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AMELIORATIVE EFFECTS OF POLYHERBAL EXTRACT ON HIGH FAT DIET HYPERLIPIDEMIA IN RATS

Mangrulkar Shubhada[1*], Korewar Sukanya[2], Gadkari Pratik[2], Mazumdar Priyanka[2], Charde Twinkle[2], Chaple Dinesh[3]

[1]Assistant Professor, Department of Pharmacology, Priyadarshini J. L. College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India.

[2] Research Scholar, Department of Pharmacology, Priyadarshini J. L. College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India.

[3]Principal, Priyadarshini J.L. College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India.

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Corresponding author: Mrs. S.V. Mangrulkar
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Abstract

Hypercholesterolemia is extremely common in the general population, is regarded as a high-risk factor for several health problems in this 21st century. This may be due to increased trends towards the western lifestyle and high cholesterol-rich diet intake. Drug based cholesterol control is a common norm in allopathic treatment, though there are several side effects. While many efficient lipid-lowering synthetic drugs exist, none is in effect for all lipoprotein disorders, and all such agents are accompanying with some adverse effects. The present study was intended to investigate the anti-hyperlipidemic activity of polyherbal extract in High Fat Diet (HFD) induced hyperlipidemia in wistar rats in an attempt to establish traditional use of the different components of this polyherbal extract. In hyperlipidemic rats, it has been observed that there is a gradual increase in the body weight of animals. Moreover, HFD fed group showed significant change in lipid profile and serum liver biomarker enzyme levels. However, high dose of polyherbal extract showed significant improvement in liver biomarker enzyme levels, lipid profile and liver histopathology. In this study, the mechanism of action of polyherbal extract correlates with anti-hyperlipidemic drugs that decrease the synthesis of triglycerides which ultimately decreased the lipid level in blood. Hence, polyherbal extract (PHE) at higher dose (1500mg/kg) can be used to treat hyperlipidemia and related complications.

Keywords: Hyperlipidemia, polyherbal extract, High fat diet, lipid profile, liver histopathology

Introduction

In 2002 cardiovascular diseases (CVD's) contributed to around a third of entire global deaths, whereas by the year 2020. It is expected that CVD's will become the major cause of death and debility worldwide [1]. Hyperlipidemia is a major risk factor for atherosclerosis. Other complications are coronary heart disease, ischemic cerebrovascular disease, hypertension, obesity, and diabetes mellitus (Type -II). Though many efficient lipid-lowering synthetic drugs exist, none is in effect for all lipoprotein disorders, and all such agents are allied with some antagonistic effects [2]. Therefore it is a necessity of the day to search other constituents from natural sources that are less toxic, less expensive, which can offer better safety and efficacy on long term usage. Natural products from plants are a high source used for centuries to therapy for various diseases. Over the last few years, the variations in the lifestyle, predominantly the westernization of the diet and a relatively inactive lifestyle have led to an increased frequency of lifestyle-related disorders such as hyperlipidemia, diabetes mellitus, and atherosclerosis [3]. The principal metabolic causes of atherosclerosis include hyperlipidemia, hypertension, obesity, insulin resistance, and diabetes mellitus [4]. Risk factors for the above are the following, Smoking, hypertension, serum cholesterol, genetic factors, physical activity, hormones, renal disease, alcohol, thyroid disease, and liver disease [5]. Since synthetic drugs have been shown to have side effects, the clinical importance of herbal drugs in the management of hyperlipidemia has received significant attention in recent years [6]. Various medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesterolemic properties [7, 8]. WHO has recommended the use of indigenous plants as an alternative remedy especially in developing countries [9]. Agreeing to the traditional system of Indian remedy, a combination of constituents is used to enrich the desired

activity and eradicate unwanted side effects. Hence polyherbal formulations such as D-400[10], Trasina [11], Cogent db [12], Diasulin [13], and Garlip [14] are commonly used for their antihyperlipidemic effect. The hypolipidemic effect was observed in the plants such as Commiphora Mukul, Hibiscus Rosa Sinensis, Emblica Officinalis, Terminalia arjuna, Trigonella foenum graecum.

Materials and Methods

Animals

The animal experiments were conducted at the animal house of Priyadarshini J. L College of Pharmacy, Nagpur, India. CPCSEA approval as per the internationally accepted principles for laboratory animal use and approved by the Institutional Animal Ethics Committee. Adult Swiss Albino rats of either sex (150- 200g each) were housed separately and maintained under standardize condition (12 hrs light-dark cycle), and provided free access to direct and purified drinking water ad libitum. All experimental procedures were carried out under strict compliance with the institutional Animal Ethics Committee and Committee for the purpose of control and supervision of experimental, Animals (CPCSEA) (Proposal No. PJLCP/2017/10), Ministry of environment and forest, Government of India, New Delhi. Every possible effort was made to reduce the suffering of animals in all experimental designs.

Procurement of plant materials

Crude drugs were procured from Wagh Brothers, Nagpur. It was dried under shade at room temperature and coarsely powdered with the help of a grinder.

Preparation of crude extract

Different parts of herbs *i.e. Hibiscus Rosa Sinensis* (flowers), *Emblica Officinalis* (fruit), *Terminalia arjuna* (bark), *Trigonella foenum-graecum* (seeds) were separately weighed. About 50 g of the coarsely powdered drug and kept in a beaker to which 250 ml of ethanol was added. The mixture was shaken properly for 6 hrs and kept at room temperature for 7 days for extraction. The mixture was filtered through filter paper and the filtrate was evaporated using evaporator at 40-45ºC.The finely powdered *Commiphora Mukul* was suspended in tween 80 at the time of administration with another mixture of extract. Percentage yield was calculated for each extract.

Preliminary phytochemical screening

Preliminary screening tests of the extract was carried out for various plant constituents. The crude extract was tested for the presence or absence of secondary metabolites such as alkaloids, steroids, phenolic compounds, flavonoids, saponins, Tannins, and Anthraquinone glycosides using standard procedure [15-18]

Composition of polyherbal extract

The polyherbal extract contains a particular quantity of herbal drugs given in table 1 were weighed and made by using tween 80 as suspending agent and volume were made of by using saline and used further for animal study.

Table 1: Composition of Polyherbal extract

Sr. No.	Botanical name	Part used	Concentration
1	*Commiphora mukul*	Gum resin	31.25mg/g
2	*Terminalia arjuna*	Bark	31.25mg/g
3	*Hibiscus rosa sinensis*	Flowers	312.5mg/g
4	*Emblica officinalis*	Fruits	312.5mg/g
5	*Trigonella foenum graecum*	Seed	312.5mg/g

Acute Oral Toxicity Studies

An acute oral toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines (acute toxic class method).Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which single dose of PHE was administered orally up to the dose level of 5000 mg/kg body weight per oral [19]. Animals were observed for any sign of discomfort or mortality for 14 days.

Experimental design

Animals were randomly divided into six groups of six animals each and were treated as follows.

Group 1: Normal Pellet diet (NPD)

Group 2: High Fat Diet (HFD)

Group 3: Atorvastatin 10mg/kg, p.o (Standard)

Group 4: Polyherbal extract 500mg/kg, p.o (PHE1)

Group 5: Polyherbal extract 1000mg/kg, p.o (PHE2)

Group 6: Polyherbal extract 1500mg/kg, p.o (PHE3)

Group 2 to group 6 were fed with HFD for 28 days. The drugs atorvastatin and polyherbal extract were given once daily per oral.

Induction of Hyperlipidemia

Experimental hyperlipidemia as induced by a High Fat Diet for 28 days. The High Fat Diet prepared in the laboratory as per the following composition given in Table 2. During this period, weights of all animals were noted on weekly basis to evaluate the effects of treatments on body weight as well.

Table 2: Composition of High Fat Diet

Sr. No.	Ingredients	Quantity (per kg)
1.	Normal Pellet Diet	365g
2.	Lard	310g
3.	Casein	250g
4.	Cholesterol	10g
5.	Vitamins & Minerals	60g
6.	Yeast powder	1g
7.	Sodium chloride	1g
8.	DL-Methionine	3g

Collection of Blood Samples

Blood samples after 24 hours of the last dose were collected from retro-orbital plexus and allowed to coagulate at room temperature which was then centrifuged at 3000 rpm for 10 minutes (cooling centrifuge, REMI LXCI-7807). The serum was separated and used for the biochemical estimations.

Estimation of Lipid Profile

Total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), very low density lipoproteins (VLDL), and high-density lipoproteins (HDL) were measured using commercially available diagnostic kits according to procedure provided by manufacturer (Dhiti Diagnostics Private Limited) [36].

Estimation of the serum Liver biomarker enzyme

Serum analyses for various biomarkers of LFTs were done using commercial kits (Dhiti Diagnostics Private Limited.). These biomarkers include alanine aminotransferase (ALT), aspartate aminotransferase (AST). Results were obtained following the colorimetric method. Data values were presented in international units per litter (IU/L).

Histopathological studies

At the end of study, rats were euthanized; liver was removed. The 10 % neutral buffered formalin fixed tissue were routinely processed according to standard procedures. Then, sections (5 µm) of the different groups were mounted on slides and dried overnight at 37°C. The sections were dewaxed in xylene, hydrated in a graded series of alcohol solution and then stained with haematoxylin and eosin for histological evaluation.

Statistical analysis

All the values were expressed as mean ± standard error of the mean (SEM). The data was analyzed by one-way analysis of variance followed by Dunnett's t-test. $p<0.05$ were considered to be significant [21].

Results

Percentage yield of crude drugs

After individual extraction of crude drugs, percentage of yield was calculated and according to obtain weight and the results were given in table 3.

Table 3: Percentage yield of crude drug

Drugs	**% Yield**
Terminalia arjuna	4.7 % w/w
Hibiscus rosa sinensis	2.4 %w/w
Emblica officinalis	5.8 % w/w
Trigonella foenum graecum	2. 45% w/w

Preliminary phytochemical evaluation of extracts

Preliminary phytochemical screening was done and found the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins, glycosides, tannins and phenolic compounds. The results were given below in Table 4.

Acute toxicity studies

Administration of PHE at a dose of 5000 mg/kg resulted in no mortalities or evidence of adverse effects implying that PHE is non-toxic. Throughout 14 days after single dose treatment of PHE, no changes in behavioral pattern, clinical signs in rats were observed. This shows that PHE was safe up to a dose of 5000 mg/kg.

Table 4: Preliminary phytochemical evaluation of different extract

Phytochemical constituents	*Hibiscus rosa sinensis*	*Terminalia arjuna*	*Commiphora mukul*	*Fenugreek*	*Embilica officinalis*
Alkaloids	+	+	_	+	+
Flavonoids	_	+	+	+	+
Carbohydrates	+	_	+	+	+
Saponins	+	+	_	+	+
Tannins	_	+	+	+	+
Glycoside	_	+	+	+	+
Steroids	+	_	+	+	+
Phenolic compounds	_	+	+	+	+

+ Present - Absent

Evaluation of body weight and anti-hyperlipidemic activity

Since, animals in the normal control group were kept on a normal diet; they did not attain any significant gain ($p > 0.05$) in their body weights throughout the experiment. All animals of the HFD group significantly achieved marked increases in their body weights as compared to normal control group and treatment with PHE showed significant reduction in body weight as compared to HFD group (Table 5). The PHE was evaluated for its anti-hyperlipidemic activity against HFD induced hyperlipidemia model in rats. After 28 days of HFD feeding, the levels of TC, TG, LDL, and VLDL in the HFD group were significantly higher and the level of HDL was significantly decreased than those in NPD group ($P < 0.001$). The liver biomarker levels were also increased in serum in HFD-induced group, when compared

to the normal group. The effects of different treatments on serum lipid profiles in the experimental rats are shown in table 5. Treatment with polyherbal extracts showed significant decrease in the levels of TC, TG, LDL, and VLDL as compared to the HFD group and increase level of HDL. PHE3 was found to be most potent in reducing the increased serum lipid levels.

Table 5: Effect of PHE on body weight and serum lipid profile

Parameters	NPD	HFD	STANDARD	PHE1	PHE2	PHE3
Body weight	244±4.3***	356±7.3	253±3.2***	306±4.1*	263±3.7***	259±3.6***
TC	172.66±8.3***	301.83±7.22	254.66±9.96***	299.33±9.65	264.66±8.8**	244.66±9.6***
TG	121.16±10.4***	280.5±9.69	193.33±9.69***	279.83±10.4	255.33±10.19*	210.16±10.8***
HDL	56.86±6.73***	33.63±7.7	49.21±5.4**	39.85±0.9	43.33±6.8**	54.83 ±5.54***
LDL	91.8±6.3***	201.19±11.6	152.87±7.4***	195.17±10.2	167.83±9.1**	161.76±7.23***
VLDL	24±1.52***	55.33±4.8	34.26±2.64***	40.83±9.64*	42±8.63**	32.05±4.68***

All values are represented as Mean±SEM, (n = 6). Data were analyzed by one way ANOVA followed by Dunnet Test.* P<0.05; ** P<0.01; *** P<0.001; as compared to HFD group.

Effect on liver biomarker enzymes

Groups kept on a high-fat diet (HFD) appeared to have a significant increase in plasma ALT and AST level ($p < 0.001$) compared to that of NPD group (Figure 1). After treatment with polyherbal extract, the levels ALT and AST were significantly lower in PHE2 and PHE3 groups than in the HFD group ($P < 0.001$).

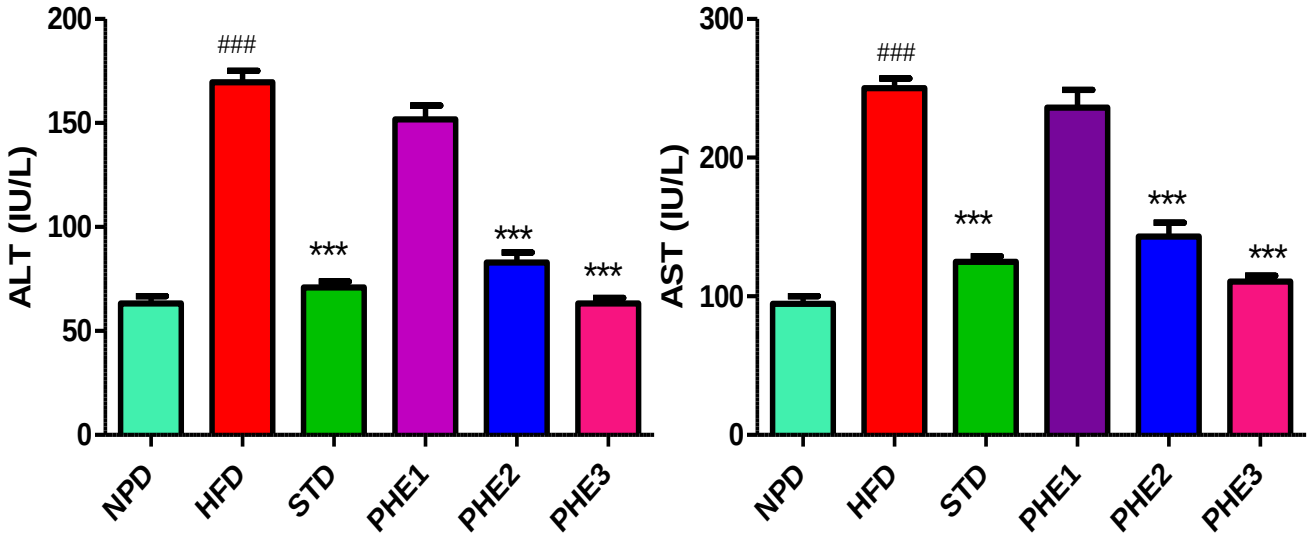

Figure 1: Effect of Polyherbal extract on ALT and AST levels. All values are represented as Mean±SEM, (n = 6). Data were analyzed by one way ANOVA followed by Dunnet Test. ***P<0.001; as compared to HFD group; ###P<0.001; as compared to NPD group.

Effect of PHF on Liver Histopathology

In HFD model mild to moderate sinusoidal space dilatation along with hemorrhages noticed in the sinusoidal space of liver and multiple foci of inflammation along with infiltration of inflammatory cells noticed in the centrilobular region of liver. The histopathological study showed recovery of the damaged liver cells in the PHE treated group. The degree of vascularization was also reduced as compare to the hyperlipidemic group. Furthermore, there was reduced infiltration of inflammatory cells was noticed in the inflammatory region of liver. Interestingly, PHE3 treated group imparted magnificent curative results, as liver sections of these groups displayed a well-organized structure of hepatocytes along with established cytoplasmic material. The results of liver histopathology were shown in Figure. 2.

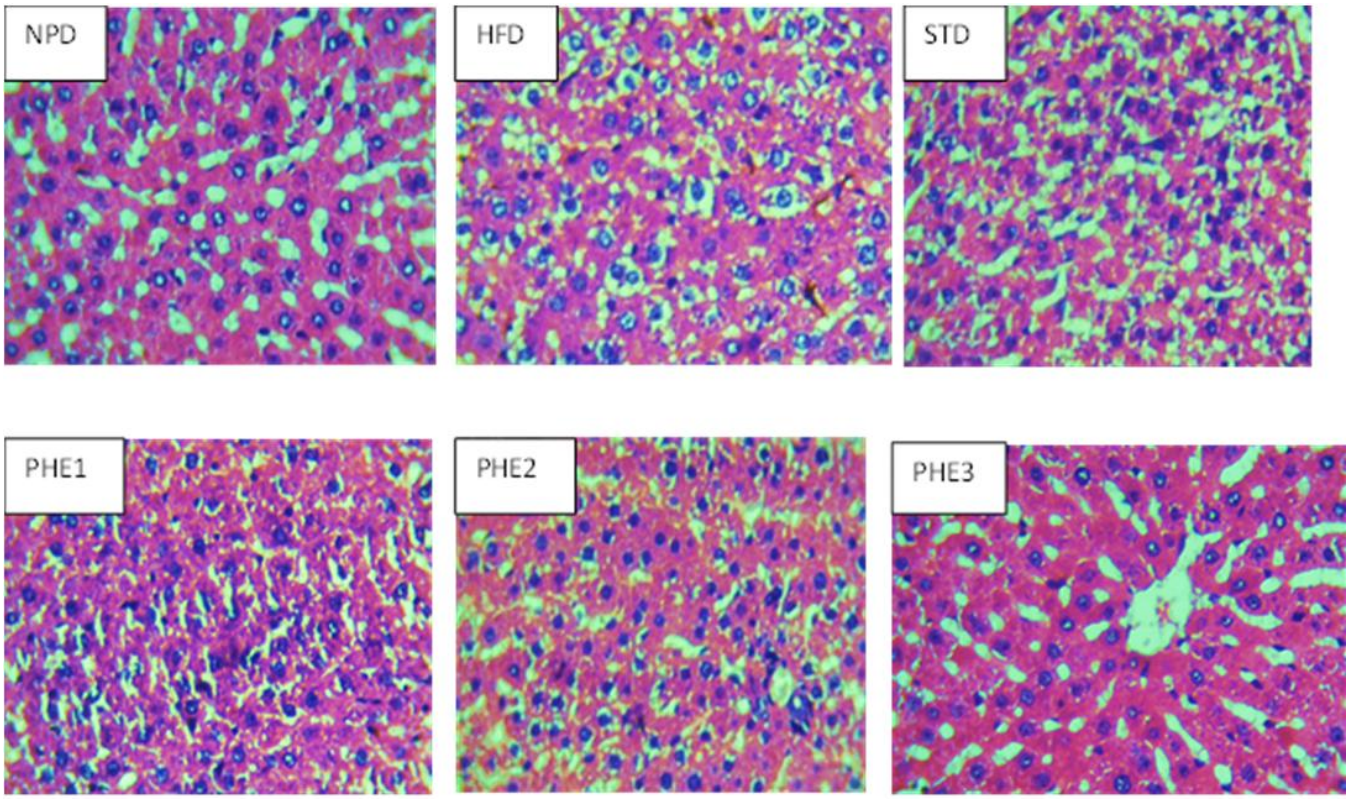

Figure 2: Histopathology liver of high-fat diet (HFD) induced hyperlipidemia model. Normal pellet diet (NPD); High fat diet (HFD); STD (Atorvastatin); Polyherbal Extract (PHE) PHE1 500mg/kg, PHE2 1000mg/kg, PHE3 1500mg/kg

Discussion

In this study, we have observed that administration of polyherbal extract decreases the blood lipid levels in high fat diet induced hyperlipidemic rats. The mechanism of action of polyherbal extract correlates with antihyperlipidemic drugs that decrease the synthesis of triglycerides which ultimately decreased the lipid level in blood. In our study we have chosen fat diet which contains the common ingredients in our daily food and some chemicals. During hyperlipidemia it has been observed that there is a gradually increase in the body weight of animals. However, on treatment with polyherbal extract there was also a significant decrease in the body weight of the treated animals.

The possible mechanism of polyherbal formulation may involve increase in activity of the endothelium bound lipoprotein lipase which hydrolyses the triglycerides into fatty acids or due to inhibition of lipolysis so that fatty acids do not get converted to triglyceride.

The preliminary phytochemical studies identified the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins, glycosides, tannins and phenolic compounds in PHF. Antihyperlipidemic activity of polyherbal extract may be due to the effect of active constituents of different plants Viz, *Hibiscus rosa sinensis*, Alkaloids from *Terminalia arjuna*, Guggulsterone from *Commiphora mukul*, saponins from *Trigonella foenum graecum* and phenolic compounds from *Emblica officinalis*.

On the basis of above result, it could be demonstrated that Polyherbal extract, a combination of five herbal plants exert significant antihyperlipidemic activity. Moreover, the polyherbal extract at higher dose imparted the most promising results in reducing the hyperlipidemia and associated complications.

Conclusion

Thus from the experimental results of present study concluded that this polyherbal extract exhibits significant hypolipidemic activity and help to maintain good lipidemic and metabolic control. High dose of extract also shows significant improvement in liver enzyme level, lipid profile, and liver histopathology hence PHF (1500mg/kg) can be used to treat hyperlipidemia and related complications.

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