Protective effects of setarud (IMOD™) on development of diet-induced hypercholesterolemia in rabbits

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ABSTRACT

Background: A new herbal drug setarud (IMOD™) containing selenium, carotene, and flavonoids, was expected to have positive effects on lipid metabolism and liver functions, due to the nature of its primary components. This study was designed to determine effectiveness of the drug in reducing the risk of development of diet-induced hypercholesterolemia in laboratory animals.

Methods: Two groups of male rabbits (n=10 per group) as: intact and control groups on regular chow, were fed a high-cholesterol diet, and two experimental groups were maintained on the same diet and treated with different daily doses (0.02 g/kg and 0.04 g/kg) of setarud (brand name IMOD®, Pars Roos, Iran). The treatment groups were then compared with the intact and control groups and with one another for the effects of the drug which was determined by changes in blood sugar, serum lipid levels, and liver function tests.

Results: Results showed that drug had important benefits in alleviating the impact of high-cholesterol diet on serum lipids and liver function markers in drug-treated groups relative to hyperlipidemic controls ($p < 0.001$). A more favorable modification of total cholesterol and triglyceride levels and the atherogenic index was found in animals, which received 0.04 g/kg drug, as compared to the 0.02 g/kg dose group ($p < 0.05$). Assessment of serum total protein, albumin, transaminases, and bilirubin levels showed that no changes in liver function of control and drug-treated animals during the period of the study.

Conclusion: From the results of this study it may concluded that setarud has dose-dependent positive effects on liver and lipid metabolism and may acts as an effective anti-hyperglycemic agent.

Keywords: Hypercholesterolemia, IMOD™, Setarud

INTRODUCTION

The drugs that are currently in use to treat hypercholesterolemia, including HMG-CoA reductase inhibiting agents (statins) are generally well tolerated but can cause dose-dependent adverse reactions, especially in patients suffering from chronic disease complications (1). In view of the growing recognition of the potential role of the selenium (Se), as an essential micronutrient in metabolism, some selenium compounds have been effective for the treatment of hyper-cholesterolemia and cardiovascular disease (2,3). Selenium is involved in the metabolism of lipids, carbohydrates and proteins and protects cells from oxidative stress (2-4). Some illnesses such as cardiovascular disorders, immune deficiency, and cancer have been linked to dietary Se deficiency. A low serum Se concentration has been reported to reduce liver microsomal enzymes activity and lower high density lipoprotein cholesterol (HDL) level in animals (5). Dietary supplementations of Se have resulted in higher hepatic concentrations of reduced glutathione (6,7), a potent intracellular inhibitor of lipid peroxidation, and decrease in the

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plasma total cholesterol (TC), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels (8).

Protective action of dietary Se against high-fat diet induced hypercholesterolemia in animals have previously been documented (9,10). Other nutrients like polyphenolic flavonoids and pigmented carotenoids have also been reported to have potent hypolipidemic and hypoglycemic effects in animals (11,12).

In the search for new medicines with the capacity to correct nutritional deficiencies and to treat immune disorders, the drug namely setarud containing Se, carotene, and flavonoids was designed. According to the nature of its primary ingredients, the formulation may have positive effects on liver and lipid metabolism. This study was performed to determine the effectiveness of the drug against diet-induced hyper-cholesterolemia and liver metabolism changes in animals.

MATERIALS AND METHODS

Experimental Animals and Diets

This preclinical study was conducted at Pasteur Institute of Iran, Tehran. Forty male New Zealand White rabbits, with a mean age of 3.5 months and average weight of 2000 g, were selected for experiments. Animals were handled in accord with the guideline of the laboratory animal welfare (13). The selected rabbits were housed individually in aluminium cages and kept in a room maintained at 20-22°C with a 12 hrs light/dark cycle. The animals were fed with standard laboratory rabbit food (Behparvar, Iran). Food and water were given ad libitum during the entire one-month period of the study.

The animals were randomized equally (n=10 per group) into four groups: (1) intact, which were fed a standard chow supplemented with 2 ml/kg of sunflower oil daily by a nasogastric (NG) tube; (2) control fed via NG tube a hypercholesterolemic diet (0.5 g/kg body mass/day): of 5% cholesterol, plus 1% cholic acid, 0.5% thiouracil – designed as CCT diet (14); and two treatment groups given by NG tube CCT diet along with 0.02 (3) or 0.04 (4) g/kg body mass/day setarud (IMOD®, Pars Roos Co., Tehran, Iran) during subsequent 30 days. The doses were selected based on the results of the earlier subchronic toxicity study (unpublished data).

Biochemical Assays

Blood samples were collected from the marginal left ear veins of 12-hrs fasting animals. Laboratory tests, including serum cholesterol lipids, triglyceride, total protein, albumin, blood sugar, bilirubin, transaminases and alkaline phosphatase (ALP), were carried out, using commercial kits (Bayer Diagnostics GmbH, Munich, Germany).

Data analysis

Results are expressed as means ± SEM (n=3). Data were analyzed by SPSS software 13.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to compare mean values among the groups. Post-hoc multiple comparison tests (Bonferroni method) were carried out. p values <0.05 were considered significant.

RESULTS

All animals survived during the study. Hypercholesterolemia was induced in control cholesterol-fed group as proved by the results of biochemical tests (Table 1). Analysis of the changes of serum lipid levels in this group showed two times increase in total cholesterol (TC) and triglycerides and about three times elevation in VLDL, LDL and atherogenic index (TC–HDL/HDL) compared to those of intact group ($p < 0.01$).

In animals treated with 0.02 g/kg of setarud, total cholesterol, triglyceride, VLDL-C and LDL levels were lowered by 36.6%, 22.6%, 33.7%, 41.3%, respectively, while HDL level was higher by 11.2% in comparison with corresponding values of the control group. As a result, the atherogenic index was reduced by 63.4% in the group which received a dose of 0.02 g/kg. A more pronounced decrease in atherogenic lipid fractions compared to controls was registered in the serum of the animals, which received 0.04 g/kg drug as related to the 0.02 g/kg group: total cholesterol by 48.0% vs. 36.6%, triglyceride by 39.1% vs. 22.6%, and atherogenic index by 70.4% vs. 63.4% ($p < 0.01$), respectively.

Liver function tests in control cholesterol-fed animals displayed a mild to moderate increase in serum total protein (13.3%), albumin (11.1%), total bilirubin (64.5%), and blood sugar (40.0%) levels in comparison with intact rabbits (Table 2), while in drug groups, all records were at intact level ($p > 0.05$).

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ALP were significantly augmented in control animals compared to the corresponding values of the intact group (Table 3). However, in animals treated with 0.02 g/kg of setarud, serum ALT level was lowered by 18.6%, AST was lowered by 15.4%, and ALP was lowered by 17.7% compared to controls ($p < 0.05$). Furthermore, the 0.04 g/kg group showed minimal alterations in liver function tests, with the activity of ALT, AST and ALP being closer to that of intact rabbits when compared to the 0.02 g/kg group ($p < 0.05$).

Table 1. Protective effects of setarud (IMOD^{TM}) against diet-induced hyperlipidemia in rabbits

Study group	Cholesterol mmol/l	HDL mmol/l	LDL mmol/l	VLDL mmol/l	TG g/l	Atherogenic index
Intact	2.61±0.74	1.62±0.04	0.40±0.006	0.33±0.01	0.61±1.21	0.61±0.004
Control	6.31±0.47**	2.00±0.04*	1.21±0.004**	1.01±0.03**	1.15±0.04**	2.16±0.010**
Setarud 0.02 g/kg	4.00± 0.37$^{\#\#}$	2.24±0.01$^{\#}$	0.71±0.001$^{\#\#}$	0.67±0.01$^{\#\#}$	0.89±0.03$^{\#\#}$	0.79±0.042$^{\#\#}$
Setarud 0.04 g/kg	3.28±0.04$^{\#\#}$	2.00±0.04	0.70±0.004$^{\#\#}$	0.60±0.01$^{\#\#}$	0.70±0.03$^{\#\#}$	0.64±0.001$^{\#\#}$

Each value is represented as mean ±SEM; $*p < 0.05$, $**p < 0.01$ compared to intact group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to control group; ns= not significant, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, VLDL=Very Low Density Lipoprotein, TG=Triglycerid.

Table 2. Influence of setarud on changes (IMOD^{TM}) in biochemical tests in rabbits on CCT diet

Study group	Total Protein mmol/l	Albumin mg%	Blood Sugar mmol/l	Bilirubin Total mmol/l	Bilirubin Direct mmol/l
Intact	6.0±0.60	54.0±6.1	5.0±0.4	7.05±2.6	1.40±0.36
Control	6.8±0.66**	60.0±4.6**	7.0±0.4**	11.60±1.7**	4.72±0.18**
Setarud, 0.02 g/kg	6.1±0.13$^{\#}$	56.6±2.1$^{\#}$	5.0±0.1$^{\#\#}$	7.44±1.1$^{\#\#}$	1.99±0.01$^{\#\#}$
Setarud, 0.04 g/kg	6.0±0.17$^{\#\#}$	56.0±1.6$^{\#\#}$	5.1±0.1$^{\#\#}$	7.05±1.6$^{\#\#}$	1.90±0.13$^{\#\#}$

Each value is represented as mean ±SEM; $*p < 0.05$, $**p < 0.01$ compared to intact group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to control group.

Table 3. Effects of setarud (IMOD^{TM})on liver enzymes activity in rabbits on CCT diet

Study group	ALT mmol/L	AST mmol/L	ALP mg%
intact	56.6 ± 1.14	80.4 ± 1.11	501.6 ± 16.6
control	79.0 ± 2.14**	118.6 ± 1.17**	700.0± 23.1**
setarud, 0.02 g/kg	64.3 ± 3.40$^{\#\#}$	100.3± 3.60$^{\#}$	576.0± 11.1$^{\#}$
setarud, 0.04 g/kg	61.5 ± 5.20$^{\#\#}$	90.4 ± 5.20$^{\#\#}$	540.0± 11.1$^{\#\#}$

Each value is represented as mean ±SEM; $**p < 0.01$ compared to intact group; $^{\#}p < 0.05$, $^{\#\#}p < 0.05$ compared to control group. ALT=alanine aminotransferase, AST=aspartate aminotransferase, ALP=alkaline phosphatase.

DISCUSSION

Results of this study show that setarud, a combination of Se, carotenoids and flavonoids, hinder development of hyperlipidemia in rabbits by stabilizing both blood sugar and serum cholesterol and triglyceride levels. In the previous studies it has been reported that, Se supplementation led to a major decrease in plasma concentrations of LDL, VLDL, total cholesterol, and triglycerides both in human (15) and animals (8,16,17). Thus, it may be assumed that Se present in the drug was responsible for a large part of the observed effects.

Selenium, when supplied exogenously, inhibited both high-fat and alcohol-induced hyperlipidemia in animals that is consistent with data of this study (9,10,18). This suppression was due to decrease in lipid synthesis as evidenced by the lower activity of HMG-CoA reductase in liver microsomes. In contrast, elevated cholesterol in rats with Se deficiency was related to the increase in HMG-CoA reductase activity (19). Selenium intake may lower plasma cholesterol concentration or stabilize it at low levels via its key role in metabolism of thyroid hormones (20), which are among the most potent agents known to reduce plasma LDL concentrations. Selenium is a critical element of selenoenzyme, type I iodothyronine deiodinase (5'-DI), which is required for hepatic conversion of T4 to 3,3,5-tri-iodothyrosine (T3) and also takes part in regulation of apolipoprotein B (apo B) expression. Thus, Se deficiency may cause an inhibition of 5'-DI activity, leading to a reduction in T3 level and related decrease in LDL-cholesterol elimination from blood through down regulation of LDL receptor activity by reduction of LDL receptor mRNA expression. Selenium supplementation may lead to a decrease in apo B expression through increase in LDL receptor mRNA level by modulation of 5'-DI expression which, in turn, may have the protective role against hypercholesterolemia (21,22). In addition, Se is a main component of glutathione peroxidase protecting tissues against free radicals (6-8). Therefore, some beneficial effects of setarud, namely normalizing the levels of liver enzymes which were observed in experimental groups, may be due to Se antioxidant activity in liver cells, although the exact mechanisms of its effects are not well understood.

Setarud has been especially formulated with extracts of Rosa sp., *Urtica dioica* and *Tanacetum vulgare*, and Se (23). Beside of Se, other substances present in setarud, such as carotene and flavonol quercetin (unpublished data), might account for anti-atherogenic and anti-glycemic action of the drug. Both carotene and quercetin have been reported to be effective in impeding the increase in serum cholesterol and glucose (11,12, 24). The protective activity of the drug was considerable in the 4-week study with more favorable effects in animals, which received higher drug dosage. The combination of all above nutrients in setarud turns out to be not less effective than individual supplements, i.e, Se, carotene or flavonol, in counteracting the cholesterolemia and hyperglycemia.

According to its ingredients and dose-dependent profoundly positive effects on liver and lipid and sugar metabolism, setarud, branded as IMOD, was active as an anti-hyperlipidemic and anti-hyperglycemic agent and the composition may be useful as nutritional support for detoxification and improvement of liver functions. The wide range of its influence on liver functions and levels of serum cholesterol and sugar suggest to study the prospective use of setarud in healthy patients.

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