



Antihyperlipedemic Activity of *Woodfordia fruticosa* Extract in High Cholesterol Diet Fed Mice

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ABSTRACT

The aim of the present study was to investigate the potential role of methanolic flower extract of *Woodfordia fruticosa* in lowering lipid parameters in mice fed a high cholesterol diet. Swiss albino mice were randomly divided into five groups of six and were administered either: 0.5 ml water (negative controls); 30 mg cholesterol (hypercholesterolemic animals); MEWF (methanolic extract of *Woodfordia fruticosa* flowers) at 400mg/kg body weight (positive control); or the same doses of both cholesterol and the extract (test animals); Atorvastatin at 10mg/kg body weight (drug control). The effects of MEWF on the lipid profile were assessed by measuring 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 ($p < 0.05$). Administration of MEWF extract in cholesterol fed mice caused a significant decrease ($p < 0.001$) in the concentrations of serum TC, LDL, VLDL TGs as well as TC: HDL-c ratio when compared with cholesterol fed control mice and results were also comparable to that of drug control group. These results suggest lipid-lowering effects of *Woodfordia fruticosa* which serves as a new potential natural product for preventing hyperlipidemia.

Key word: *Woodfordia fruticosa*, Swiss albino mice, Hyperlipidemia, Atorvastatin

INTRODUCTION

Mortality rates due to cardiovascular diseases have increased several folds in most developed and undeveloped countries. These cardiac ailments are directly related to hyperlipidemia¹. Hyperlipidemia is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease. The treatment of hyperlipidemia depends on the patient's cholesterol profile. Many antihyperlipidemic agents like statin, fibrates, niacin, bile acids, ezetimibe etc reduce cholesterol level with different condition². Hyperlipidemia characterized by elevated serum total cholesterol, low density, very low density lipoprotein and decrease high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease³. Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated using antilipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia⁴. The inhibitors

of 3-hydroxy-3-methylglutaryl coenzyme A reductase (i.e., statins) profoundly lower LDL cholesterol⁵.

Evidence from lipid lowering trials has clearly established that reduction of total cholesterol or low density lipoprotein cholesterol (LDLc) is associated with a decreased risk of atherosclerosis and coronary heart disease⁶. Furthermore, since the last two decades a strong correlation has been observed between levels of circulating lipids and mortality rates from coronary atherosclerotic heart disease⁷.

Several synthetic drugs are available; they have been reported to have serious adverse effects, particularly liver damage. A large number of synthetic antihyperlipidemic drugs are currently available in the market but these lag on the desired properties of safety on long term use and cost. These factors affect a patient's compliance⁸. Plants and herbs are mines of a large number of bioactive phytochemicals that might serve as leads for the development of effective, safe, cheap and novel drugs. A number of medicinal plants have shown their beneficial effect on cardiovascular disease by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects^{9,10}.

Therapeutic role of *Woodfordia fruticosa* has been reported in many disease conditions and reports shows its potent role as antioxidant, antimicrobial and anticancerous¹¹. But its

role in CVD is not yet investigated. Here we investigated the lipid-lowering effect of MEWF in mice model of hyperlipidemia.

MATERIALS AND METHODS

Chemicals and Reagents

Cholesterol kits were procured from Span Diagnostics Ltd. India. All the chemicals and reagents were of analytical grade.

Collection of Plant Material and Extract Preparation

Flowers of *W. fruticosa* collected from Himachal Pradesh, were shade dried at room temperature and reduced to coarse powder. The dried and powdered flowers were percolated four times with Methanol at room temperature. The combined extracts were filtered (Whatmann paper), centrifuged (3200 × g, 4°C and 30 min) and concentrated under reduced pressure in a thin film evaporator at 50 ± 5°C. The paste so formed was dissolved in methanol and concentrated in thin film evaporator. Finally, the extract was completely dried under vacuum in the dessicator. The whole procedure yielded 9-10% (w/w) of the extract in terms of dried starting material.

Phytochemical Analysis

The Methanolic extract of *Woodfordia fruticosa* (MEWF) was subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, flavonoids, carbohydrate, triterpenoids, steroid, alkaloids and glycosides¹². The preliminary phytochemical analysis was also carried out using thin layer chromatography (TLC). The TLC analysis was performed on pre-coated silica gel plates, developed with a mixture of CHCl₃: glacial CH₃CO₂H: MeOH: H₂O (64:32:12:8) for saponins¹³ (), benzene: EtOAc (7:3) for triterpenoids¹⁴ and EtOAc: HCO₂H: glacial CH₃CO₂H: H₂O (100:11:11:26) for flavonoids. Spots were revealed by the anisaldehyde sulphuric acid reagent for saponins and triterpenoids and UV 365 nm for flavonoids.

Animals

Swiss albino mice (25-30 g) maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and water *ad libitum*, housed in the departmental animal house and exposed to 12 h cycle of light and dark. The experimental protocol was approved by Institutional Animal Ethics Committee and care of the animals was carried out as per the guidelines of Committee For the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No- 107/99/CPCSEA-2011-34).

Acute Toxicity Study

Acute toxicity of *W. fruticosa* flower extract was determined¹⁵. Mice were given a single oral dose each of 100 mg to 2000 mg/kg body weight. Animals were observed for behaviour and mortality rate. Blood was tested for various parameters (TLC, DLC, and Hb) over a period of one month. No mortality, signs of toxicity and abnormalities were observed during the experimental period. In addition, no significant difference was noticed in the body and organ weights between control and treated groups (data not shown). These results show that the methanol extract of

Woodfordia fruticosa is toxicologically safe by oral administration.

Effect of MEWF on Diet Induced Hyperlipidemia (In vivo experiment)

The effect of MEWF (Methanolic extract of *Woodfordia fruticosa*) on normal and cholesterol-fed mice was studied in mice after induction of hyperlipidemia.

Groups of animals:

Group I (Control mice) - administered with 0.5 ml distilled water;

Group II (Hyperlipidemic mice) – Normal diet + 30 mg cholesterol.

Group III (Positive control) - MEWF @ 400 mg/kg body weight.

Group IV (Test group)-MEWF @ 400 mg/kg body weight + Cholesterol.

Group V (drug control) - Atorvastatin at 10mg/kg body weight.

After overnight fasting, the mice were sacrificed. Blood samples were collected directly via cardiac puncture using 23G needles and 3-ml syringes and collected into EDTA tubes. The plasma was immediately separated by centrifugation at 3000 rpm for 10 minutes. Samples were analyzed spectrophotometrically. The serum cholesterol levels of the animals were checked by using a commercial diagnostic reagent kit which was manufactured by Span Diagnostics Ltd. India. The concentration of low-density lipoprotein (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) was estimated according to Friedewald's equation¹⁶.

$$\text{VLDL-C} = \text{Triglycerides}/5$$

$$\text{LDL-c} = \text{Total cholesterol} - (\text{HDL-c}) - (\text{VLDL-c}).$$

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.

RESULTS

Phytochemical Analysis

MEWF (Table 1) showed the presence of glycosides, flavonoids, alkaloids, tannins, and saponins. MEWF gave positive reaction for anthraquinone, cardiac and saponin glycosides, flavonoids, steroids, triterpenoids, carbohydrates and tannins. The TLC results showed the presence of two spots at Rf 0.43 and 0.46 as green spot and violet spot respectively for saponins, three spots at Rf 0.28, 0.84 and 0.94 as pink, violet and violet spot respectively for triterpenoids and a single spot at Rf 0.52 as yellow fluorescence for flavonoids.

Acute Toxicity Study

Study revealed that the extract was safe up to a dose level of 1000 mg/kg body weight. Although at higher doses no lethality was observed but the animals treated with the dose range above 1000 mg/kg b. wt. showed lethargic behaviour.

Diet Induced Hyperlipidemia

Table 2 shows the values of the serum lipid profile in Groups I-V. Serum total cholesterol (TC), LDL-c, VLDL-c, and TG concentrations increased significantly ($p < 0.001$) in cholesterol fed group, and the TC: HDL-c ratio was also increased significantly ($p < 0.05$). Concurrent administration of MEWF caused a significant decrease ($p < 0.001$) in the concentrations of serum TC, LDL-c, VLDL-c, TG as well as TC: HDL-c ratio when compared with cholesterol-fed control mice.

DISCUSSION

In developing countries, the incidence of CVD is increasing at an alarming rate, and India is on the verge of a cardiovascular epidemic^{17,18}. 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^{19,20}. In the present study, we investigated the lipid-lowering effect of MEWF in mice fed a high-cholesterol diet. Notably, the mice fed 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 reports that established a correlation between dietary lipids and serum lipid profile^{21,22}. Supplementation of cholesterol in the diet rapidly results in a marked increase in the production of cholesteryl esters VLDL by the liver and intestine and a reduced rate of cholesterol removal by the hepatic LDL receptors, consequently serum levels of LDL-c and VLDL-c are increased²³. A significant increase in the ratio of TC: HDL-c indicates an increased risk of CVDs²⁴. 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 mice 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^{25,26}. Elevated serum TG is considered an independent risk factor for CVD²⁷. 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^{28,29}. A significant decline in the serum TG concentration observed in extract-treated mice supports the cardiovascular protective influence. The mechanism by which MEWF 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 absorption³⁰⁻³². Based on the present results the presence of cardiac glycoside in this plant known to slow and strengthen a failing heart may have accounted for its antihypercholesterolemia effect. Besides, it is known that tannins have diuretic effect hence its presence in this plant may have contributed to the antihypercholesterolemic effect³³. Flavonoids may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells. β -sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain³⁴. It is believed to lower cholesterol by lowering plasma concentrations of LDL³⁵. Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of sterols that results in decrease of body lipids. We suggest that MEWF might elicit beneficial effects by lowering the plasma lipid levels of the treated mice. This is a preliminary study; it is among the earliest reported work regarding the antihyperlipidemic activity of MEWF. *Woodfordia fruticosa* 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

Our study confirms the lipid-lowering effects of MEWF, in mice model of hyperlipidemia which might have role to combat against cardiovascular diseases. The active components in the MEWF 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.

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Table 1: Phytochemical analysis of MEWF

Test	Result
Tannins	Present
Saponins	Present
Flavonoids	Present
Carbohydrate	Present
Triterpenoids	Present
Steroid	Present
Alkaloids	Present
Glycosides	Present

Table 2: Effect of MEWF on Serum lipid profile in mice

Parameters	Group I	Group II	Group III	Group IV	Group V
TC(mg/dL)	85.83 ± 4.92 ^S	156.50 ± 11.10 [#]	83.2 ± 2.23	92.2 ± 6.46 [@]	87.2 ± 6.23
TG(mg/dL)	116.0 ± 13.4 ^S	212.66 ± 8.94 [#]	122.0 ± 11.7 ^{NS}	95.8 ± 2.23 [@]	99.3 ± 1.94
HDL-c (mg/dL)	57.33 ± 10.00 ^S	47.33 ± 9.42 [#]	52.3 ± 12.3 ^{NS}	72.2 ± 7.08 [@]	60.3 ± 7.13
LDL-c (mg/dL)	51.20 ± 9.07 ^S	72.2 ± 12.62 [#]	55.2 ± 13.8 ^{NS}	40.2 ± 2.99 [@]	40.2 ± 2.99
VLDL-c (mg/dL)	23.20 ± 2.69 ^S	42.5 ± 1.79 [#]	24.4 ± 2.34 ^{NS}	19.2 ± 0.44 [@]	20.1 ± 0.16
TC/HDL Ratio	1.512 ± 0.013 ^{**}	3.3 ± 0.018 [#]	1.535 ± 0.024 ^{NS}	0.635 ± 0.20 [@]	0.692 ± 0.27

Values are expressed as mean ± SEM.

Group I (Control mice) - administered with 0.5 ml distilled water;

Group II (Hypercholesterolemic mice) - dietary supplementation of 30 mg cholesterol

Group III (Positive control) - MEWF at a dose of 400 mg/kg body weight;

Group IV (Test group)-MEWF dose of 400 mg/kg body weight, in addition to oral administration of cholesterol.

Group V (drug control) - Atorvastatin at 10mg/kg body weight

Group I versus Group II: ^{**}p < 0.05; ^Sp < 0.001;

Group II versus Group IV: [#]p < 0.001;

Group I versus Group III: NS -not significant;

Group IV versus Group V: [@]p < 0.001.

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