INVESTIGATION OF EFFECT OF *GARLIC EXTRACT* ON THE HYPOLIPIDEMIC EFFECT OF EZETIMIBE

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Summary

The present work was aimed to study the influence of garlic extract on lipid profile upon simultaneous intake of ezetimibe. The dried and ground bulbs of *Allium sativum* were subjected to extraction with alcohol using a Soxhlet apparatus for 72 hours. The obtained garlic alcoholic extract was suspended in distilled water and administered orally to *Swiss albino* rats (fructose induced hyperlipidemia) through oral feeding tube till a period of 7 days. Similarly, ezetimibe suspension and vehicle (control) were administered. Thereafter the sampling of blood from animal was performed and the serum was analyzed for lipid profile. Data were expressed as the mean ± SD and "t" test was applied to determine statistical significance of results ($p<0.05$ and $p<0.01$). It was observed that the combination of ezetimibe with garlic extract resulted in lowering of serum total body cholesterol from 103.7±0.92 mg/dl to 90.5±0.27mg/dl, triglycerides from 52.4±0.21 mg/dl to 36.8±1.2 mg/dl, LDL-cholesterol from 62.3±1.52 mg/dl, to 41.06±1.43mg/dl,VLDL-cholesterol from 28.7±0.04 mg/dl to 22.03±0.8 mg/dl along with increase in serum HDL-cholesterol from 13.4±0.39 mg/dl to 19.1±0.23mg/dl after treatment. The inferences drawn from lipid profile analysis suggest potentiation of hypolipidemic by co-administering garlic extract with ezetimibe.

Key Words: Dyslipidemia, Atherosclerosis, fasting lipoprotein profile (FLP).

Introduction

Hyperlipidemia is defined as an elevation of one or more of the following: cholesterol, cholesterol esters, phospholipids, or triglycerides. It describes an increased concentration of the lipoprotein macromolecules that transport lipids in the plasma. Abnormalities of plasma lipids can result in a predisposition to coronary, cerebrovascular, and peripheral vascular arterial disease [1]. Keys to prevention and treatment are the elimination or modification of risk factors, if possible, in conjunction with treatment of the specific lipid disorder [2]. Ezetimibe (Eze) is a newly Food and Drug Administration (FDA)-approved medication that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. It is approved for primary hypercholesterolemia (heterozygous familial and nonfamilial hypercholesterolemia) as monotherapy or in combination with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). It is also indicated in combination with atorvastatin or simvastatin for homozygous familial hypercholesterolemia and as an adjunct to diet for homozygous sitosterolemia. It localizes to the brush border of the small intestine where it inhibits absorption of cholesterol thereby decreasing the delivery of intestinal cholesterol to the liver. This decreases cholesterol stores within the liver and ultimately increases clearance of cholesterol from the blood [3]. It has been reported that variety of chemical moieties derived from plants has been investigated for the treatment of hyperlipidemia either alone or in combination with standard drugs [4,5,6,7,8].

The present study was designed to ascertain the improvement in lipid profile of selected animals while co-administering ezetimibe and garlic extract.

Materials and Methods

Materials

The Ezetimibe was a gift sample from Hetero Labs Mumbai. The Diethyl ether, carboxymethyl cellulose (CMC) and Fructose were purchased from CDH Laboratories, New Delhi, India. Alcohol was purchased from SD Fine Chemicals, Mumbai, India. The kits for testing of lipid profile were purchased from Qualigens diagnostics, Mumbai, India.

Methods

Extraction of plant material

The fresh fruits of *Allium sativum* were obtained from the local market in Bhopal, India. Dried and ground bulbs (approx.100 g) were subjected to extraction with 300 mL ethanol (80%) in a Soxhlet apparatus for 72 h. After extraction, the solvent was filtered and evaporated using rotavapor (Heidolph, UK).The obtained garlic alcoholic extract was stored at -20^{o}C until use.

Animal studies

Garlic extract was suspended in distilled water to give 0.4 g garlic extract per mL of the suspension and administered orally through feeding tube to *Swiss albino* rats. The animals were kept in quarantine as per CPCSEA guideline and fed on standard pellet diet and water was provided *ad libitum*. The study design was in compliance with guidelines of institutional animal ethical committee (IAEC). The volume of administrated extract was 1 mL for each animal [9]. The animals were selected of either sex on basis of their body weight 135-160 g randomly and divided into seven groups comprising six animals each. The dosage regimen was as follows: Group I-control, II-fructose (5 g/kg), III-fructose and ezetimibe, IV- fructose and garlic extract, V-fructose, Ezetimibe and garlic extract, VI-Ezetimibe (30 μg/kg) and VII-garlic extract (10 mg/kg).

Sampling and measurement of lipid Profile parameter from serum

The sampling of blood from animals at was done after the treatment. Prior to sampling rats were fasted for 18 h. Then animals were anaesthetized with diethyl ether and blood was withdrawn from retro-orbital sinus in EDTA Coated tubes. The blood was centrifuged and analyzed for lipid profile. The total body cholesterol-TBC, total triglyceride-TG, total high density lipoprotein-HDL, Low density lipoprotein-LDL, very Low density lipoprotein-VLDL, were quantified using respective diagnostic commercial kits.

Statistical analysis

All the data were analyzed by using one way ANOVA with Dunnett's test using SYSTAT12 software. The statistical significance of results was tested at two confidence levels viz. $p<0.05$ and $p<0.01$.

Results and Discussions

The alcoholic extract resulted from processing of fresh fruits of *Allium sativum* was fed orally to the *Swiss albino* rats accompanied by standard treatment. The change in the lipid profile was compared against control. It is apparent from findings that fructose successfully induced hyperlipidemia in the animals under study (fig. It caused rise in blood serum levels of TBC, TG, LDL and VLDL simultaneously decrease in HDL levels. The animal groups (hyperlipidemic) were then subjected to treatment with eze and garlic extract in combination. The influence of eze and garlic extract alone was also ascertained on animals having normal lipid profile.

The lowering of TBC was more pronounced upon in case of animals fed on eze and garlic extract plain having normal lipid profile. Whereas the eze caused lowering in TBC levels

at higher magnitude as compared to garlic extract in hyperlipidemic animals. But the decrease in TBC levels was found to greater extent when garlic extract and eze were co-administered as compared with eze in animals with hyperlipidemia. This suggests that garlic extract augments the hypolipidemia by eze. Similar findings were observed in other lipid parameters.

The treatment resulted in rise in blood serum levels of HDL (good cholesterol). It was obvious that eze plain yielded highest rise in HDL levels. The co-administration of eze and garlic extract had no significant impact over the HDL values (Table1).

Table 1 Lipid Profile (mg/dl) of animals at the end of treatment (mean ±SD, n = 6)

Group	TBC	TGA	LDL	HDL	VLDL
I	84.3±1.62	27.1±0.43	34.9±2.15	17.12±1.73	23.4±1.03
II	103.7±0.92	52.4±0.21	62.3±1.52	13.4±0.39	28.7±0.04
III	92.1±1.37	45.6±1.4	51.0±0.91	18.7±0.59	22.3±1.67
IV	99.6±0.98	49.9±0.76	55.7±1.52	16.4±2.13	26.5±1.09
V	90.5±0.27	36.8±1.2	41.06±1.43	19.1±0.23	22.03±0.8
VI	78.53±0.45	24.3±0.37	31.9±0.48	21.05±0.32	25.2±0.29
VII	85.1±1.7	26.8±0.5	29.7±1.34	19.67±1.22	24.1±2.3

Conclusions

The present study was an effort to demonstrate the effect of garlic extract on hypolipidemia by ezetimibe. The varieties of herbal constituents are being tried towards improvement in lipid profile. There is need to elucidate the exact mechanism by which such plant products provides lowering of bad cholesterol. The studies on hyperlipidemia have immense potential for exhaustive research.

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