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EFFECT OF POLYHERBAL FORMULATION (OB-6) ON HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

From time immemorial plants have been used as a source for health care by human society. In this study, a polyherbal formulation (OB-6) was prepared using extracts of six medicinal plants such as *Cassia angustifolia, Nigella sativa, Phyllanthus amarus*, *Emblica officinalis, Zingiber officinale* and *Terminalia chebula*. The present study is undertaken to evaluate Anti-hyperlipidemic potentials through *in-vivo* methods of the polyherbal formulation prepared in-house. The rats were fed with high fat diet for the induction of hyperlipidemia for two weeks. Upon confirmation of disease induction the animal experiments were conducted as per standard protocols. The hyperlipidemic rats were administered three graded doses of OB-6 and the standard control group was treated with Atorvastatin (30 mg/kg) orally for 14 days. After treatment for ten days, blood samples from the OB-6/standard/vehicle were collected and lipid profile, atherogenic index and atherogenic ratio were determined. After sacrificing the animal, histopathological study suggested that OB-6 produced significant hypocholesterolemic effect.

Keywords: High fat diet, Hyperlipidemia, Lipid profile, Polyherbal formulation.

INTRODUCTION

Cardiovascular diseases (CVD) account for 29% deaths worldwide in 2005[1]. The major risk factor for CVD is hypertension, hypercholesterolemia, diabetics and obesity. Hypertensive individual number in India is projected to increase two fold by 2025[2]. Hyperlipidemia contributes drastic threat towards the spread and expansion of atherosclerosis and coronary heart diseases (CHD). Significant impairment of lipid profiles is responsible for the onset of CHD. Lipid lowering drugs, mostly statins and fibric acid derivatives have been widely used to manage the elevated levels of various forms of lipids in hyperlipidemia patients. Due to its serious complications, these drugs have to be used safely or avoided when possible[3]. In recent years, the development of lipid lowering drug or formulation from natural source has gained importance. Hence an attempt is made to develop a polyherbal formulation (OB-6) containing the extracts of *Cassia angustifolia, Nigella sativa, Phyllanthus amarus*, *Emblica officinalis, Zingiber officinale* and *Terminalia chebula* and to evaluate its anti-hyperlipidemic action.

MATERIALS AND METHODS

Plant materials collection and extraction

All the six plant materials used in the formulation were collected from an authorized raw Drug dealer, Thanjavur, Tamilnadu, India. The plant materials were properly authenticated with the help of Pharmacognosy department of Drug Testing Laboratory, CARISM, SASTRA University, Thanjavur and shade dried, coarsely powdered using mechanical grinder. The rhizome of Zingiber officinale was procured from vegetable market, Thanjavur. The Cassia angustifolia leaves, Zingiber officinale rhizome, fruits of Emblica officinalis and Terminalia chebula were extracted with distilled water individually by cold maceration process for three days. The roots and leaves of Phyllanthus amarus and Nigella sativa seeds were soaked individually with ethyl alcohol for three days by cold maceration method. The extract was filtered and concentrated under vacuum. The crude extract obtained was stored in refrigerator for further use. The formulation prepared by mixing extracts of six plant ingredients as specified in Table 1.

Drugs and chemicals

The cholesterol and cholic acid were purchased from Himedia Laboratories, Mumbai. The biochemical kits used for the estimations were brought from Reckon Diagnostics Pvt. Ltd., Vadodara, India.

Preparation of Polyherbal Formulation (OB-6)

Preliminary phytochemical analysis

A portion (2 g) of the polyherbal formulation was used for the preliminary phytochemical analysis, which includes tests for saponins, tannins, glycosides, alkaloids, volatile oils, flavonoids, steroids, terpenoids, vitamin c, aminoacids and carbohydrates in accordance with the methods of Trease and Harborne[4,5].

Table 1: The ingredients of the polyherbal formulation (OB-6) under study

Sl. No	Plant name	Part used	Extract used	% g
1	*Cassia angustifolia Vahl.*	Leaves	Aqueous	17.1
2	*Nigella sativa Linn.*	Seeds	Ethanolic	1.7
3	*Phyllanthus amarus Linn.*	Roots &leaves	Ethanolic	1.7
4	*Zingiber officinale Rosc.*	Rhizome	Aqueous	34.5
5	*Emblica officinalis Gaerth.*	Fruits	Aqueous	22.5
6	*Terminalia chebula Retz.*	Fruits	Aqueous	22.5

The reducing power of the formulation

The reducing power of the polyherbal formulation (OB-6) was evaluated by the procedure of Oyaizu[6].

Estimation of total phenol

The amount of total phenol in the polyherbal formulation (OB-6) was determined using Folin-Ciocalteu reagent as per the method of Spanos[7].

Experimental animals

Wistar rats weighing 180±20 g were used as experimental animals and maintained as per standard guidelines in accordance with Institutional Animal Ethics Committee (IAEC) regulations and approved by CPCSEA. (IAEC approval number: 86/SASTRA/IAEC/RPP)

Induction of hyperlipidemia

The high fat diet (HFD) was prepared with a mixture of 2% (w/w) cholesterol and 0.5% (w/w) cholic acid in standard animal chow and administered for 4 weeks except for the normal control group which was fed with standard chow only. At the end of 4th week, total cholesterol level in serum was estimated and the animal with greater than 250 mg/dl level was selected and considered as hyperlipidemic rats[8].

Experimental design

The animals were grouped into 6 groups each containing 8 rats.

Group I: Normal control fed with standard diet.

Group II: Hyperlipidemic rats

Group III: Hyperlipidemic rats treated with Atorvastatin (30 mg/kg) orally for 2 weeks.

Group IV: Hyperlipidemic rats treated with OB-6 (100 mg/kg) orally for 2 weeks.

Group V: Hyperlipidemic rats treated with OB-6 (200 mg/kg) orally for 2 weeks.

Group VI: Hyperlipidemic rats treated with OB-6 (400 mg/kg) orally for 2 weeks.

Blood samples after 24 hours of last dose was collected from retro-orbital plexus and allowed to coagulate at room temperature which was then centrifuged at 3000 rpm for 10 minutes. The serum was separated and used for the biochemical estimations. Finally, the rats were sacrificed, liver isolated, washed with cold saline, weighed and finally fixed in 10% buffered formalin solution for histopathological studies. The fixed tissue was embedded in paraffin and the sections were cut in 3-5µm slices and were stained using haematoxylin and eosin. The stained tissue observed under light microscope.

Estimation of serum lipid profile

Serum lipid profile was carried out using standard protocols[9,10].

Statistical Analysis

Statistical analysis was done using ANOVA[11].

RESULTS

The data obtained on the preliminary phytochemical analysis was given in Table 1.

Table 2: Preliminary phytochemical analysis of polyherbal formulation (OB-6)

Tests	Result
Saponins (Froth test)	-
Tannins (Ferric chloride test)	+
Phenol	+
Glycosides (General test)	+
Cardiac glycosides	+
Alkaloids (Hager's test)	-
Volatile oils	+
Hydrosable Tannins	-
Flavonoids (Alkaline reagent test)	+
Steroids (Libermann Burchard Test)	-
Terpenoids (Salkowski test)	+
Vitamin-C (Sodium nitroprusside test)	+
Amino acid & Proteins (Ninhydrin test)	-
Carbohydrates (Fehling's test)	+

The potential of the OB-6 formulation and vitamin C in the manifestation of reducing power were evaluated and presented in Fig. 1

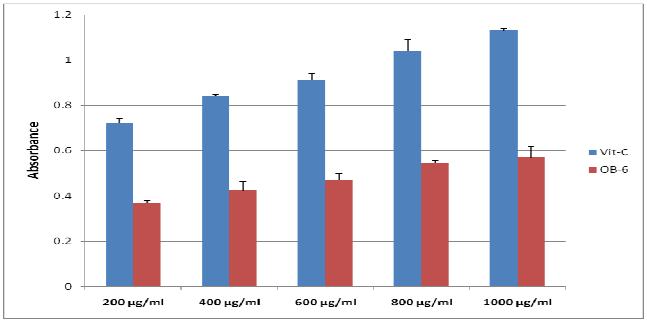


Fig 1: The reducing power potential of the polyherbal formulation (OB-6)

Table 3: Effect of polyherbal formulation (OB-6) on lipid profile in HFD induced hyperlipidemic rats

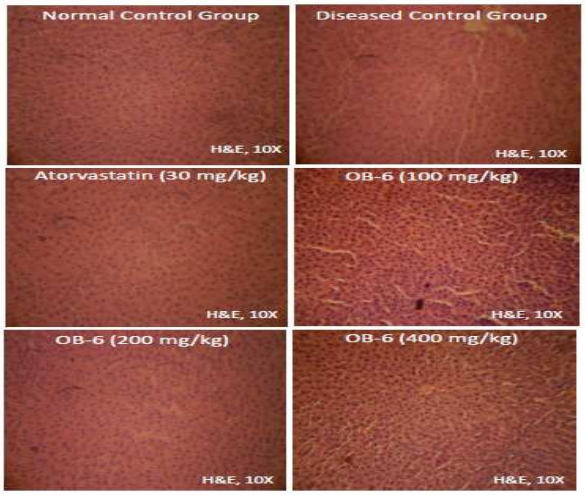
Treatment	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control	179.03±3.40	11.08±0.32	52.00±0.57	102.70±1.70	19.50±0.22
HFD induced Hyperlipidemia	426.70±16.05*,#	6.16±0.33*,#	67.83±0.94*,#	180.24±2.34*,#	32.83±0.60*,#
Atorvastatin (30 mg/kg)	201.70±4.66	10.68±0.17	54.17±1.07	110.30±8.10	21.67±0.66
OB-6 (100 mg/kg)	345.20±14.97*,#	9.50±0.27*,#	64.00±1.21*,#	153.50±3.43*,#	30.00±0.57*,#
OB-6 (200 mg/kg)	204.70±5.07	10.40±0.17	61.17±1.47*,#	119.28±3.25*	23.33±0.55*
OB-6 (400 mg/kg)	241.50±12.77*	9.71±0.28*	57.17±0.47*	131.57±2.01*,#	25.50±0.76*,#

Values as mean ± SEM. n=8, One Way ANOVA followed by Dunnett's multiple comparison tests.*p<0.05 when compared with normal control group, #p<0.05 when compared with standard drug (Atorvastatin) treated group.

Table 4: Effect of OB-6 on atherogenic index, atherogenic ratio and hepatic index in HFD induced hyperlipidemic rats

Treatment	Atherogenic index	Atherogenic Ratio	Hepatic index
Normal control	0.25±0.04	16.20±1.02	3.00±0.1
HFD induced Hyperlipidemia	0.73±0.07*,#	70.18±10.67*,#	3.71±0.4
Atorvastatin (30 mg/kg)	0.31±0.04	18.90±1.30	3.50±0.8
OB-6 (100 mg/kg)	0.50±0.04*,#	36.33±2.75*	3.14±0.3
OB-6 (200 mg/kg)	0.35±0.04	19.69±1.16	3.16±0.9
OB-6 (400 mg/kg)	0.42±0.04	24.98±3.98	2.7±0.5

Values as mean ± SEM. n=8, One Way ANOVA followed by Dunnett's multiple comparison tests.*p<0.05 when compared with normal control group, #p<0.05 when compared with standard drug (Atorvastatin) treated group.



Histopathological studies

Fig. 2: Histology of liver tissues (H&E Staining) with 10X magnification in rats at the terminal of 5th week treatment.

RESULTS AND DISCUSSIONS

Hyperlipidemia is a major contributor for health problems worldwide and leads especially to atherosclerosis, resulting in coronary heart diseases (CHD). According to WHO by 2020, 60% of the cardiovascular cases will be of Indian origin[12]. Hyperlipidemia induces the damages in various tissues, which in turn, alters the cellular functions leading to cell damage and many pathological conditions[13]. A high-fat diet may cause elevated levels of cholesterol, which ultimately leads to obesity. Elevated cholesterol level particularly LDL, VLDL increases the risk of cardiovascular diseases particularly coronary heart disease (CHD)[14]. Increase in HDL cholesterol reduces the risk of CHD[15,16]**.** Reduction of 1% cholesterol can lead to 2-3% reduction of CHD risk[17]. The importance of medicinal plants in the treatment of hyperlipidemia was experimentally studied in recent years, where oxidative stress induced apoptosis in adipose tissue was noticed[18,19]. Medicinal plants have been used to control the lipid level and also used as an inhibitor to trigger oxidative stress which reduces adipose tissue apoptosis.

Cassia angustifolia has been accepted as an official species in the British pharmacopoeia for its strong purgative property[20]. *In-vitro* and *in-vivo* studies on flavonoids from *Emblica officinalis* revealed reduction in serum and tissue lipid levels of hyperlipidemic rats. Besides they also possess good anti-oxidant and cardio-protective properties[21,22,23,24]. *Nigella sativa L.* has been scientifically reported for its hepatoprotective, neuroprotective and antioxidant effects[25,26]. Aqueous alcoholic extract of *Phyllanthus amarus* contains alkaloids, flavonoids, saponins and tannins which are responsible for the application of wide range of pathological conditions and these include hypoglycemic and hypolipidemic[27,28]**.** Nail et al., have published the antioxidant property of water extract of *Terminalia chebula*[29]. Preclinical evaluation with ginger has revealed antioxidant and hypolipidemic effects[31,32]. Previous studies have also confirmed that the presence of phytoconstituents like flavonoids, alkaloids, saponin and tannins in extract or as isolated compound might contribute towards hypolipidemic activity[33]. Based on these informations, the polyherbal formulation was prepared using extracts of above mentioned six medicinal plants which are known to possess hypolipidemic potentials and antioxidant properties and evaluated scientifically for these potentials using Wistar rats as experimental animal models.

The presence of tannin, flavanoids and vitamin C might be responsible for the reducing capacity of the formulation (Table 2). Further the total phenolic content in polyherbal formulation (OB-6) was determined as 102 mg GAE/g of dry extract which further provided chemical evidences in proving that the formulation is capable of donating electrons.

Joris et al. reported that rats were hyporesponsive to dietary cholesterol alone, so cholic acid is added in order to induce hyperlipidemia[34]. The high fat diet administered in present study includes HFD for effective hyperlipidemia induction. The significant ($p<0.05$) change in lipid profile noticed in the experimental animals confirmed the induction of hyperlipidemia in HFD fed rats (Table 3). High fat diet increased triglycerides level and leads to hardening of arteries[34,35]. The present study showed that HFD significantly ($p<0.05$) increased TG level when compared with standard pellet treated rats. Treatment with OB-6 at the different dose levels (100, 200 and 400 mg/kg) for 14 days showed significant ($p<0.05$) decrease in triglyceride level. The polyherbal formulation at the dose level of 200 mg/kg, b.wt reduced significantly the serum TG level in hyperlipidemic rats compared to Atorvastatin treated animals.

HDL is a beneficial lipoprotein synthesized in intestine and liver which protects the system from the pathogenesis of atherosclerosis[36]. In the present study, it is noticed that HDL cholesterol level in serum increased significantly ($p<0.05$) in OB-6

treated hyperlipidemic rats. Increase in LDL level causes deposition of cholesterol in the arteries and aorta and hence is a leads to CHD. LDL transports cholesterol from the liver to the periphery[37,38]. The fortification of LDL from oxidation and decrease in oxidative stress might therefore be useful for prevention of atherosclerosis associated CVD[39]. In the present study administration of OB-6 at three different dose levels effectively reduced LDL cholesterol content of hyperlipidemic rats. For a good lipid lowering therapy, a drug should be able to significantly lower LDL and increase HDL cholesterol concentration and this appreciably decreases the fatty cytoplasmic vaculated cells in liver parenchyma and prevents hepatic necrosis and this correlates with the present study[40]. Reduced LDL and increased HDL concentration were observed in the present study, thereby suggesting that this formulation could be used as a good lipid lowering therapeutic agent.

VLDL particles contain less protein and are rich in triglycerides. VLDL is less harmful but still can damage the arterial lining and their production in body is directly related to the body fat, as their elevation in blood leads to hypercholesterolemia[41]. The level of VLDL of hyperlipidimic rats increased significantly ($p<0.05$) compared with standard diet fed rats. After treatment with polyherbal formulation, the VLDL level reduced significantly ($p<0.05$). Atherogenic index (AI) signifies the deposition as foam cells, plaque or fatty infiltration in circulatory system. An increased atherogenic index indicates high risk of susceptibility of heart and kidney to oxidative damage[42]. In the present study, treatment with OB-6 at the dose of 200 and 400 mg/kg indicated significant ($p<0.05$) decrease in both atherogenic index and ratio, thus indicating the protective role of test formulation against atherogenesis.

The histopathological observation of liver revealed the accumulation of triglycerides and fatty changes. The administration of OB-6 reversed the pathological changes and brought back the normal architecture of the liver.

To sum up, the effect of polyherbal formulation (OB-6) was studied in experimental rats, where hyperlipidemia was induced through high fat diet. The administration of OB-6 to the hyperlipidemic rats significantly reduced total cholesterol, TG, VLDL, LDL and atherogenic index. The formulation (OB-6) revealed maximum protective effect at a dose of 200 mg/kg in comparison with 100 and 400 mg/kg and the results were comparable with standard drug. Further in-depth studies can result in the development of an effective herbal anti-obesity drug.

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