrease in TC, TG, LDL-c, VLDL-c, CRI (TC/ HDL-c ratio), HMG-CoA reductase activity (HMG-CoA/Mevalonate ratio) and increase in HDL-c in their respective test groups V, VI and VII as compared to hyperlipidemic group II and III (Figure 1 & 2).



*$p<0.05$ when compared with respective group II and III.

Fig. 1. Effect of MFEt on lipid profile.

3.4 Antioxidative Activity of MFEt

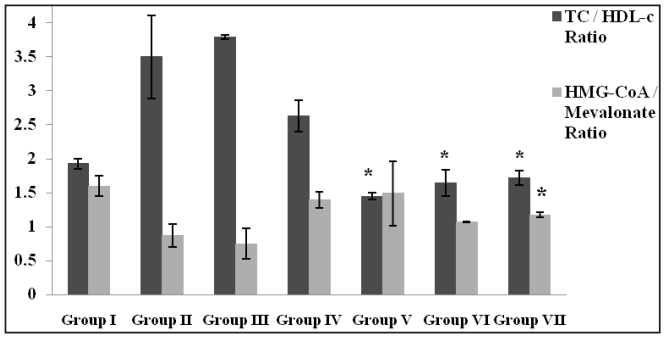
All the three doses of MFEt significantly ($p<0.05$) induced reduction in LPO and percent inhibition of CAT and SOD in the test groups V, VI & VII as compared to hyperlipidemic groups II & III (Figure 3).

3.5 Hepato and Cardio Protective Activity of MFEt

MFEt showed no toxic effect on liver and heart by demonstrating normal levels of ALT and CK respectively in all tree test groups as same as found in normal control group (Table 1).

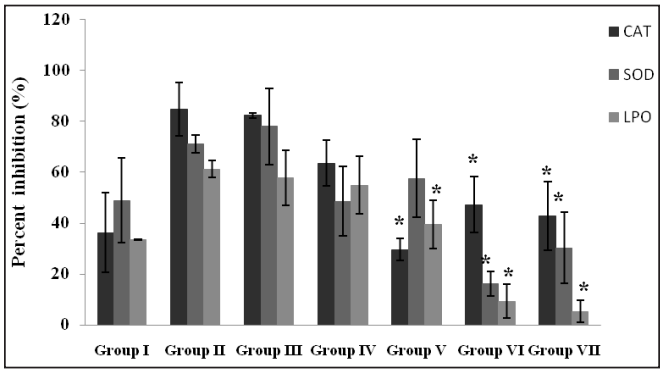
4. Discussion

Herbal medicines are gaining fame in the treatment of number of health problems including hyperlipidemia



*$p<0.05$ when compared with respective group II and III.

Fig. 2. Effect of MFEt onTC/HDL-c Ratio) and HMG-CoA/Mevalonate ratios.



*$p<0.05$ when compared with respective group II and III.

Fig. 3. Effect of MFEt on antioxidant parameters.

especially in South Asian countries like Bangladesh, Pakistan and China [7]. Among medicinal plants, two species of genus *Withania* including *W. somnifera* and *W. coagulans* are well-reputed for many medicinal purposes especially antidiabetic and antihyperlipdemic activities in alloxan and streptozotocin-induced diabetic animal models [9]. It has been previously reported that fruits of *W. coagulans* are rich in Withanolides [8]. However, our study estimated high quantity of total phenols especially flavonoids in MFEt of same plant as compared to the amounts of alkaloids and saponins whereas other constituents including carbohydrates, cardiac glycosides, glycosides, phlobatannins, resins, steroids, tannins and triterpenoids have also been detected in same extract. Several studies described that saponins and steroids present in few plants are known to have lipid lowering activities whereas flavonoids and total phenols are active antioxidants and provide protection against CVD and age-related degenerative diseases [25,26].

Addition of high fat content like cheese, butter and sausages in diet serves as a slow poison that gradually and slowly makes individuals susceptible to life threatening problems like weight gain, hypertension, diabetes and CVD [27]. Interestingly, such types of inducers are also affecting our country. However, people are adopting natural lipolytic agents like green tea, lime water, *Allium sativum,* etc., for minimizing the risk of obesity and other heart problems [28, 29]. Aqueous extract of fruits of *W. coagulans* have also been reported as antihyperlipidemic agent in diabetic animal model but at 1000 mg/kg. In the present study, three doses (200, 400 & 600 mg/kg) of MFEt of *W. coagulans* found effective and induced a significant ($p<0.05$) decrease in serum TC and TG levels in their respective test groups and confirming its antihyperlipidemic activity. The decrease found in TC

Table 1: Effect of MFEt on CK and ALT

Groups	CK(U/l)	ALT(U/l)
Group I	14.16 ± 7.74	12.26 ± 1.27
Group II	21.85 ± 14.56	19.73 ± 4.34
Group III	28.31 ± 19.24	11.06 ± 0.11
Group IV	12.14 ± 8.09	12.53 ± 4.14
Group V	18.21 ± 12.14	11.93 ± 0.46
Group VI	18.21 ± 15.32	11.90 ± 0.81
Group VII	18.21 ± 15.32	11.06 ± 1.40

Each value is the mean ± SD (n=6).

level may be due to the inhibitory effect of MFEt on HMG-CoA reductase activity, the rate-limiting enzyme of cholesterol biosynthesis [30]. This possibility was proved by observing significantly ($p<0.05$) improved HMG-CoA/mevalonate ratio in all three test groups treated with MFEt. Similarly, a significant ($p<0.05$) decrease in CRI was observed in alltest groups indicating the cardio-protective effect of extract. Simvastatin (positive control) is well-known HMG-Co reductase enzyme inhibitor showed high values of HMG-CoA/Mevalonate ratio and found effective in improving CRI by reducing serum TC, TG, LDL-c and VLDL-c ($p<0.05$) but did not elevate the HDL-c. The hypotriglyceridemic effect of MFEt may be due to enhance activity of lipase enzyme which accelerates lipolysis [31]. This cardio-protective possibility of extract was also supported by observing the elevation in HDL-c and reduction in LDL-c & VLDL-c levels in test groups. HDL-c transports cholesterol away from the peripheral tissues to liver thus prevents atherosclerosis by antagonizing the actions of LDL-c and VLDL-c, which are involve in transporting newly synthesized cholesterol and TG from liver to peripheral tissues [32].

Literature described that hyperlipidemia induces oxidative stress by generating Reactive Oxygen Species (ROS) which play a key role in pathogenesis of atherosclerosis by inducing Lipid Peroxidation (LPO) especially in coronary arterial walls [33]. Endogenous enzymatic and non-enzymatic antioxidants prevent body cells and tissues from these harmful effects of oxidative stress [34]. In the present study, all the three doses of MFEt significantly ($p<0.05$) induced reduction in LPO and percent inhibition of antioxidant enzymes CAT and SOD in the test groups. Even positive control was not found as effective as MFEt in increasing the efficiency of antioxidant enzymes. Increase in antioxidative status of test groups by fruit extract may be due to the presence of large quantity of flavonoids and polyphenolic compounds in same extract which are natural antioxidants and well-reported for increasing the activity of SOD and CAT [35]. Similarly, MFEt also showed no toxic effect of liver and heart by showing normal levels of ALT and CK in test groups. This finding was also supported by observing no sign of acute toxicity in rabbits when they were administrated MFEt from 10–2000 mg/kg in initial phase of study. Therefore, the present study concludes the antihyperlipidemic, antioxidative and cardio-protective effects of MFEt and these effects may be due the presence of different constituents including total phenols, flavonoids, steroids, saponins, cardiac glycosides in extract and/or withanolides which were previously reported. It can be suggested that other constituents of extract beside withanolides could be targeted for evaluating their antihyperlipidemic property in future.

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