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RESEARCH ARTICLE
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"Evaluation of Antihypercholesterolemic Activity of Aqueous Methanolic Extract of Polyherbal Formulation comprising *Plantago ovate* husk, *Trigonella foenum-graecum* seeds, *Nigella sativa* seeds and *Camellia sinensis* leaves"

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Abstract

Hyperlipidemia, a significant risk factor for cardiovascular diseases, is highly prevalent in many parts of the world, including Pakistan. The condition is characterized by elevated serum cholesterol and low-density lipoprotein (LDL) levels, alongside decreased high-density lipoprotein (HDL) levels, leading to an increased risk of coronary heart disease. Recent successes in developing antihyperlipidemic drugs from plant sources have spurred further exploration of botanicals with potential lipid-lowering properties. Ethnopharmacological approaches in particular have proven effective in identifying new plant-based antihyperlipidemic agents.The present study was conducted to evaluate and validate the antihyperlipidemic effects of a polyherbal formulation consisting of Plantago ovate husk, Trigonella foenum-graecum seeds, Nigella sativa seeds, and Camellia sinensis leaves. These plants were chosen based on their diverse chemical constituents, which have been reported to possess lipid-lowering activities. A crude extract of the polyherbal formulation was prepared and tested on hyperlipidemic Sprague-Dawley albino rats fed an atherogenic diet for 28 days.The study involved seven groups of six rats each. Group I served as the Normal Control, receiving normal feed and normal saline (1 ml/kg/day). Group II, the Positive Control, received a high-fat diet (HFD) along with normal saline. Group III, the Standard Control, was given an HFD plus Atorvastatin (5 mg/kg/day). Groups IV to VII received the HFD along with varying doses of the polyherbal crude extract (30 mg/kg, 100 mg/kg, 200 mg/kg, and 300 mg/kg body weight, respectively). After 28 days of treatment, blood samples were collected via cardiac puncture under

anesthesia, and serum lipid profiles were assessed using standard methods. The results showed that rats fed the atherogenic diet exhibited significantly elevated levels of total cholesterol (TC), triglycerides (TG), and LDL, while HDL levels were significantly decreased. The polyherbal formulation, administered at different dose levels, successfully attenuated these lipid parameters toward normal values in a dose-dependent manner. The highest dose (300 mg/kg) produced the most pronounced lipid-lowering effects.In addition to its efficacy, the polyherbal crude extract was evaluated for acute toxicity in albino mice, demonstrating safety up to an oral dose of 5 g/kg body weight. The study also explored the possible phytochemicals responsible for the antihyperlipidemic activity and discussed potential mechanisms of action. In conclusion, the 70% aqueous methanolic extract of the polyherbal formulation exhibited significant antihyperlipidemic activity, as evidenced by reductions in TC, TG, and LDL-C levels, along with an increase in HDL-C levels and a reduction in body weight gain compared to the positive control group. These findings suggest that the polyherbal formulation could serve as a basis for developing effective therapeutic combinations with reduced side effects and toxicity. However, further studies are warranted to isolate the active constituents and to fully elucidate the exact mechanisms underlying the antihyperlipidemic and anti-obesity effects of this polyherbal formulation.

Keywords: Hyperlipidemia, Antihypercholesterolemic activity, Polyherbal formulation, Atorvastatin, Sprague Dawley Rats, High fat-diet

INTRODUCTION

There is no doubt that herbal medicines have been utilized since antiquity in health care. The oldest prescription found on Babylonian clay tablets and the hieratic writing of ancient Egyptians on papyrus, archive numerous ancient pharmaceutical and medicinal uses of hundreds of botanicals and foods e.g., olive oil, turpentine, myrrh, opium, castor oil, garlic etc. The earliest Chinese book on medicinal herbs was written by Shen-Nung about 3000 years B.C (1). The Persians, Romans, Greeks, Hebrews, Arabs and other races were all familiar with the use and practice of herbal medicine. In the Indopak subcontinent, about 2000 plants are listed in the ayurvedic, unani and tibbi systems of medicine, and these herbal remedies are still providing the most widely used treatment for the people of this area (2). Lipoproteins are macromoleculer complexes that carry hydrophilic plama lipids, particularly cholesterol and triglycerides, in the plasma. Lipoproteins are spherical in nature made up of hundreds of lipid and protein molecules. They are smaller than red blood cells and visible only in the electron microscopy (3). However,when the larger, triglyceride-rich lipoproteins are present in high concentration, plasma can appear turbid or milky to the naked eye. The major lipids of the lipoproteins are cholesterol, triglycerides and phospholipids. A family of proteins, the apolipoproteins, also occupies the surface of lipoproteins; the apolipoproteins play crucial role in the regulation of lipid-transport and lipoprotein metabolisms (4). Hyperlipidemia a broad term, also called hyperlipoproteinemia, is a common disorder in developed countries and is ranked as one of the major risk factors contributing to the prevalence and severity of coronary heart diseases (5). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (6). Hyperlipidemia is characterized by elevated serum total cholesterol, low-density lipoprotein and very low-density lipoprotein and decreased high-density lipoprotein levels (7). It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins. An other related term "dyslipidaemia" indicating disorders of lipoprotein metabolism, including lipoprotein overproduction or deficiency is increasingly being used now a day to describe abnormal changes in lipid profile, replacing the old term hyperlipidaemia. Any abrupt change in various blood fats and lipoproteins and their deposition in arterial walls of the circulatory system may lead to atherosclerosis and arteriocsclerosis (8). These fats include cholesterol and triglycerides. These are important for our body function but when they

are high, they can cause heart disease and stroke. Hyperlipidemia is manifested as hypercholesterolemia and/or hypertriglycerolemia.

Table 1. Major classes and characteristics of lipopropteins

Lipoprotein Class	Density of flotation (gm/ml)	Major lipid constitutent	TG:TC ratio	Significant Apoproteins	Site of synthesis	Mechanism of catabolism
Chylomicrons and remnants	<<1.006	Dietary triglyceride and cholesterol	10:1	B-48, E, A-I, A-IV, C-I, C-II, C-III	Intestine	Triglyceride hydrolysis by LPL
						ApoE-mediated remnant uptake by liver
VLDL	<1.006	"Endogenous" or hepatic triglycerides	5:1	B-100, E, C-II, C-III	Liver	Triglyceride hydrolysis by IPL
IDL	1.006 - 1.019	Cholesteryl esters and "indugenous" triglycerides	1:1	B-100, E, C-II, C-III	Product of VLDL catabolism	50% converted to LDL mediated by HL, 50% ApoE-mediated uptake by liver
						50% ApoE-mediated uptake by liver
LDL	1.019 - 1.063	Cholesteryl esters	NS	B-100	Product of VLDL catabolism	ApoB-100 mediated uptake by LDL receptor (75 % in liver)
HDL	1.063-1.21	Phospholipids, Cholesteryl esters	NS	A-I, A-II, E, C-I, C-II, C-III	Intestine, Liver, Plasma	Complex
						Transfer of cholesteryl ester to VLDLand IDL
						Uptake of HDL cholesterol by hepatocytes
Lp(a)	1.05-1.09	Cholesteryl ester	NS	B-100, Apo(a)	Liver	Unknown

Hyperlipidemia has been found to be the major risk factor for various diseases such as type II diabetes mellitus, hypertension, cardiovascular diseases, stroke and metabolic syndrome etc. which may be life-threatening in many, if not all, patients (9). The currently available drugs for the treatment of hyperlipidemia include HMG-CoA reductase inhibitors, fibrates, bile acid sequestrants, niacin and

choleaterol absorption inhibitors etc. but these are very costly and are associated with a number of side effects including hyperuricemia, nausea, vomiting, diarrhea, gstric irritation, myositis, flushing, dry skin and abnormal liver functions (10). Research has proved that traditional system has immune potential against various diseases. Thousands of medicinal plants have been evaluated scientifically for their pharmacological properties. Due to severe adverse effects associated with synthetic antihyperlipidemic drugs, physicians as well as patients prefer natural therapeutic agents for the treatment of hyperlipidemia, among which medicinal herbs or plants are more commonly used. The preference for herbal therapy is mainly due to fewer side effects associated with medicinal plants as well as their low cost and easy availability (11).

The aim of the present study is to scientifically evaluate some of the folk remedies commonly used for hyperlipidemia and obesity, thereby leading to discovery of new therapeutic options that would be economical and having lesser side effects. Therefore, a polyherbal formulation composed of *Plantago ovate husk, Trigonella-foenumgraecum seeds, Nigella sative seeds and Camellia sinensis leaves* was selected. Although, individually these medicinal plants have already been studied and evaluated scientifically for their antihyperlipidemic activity but their combined antihyperlipidemic and antiobesity activities in the form of compound formulation have not been studied scientifically still now, which is the objective of this study. One of the reasons for the selection of plants in the polyherbal formulation is based on the fact that these plants, in the form of compound formulation, are most commonly used as antihyperlipidemic and antiobesity agents by different hakims and in different slim centers of District. Bahawalpur. After experimentally evaluating the antihyperlipidemic and antiobesity activities of the polyherbal formulation, we will be having scientific proof for the therapeutic use of this compound formulation as antihyperlipidemic and antiobesity agent, which is the ultimate objective of this study. However, further studies regarding isolation of active constituents of the polyherbal formulation will be very beneficial and fruitful for further research in this field.

METHODOLOGY

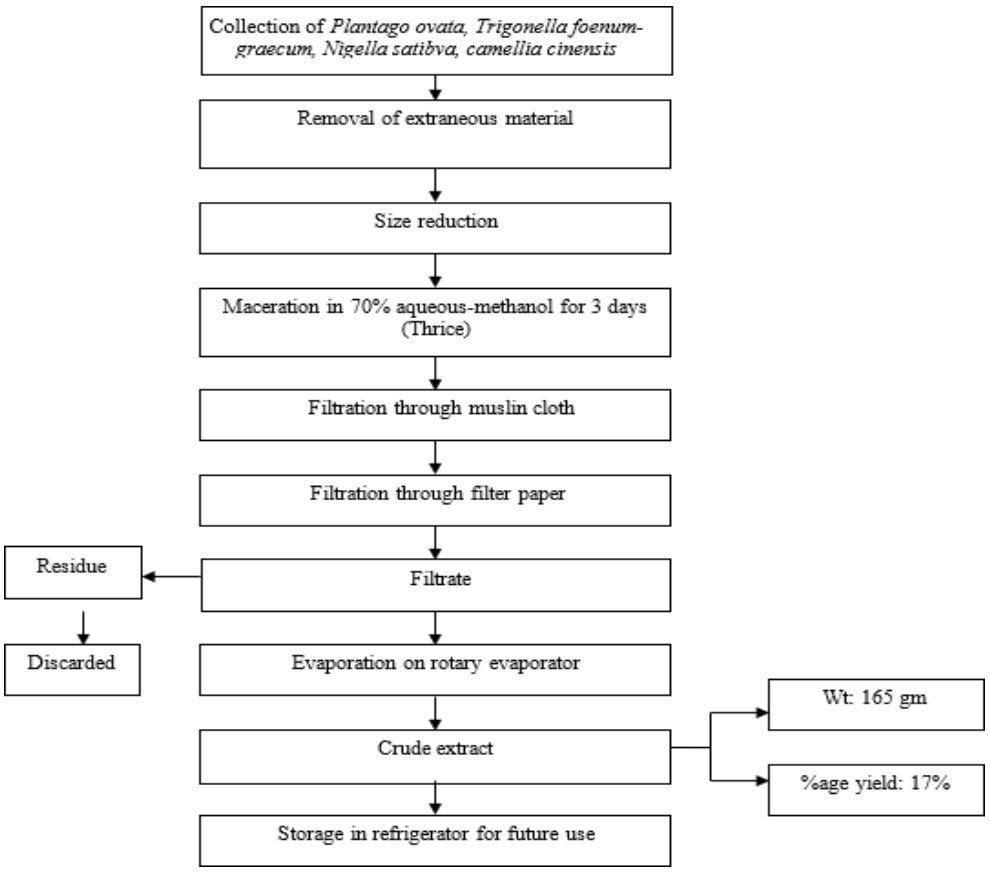
This is a Laboratory based in-vivo experiments involving quantitative and descriptive analysis of data conducted at the Pharmacology Research Laboratory, Facuty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur. Following drugs, organic and in-organic solvents, chemicals and reagents were used during research work. Atorvastatin (Lipitor; Pfizer-USA), Diazepam (Inj.Valium; Roche Pharmaceuticals-Germany) and Ketamine (Inj.Ketalar; Abbott Laboratories-USA) were purchased from a reputed pharmacy in Bahawalpur. Chloroform, methanol, ethanol, petroleum-ether, petroleum-spirit, benzene, hydrochloric acid, sulphuric acid, acetic acid and glacial-acetic acid, sodium chloride, aluminium chloride, ferric chloride, gelatin, ammonium hydroxide, ammonium chloride, potassium chloride and potassium hydroxide. All these chemicals were of analytical grade and were purchased from Merk, Germany. Spectrophotometer, model U2020 (IRMECO, Germany), Vortex mixer, model MyLab SLV6 (seouLin Bioscience, Korea), Centrifuge machine, model EBA-20 (Hettich, Germany), Digital Electronic Balance, model AY62 (Shimadzu, Japan), Rotary evaporator, model Heidolph Laborata-efficient-4000, $_{pH}$ meter, model Inolab $_{p}$H 720 (Germany) and Lab incubator (Gemmy Industrial Corp. Taiwan).

Male and female Sprague-Dawley albino rats of 3month age and weighing about 200-250g and albino mice of either sex weighing between 15-30 g were housed in the animal house of Pharmacology Research Lab, Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur. Animals were grouped and housed in poly-carbonated cages (47x34x18 cm) with not more than six animals per cage under standard laboratory conditions (temperature: 25±2C; humidity: 60±5%) with dark and light cycle of 12hr, were divided into 7 groups of 6 animals in each group. They were allowed free access to standard dry pellet diet and water *ad libitum.* The rats were acclimatized to laboratory conditions for 10 days before commencement of experiment (12). Our experimental protocol was conducted in accordance with the guide-lines approved by Institutional Animal Ethics

Committee of the animal house of Pharmacology Research Lab, Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur.

Different parts of plants were purchased from the vegetable market of Distt:Bahawalpur. Plants were taxonomically identified by authentic botanist. A voucher specimen of each plant was deposited at the herbarium of laboratory of Pharmacology, The Facuty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, for future reference. The voucher numbers are PO-HK-03-11-13, NS-SD-03-11-14, TF-SD-03-11-15 and CS-LS-03-11-16 for *Plantago ovata, Nigella sativa, Trigonella foenumgracum and Camellia sinensis* respectively. After the process of extraction, preliminary phytochemical screening of the poly-herbal crude extract, to detect the presence of secondary metabolites such as alkaloids, saponins, anthraquinones, coumarins, flavonoids, tannins and phenolic compounds and glycosides, was carried out according to standard procedures as reported by Kokate and J.B Harbone.

Fig. 1. Schematic diagram for the preparation of 70 % aqueous-methanolic crude extract of poly-herbal formulation (Ph.Cr)



The normal diet was prepared at animal house of Pharmacology Research Lab, Department of Pharmacology, The Islamia university of Bahawalpur.

Table 2. Composition of 01 Kg of normal diet

Ingredients	Quantity
Poultry feed	500 gm
Chokar	300 gm
Needo	200 gm

The high fat diet was prepared by adding 1% w/w cholesterol (Riedel-De-Haen, Germany) in normal diet.

Table 3. Compopsition of 01 Kg of high fat diet

Ingredients	Quantity
Poultry feed	500 gm
Chokar	300 gm
Needo	200 gm
Cholesterol	10 gm

Animal study Design

For antihyperlipidemic effect of poly-herbal formulation, rats were divided into 7 groups, each group consisting of 6 animals- 3 males and 3 females. The description of each group is as follws:

Group-I: Normal Control Rats were fed on normal diet and normal saline (1ml/Kg/day p.o.) throughout the whole experimental period.

Group-II: Positive Control Rats were fed on atherogenic (High fat) diet and normal saline (1ml/Kg/day p.o.) throughout the whole experimental period.

Group-III: Standard Control Besides the atherogenic diet, rats also received Atorvastatin (5mg/Kg/day p.o.) throughout the whole experimental period.

Group-IV: Treated Group-1 Besides the atherogenic diet, rats also received Ph.Cr (30mg/Kg/day p.o.) throughout the whole experimental period.

Group-V: Treated Group-1I Besides the atherogenic diet, rats also received Ph.Cr (100mg/Kg/day p.o.) throughout the whole experimental period.

Group-VI: Treated Group-1II Besides the atherogenic diet, rats also received Ph.Cr (200mg/Kg/day p.o.) throughout the whole experimental period.

Group-VII: Treated Group-1V Besides the atherogenic diet, rats also received Ph.Cr (300mg/Kg/day p.o.) throughout the whole experimental period.

Atorvastatin was used as standard drug in this research work because Atorvastatin is more efficacious than other statins (like simvastatin) for controlling hypercholesterolemia and also for treating cardiovascular events (13). All doses were prepared in normal saline (0.9 % NaCl solution).

Collection of Blood and Serum Samples After 28 days of treatment, blood samples of all animals were collected by cardiac puncture under mild anesthesia with Ketamine 50 mg/Kg/i.p. (inj.Katalar) and Diazepam 5mg/Kg/i.p (Inj.Valium). Blood was allowed to clot at room temperature for 15 minutes. Aters 15 minutes, the clotted samples were centrifuged for 15 minutes at the speed of 5000 rpm using centrifugation machine (Hettich zentrifugen-Germany). The sera thus collected were used for various biochemical experiments.

Biochemical Analysis Biochemical assays for the determination of serum total cholesterol (TC), serum triglycerides (TG), serum low-density lipoproteins (LDL), and serum high-density lipoproteins (HDL) were done by using standard kit method.

Results

Acute toxicity test was performed on albino mice weighing about 18-26 gm. Mice were kept in poly-carbonated cages. The crude extract was given in the doses of 300mg/Kg, 1000mg/Kg and 5000 mg/Kg of body weight orally to different groups of mice, each group consisting of six mice. The behaviors of animals were observed for 2h, 4h, 6h, 8h, 12h and 24h. The no. of animals survived after 24 and 48 hr were also noted. A normal control was also run parallel which was receiving normal saline (10 ml/Kg). The mice received tap water and normal diet *ad labitum* (14, 15). It was observed that all the animals survived after 24hr and even after 48hr. Hence, it was proved from the acute toxicity test that the crude extract is quite safe and having no acute toxicity up to the dose of 5g/Kg of body-weight orally.

Table 4. Results of phytochemical analysis of crude extract of polyherbal formulation

Phytoconstituents	Results
Alkaloids	+
Saponins	++
Anthraquinones	+
Coumarins	-
Flavonoids	++
Tannins & Phenolic compounds	+
Glycosides	+

(+) & (-) signs indicate presence and absence of phytochemical constituents of the crude extract of poly-herbal formulation respectivelyThe poly-herbal formulation (containing 250 gm *Plantago ovata, 250 gm Trigonella foenum graecum, 250 gm Nigella sativa and 250 gm Camellia sinens)* was investigated for antihypercholesterolemic activity at dose level of 30 mg/Kg, 100 mg/Kg, 200 mg/Kg and 300 mg/Kg body weight in adult Sprague-dawley albino rats. The 70 % aquoues methanolic extract of poly-herbal formulation was found to result in significant attenuation in lipid-profile of all the treated rats towards the normal level, which proves the hypocholesterolemic effect of the poly-herbal formulation. The poly-herbal formulation showed significant hypolipidemic activity when compared to Atorvastatin 5 mg/Kg body weight treated group (standard group) in a dose-dependent manner.

Table 5. Effect of Aqueous Methanolic Extract of poly-herbal Formulation

Sr. #	Animal Groups	Serum Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)	Serum HDL-C (mg/dl)	Serum LDL-C (mg/dl)	Change in Body-Weight	P-Value
1	Normal Control (N.D & tap water ad labitum)	122.0 ± 2.582	125.3 ± 2.963	55.17 ± 1.682	79.00 ± 2.582	22.50 ± 0.4282	----------
2	Positive Control (HFD+Normal Saline)	153.7 ± 3.461	145.0 ± 2.352	43.50 ± 1.088 N	128.7 ± 3.461	27.17 ± 0.6009	----------
3	Standard Control (HFD+Atorvastatin 5mg/Kg)	115.0 ± 2.696	118.3 ± 2.171	70.00 ± 1.390	69.17 ± 3.361	20.17 ± 0.6009	< 0.0001
4	Ph.Cr (I) (HFD+30 mg/Kg p.o)	125.0 ± 3.011	131.0 ± 1.549	63.00 ± 2.129	82.00 ± 3.011	22.50 ± 0.4282	< 0.0001
5	Ph.Cr (II)	114.2 ± 1.887	123.5 ± 1.875	66.83 ± 2.301	71.17 ± 1.887	18.33 ± 0.4944	< 0.0001

	(HFD+100 mg/Kg p.o)						
6	Ph.Cr (III) (HFD+200 mg/Kg p.o)	106.2 ± 2.212	118.8 ± 1.249	78.00 ± 1.438	64.67 ± 1.856	16.33 ± 0.4216	< 0.0001
7	Ph.Cr (IV) (HFD+300 mg/Kg p.o)	99.50 ± 1.839	110.2 ± 2.040	79.50 ± 1.668	54.50 ± 1.204	14.33 ± 0.3333	< 0.0001

ND = Normal Diet; HFD = high Fat Diet; n = Number of animals

Conclusion of the Study And Discussion

Dietry cholesterol has been widely used to induce acute hyperlipidemia and atherosclerosis in research animals (16). This model is commonly used for a no. of different aims particularly; this has been used for screening natural as well as chemical substances as antihyperlipidemic drugs in rats. Interestingly, the results of the present study showed that the extract of our poly-herbal formulation resulted in significant attenuation of lipid profile towards normal level and also it reversed cholesterol induced hyperlipidemia in rats.

The study clearly shows that methanolic extract of poly-herbal formulation at dose level of 30mg/Kg, 100mg/Kg, 200mg/Kg and 300mg/Kg of body-weight significantly decreased total cholesterol, triglycerides and LDL-C levels, at the same time, it caused significant increase in HDL-C. From the results, it may be concluded that the significant decrease in total cholesterol and triglycerides is due to an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism. The reduction of total cholesterol was associated with a decrease of its LDL fraction, which is the target of several antihyperlipidemic drugs. This result suggests that antihypercholesterolemic activity of the poly-herbal formulation may be due to rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids (17).

It has been proved scientifically that HDL-C levels have protective role in coronary artery disease (Wilson et al., 1988). Similarly increased serum LDL-C levels are important risk factor for the development of atherosclerosis (18).The increased level of HDL-C as well as decreased level of total cholesterol along with its LDL fraction, which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastrointestinal tract.

According to Johnson et al (1986), some saponins increase the permeability of intestinal mucosal cells in-vitro, inhibiting active mucosal transport and facilitating uptake of substances that are not absorbed normally. Gee et al (1989) concluded that saponins also decrease transmural potential difference across the small intestine of the rats. Flavonoids exhibit different pharmacological properties including anti-atherogenesis and anti-oxidant effect (19). Saponins have the property of reducing hypercholesterolemia, as a consequence of inhibition of dietary cholesterol absorption (20). The result strongly suggests that the antihypercholesterolemic activity of poly-herbal crude extract could be attributed to the presence of valuable valuable flavonoids and saponins in the poly-herbal crude extract. Finally, it is concluded that administration of 70 % aquoues methanolic extract of poly-herbal formulation (containing *Plantago ovata, Nigella sative, Trigonella foenumgracum and Camellia sinensis*) showed significant hypolipidemic activity. The poly-herbal formulation would be more effective as compared to any other locally available compound herbal formulation for controllind hyperlipidemia and preventing cardio-vascular as well as other hyperlipidemia associated disorders. Also, the poly-herbal crude extract contains important secondary metabolites such as saponins and flavonoids etc. Such active ingredients may recover various disorders in lipid metabolism as noted in hyperlipidemic state. However, further research to isolate the active constituents and to explore the exact mechanism of action regarding antihyperlipidemic activity of the poly-herbal crude extract would be very beneficial in natural product research.

References

1. Rahmatullah M, Sultan S, Toma T, Lucky S, Chowdhury M, Haque W, et al. Effect of Cuscuta reflexa stem and Calotropis procera leaf extracts on glucose tolerance in glucose-induced hyperglycemic rats and mice. African Journal of Traditional, Complementary and Alternative Medicines. 2010;7(2).
2. Adhirajan N, DIXIT VK, CHANDRAKASAN G. Development and evaluation of herbal formulations for hair growth. Indian drugs. 2001;38(11):559-63.
3. Rajakumar N, Shivanna M. Traditional herbal medicinal knowledge in Sagar taluk of Shimoga district, Karnataka, India. 2010.
4. Maruyama T, Yamashita S, Matsuzawa Y, Bujo H, Takahashi K, Saito Y, et al. Mutations in Japanese Subjects with Primary Hyperlipidemia—Results from the Research Committee of the Ministry of Health and Welfare of Japan since 1996. Journal of atherosclerosis and thrombosis. 2004;11(3):131-45.
5. SALONEN JT, PUSKA P. Relation of serum cholesterol and triglycerides to the risk of acute myocardial infarction, cerebral stroke and death in eastern Finnish male population. International Journal of Epidemiology. 1983;12(1):26-31.
6. Ganji SH, Tavintharan S, Zhu D, Xing Y, Kamanna VS, Kashyap ML. Niacin noncompetitively inhibits DGAT2 but not DGAT1 activity in HepG2 cells1. Journal of lipid research. 2004;45(10):1835-45.
7. Ahmad J, Farooqui A, Ahmad S. Trianthema portulacastrum L., an herbal drug for the cure of edema. Journal of herbs, spices & medicinal plants. 2000;7(2):65-70.
8. Sanmugapriya E, Venkataraman S. Toxicological investigations on Strychnos potatorum Linn seeds in experimental animal models. Journal of health science. 2006;52(4):339-43.
9. Qureshi R, Waheed A, Arshad M, Umbreen T. Medico-ethnobotanical inventory of tehsil Chakwal, Pakistan. Pak J Bot. 2009;41(2):529-38.
10. Pal D, Mandal M, Senthilkumar G, Padhiari A. Antibacterial activity of Cuscuta reflexa stem and Corchorus olitorius seed. Fitoterapia. 2006;77(7-8):589-91.
11. Omar MA, Wilson JP, Cox TS. Rhabdomyolysis and HMG-CoA reductase inhibitors. Annals of Pharmacotherapy. 2001;35(9):1096-107.
12. Surkar A, Lavania S, Pandey D, Pant M. Changes in the blood lipid profile after administration of Ocimum sanctum (Tulsi) leaves in the normal albino rabbits. Indian Journal of Physiology and Pharmacology. 1994;38:311-.
13. Jacobson TA, Wertz DA, Hoy T, Kuznik A, Grochulski D, Cziraky M, editors. Comparison of cardiovascular event rates in patients without cardiovascular disease in whom atorvastatin or simvastatin was newly initiated. Mayo Clinic Proceedings; 2008: Elsevier.
14. Schwartz ML. Severe reversible hyperglycemia as a consequence of niacin therapy. Archives of internal medicine. 1993;153(17):2050-2.
15. Gilani AH, Jabeen Q, Khan A-u, Shah AJ. Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom. Journal of ethnopharmacology. 2008;115(3):463-72.
16. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry. 1982;28(10):2077-80.
17. Khanna A, Chander R, Singh C, Srivastava A, Kapoor N. Hypolipidemic activity of Achyranthus aspera Linn in normal and triton induced hyperlipemic rats. Indian Journal of Experimental Biology. 1992;30(2):128-30.
18. McDonald SD, Walker MC, Ohlsson A, Murphy KE, Beyene J, Perkins SL. The effect of tobacco exposure on maternal and fetal thyroid function. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008;140(1):38-42.
19. Bakker-Arkema RG, Davidson MH, Goldstein RJ, Davignon J, Isaacsohn JL, Weiss SR, et al. Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. Jama. 1996;275(2):128-33.

20. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. The American journal of clinical nutrition. 2005;81(2):397-408.