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Evaluation of the Effects of Simvastatin Alone and in Combination with Garlic on Lipid Profile and Liver Enzymes in Rats Fed Normal and Fat Rich Diet

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Abstract: Forty mature Sprague-Dawley rats were selected. The animals were divided randomly into 8 groups of 5 rats each. Different groups underwent different treatments for two months. Before commencing and at the end of the treatment period, the animals were bled from the heart and the plasma was separated. Total cholesterol (TC), HDL-C, LDL-C and triglycerides were determined in the plasma of the rats. Results indicated that garlic reduced plasma total cholesterol and LDL both in rats on normal ($p>0.05$) and those receiving fat rich diet ($p<0.05$). Although the garlic increased the level of HDL more in rats on fat rich diet, the difference was not significant when it was compared to the level of increase in those on normal diet. Simvastatin in rats on garlic + fat rich diet and on garlic + normal diet reduced total cholesterol ($p<0.05$) when compared to respective controls. Simvastatin in rats on garlic + fat rich diet reduced trigyceride and LDL significantly ($p<0.05$). HDL in rats on simvastatin in rats on garlic + fat rich diet increased significantly versus nonsignificant increase in simvastatin + garlic + normal diet group. Simvastatin in rats on garlic + fat rich diet reduced more cholesterol, triglyceride and LDL levels when compared to those in rats on garlic + fat on normal diet ($p<0.05$). A greater effect on lipid profiles was exhibited when both simvastatin and garlic powder were simultaneously given to rats on fat rich diet. It was also indicated that the effect of combined garlic and simvastatin had a greater effect on HDL increase in rats on fat rich diet.

Key words: Rat · Simvastatin · Garlic · Lipid Profile

INTRODUCTION

The risk and rates of heart attack and stroke cardiovascular risk both at the population level and at the individual level was directly and independently related to the level of total serum cholesterol. It demonstrated that the association between blood cholesterol level and coronary heart disease risk from 5 to 40 years follow-up is found consistently across different cultures [1, 2]. Merck submitted its third application to the Food and Drug Administration (FDA) to allow it to sell its cholesterol-lowering drug over the counter [3]. Perhaps the most compelling public health argument for allowing statins to be sold without a prescription is that self-management is the most sustainable option for the prevention of the many chronic diseases [3]. HMG-CoA reductase (or 3-hydroxy-3-methyl-glutaryl-CoA reductase orHMGCR) is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. Drugs such as simvastatin which inhibit HMGR are used to lower serum cholesterol as a means of reducing the risk for cardiovascular disease [4, 5]. *Allium sativum*, commonly known as garlic is widely used around the world with a history of human use of over 7,000 years for culinary and medicinal purposes [6, 7]. Garlic is also claimed to help prevent heart disease (including atherosclerosis, high cholesterol and high blood pressure [8] and animal studies and some early research studies in humans, have suggested possible cardiovascular benefits of garlic [9-11]. A 2012 meta-analysis of randomized, double-blind, placebo-controlled trials reported that garlic was superior to placebo in reducing serum total cholesterol and triglyceride levels [12]. Regular and prolonged use of garlic along with simvastatin is quite likely in different asian cultures and to the best of our knowledge, there is no study on the effect of concurrent chronic use of simvastatin and garlic on lipid profile. This study was

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carried out to investigate the effect of chronic concurrent consumption of this statin and garlic on lipid profile of rat under natural condition of hyperlipidemia.

MATERIALS AND METHODS

Forty mature Sprague-Dawley rats weighing 250-300 g were selected. The animals were housed in a well ventilated experimental animal house under constant environmental and a photoperiod of 14 h light/10 h dark per day. The animals were left for 2 weeks without interference for acclimatization. Animals received standard laboratory balanced commercial diet and water *ad libitum*. The animals were divided randomly into 8 groups of 5 rats each. All experiments on rats were carried out in compliance with the Shiraz Medical University, which consider ethical guide for care and use of laboratory animals. Groups (G) 1 to 8 were consisted as G1: rats on normal diet, G2: rats on normal diet + fat, G3: rats on normal diet + garlic, G4: rats on normal diet+ fat + garlic, G5: rats on normal diet + oral simvastatin, G6: rats on normal diet + fat + oral simvastatin, G7: rats on normal diet + fat + garlic + oral simvastatin, G8: rats on normal diet + garlic + oral simvastatin. For those groups receiving garlic, garlic tablets (Garlet, Cosar pharmaceutical Co., Iran) were grinded and emulsified in water and dosed by oral gavage (750 mg/kg/day). Fat was obtained by small cubical pieces of fat from stored fat in the tail area of fat-tailed sheep and was presented to corresponding groups freely. Simvastatin tablets were emulsified in water and were administered at the dose of 4 mg/kg p.o. Oral gavage was used to dose the animals. Different groups underwent different treatments for two months. Before commencing and at the end of the treatment period, the animals were bled from the heart under light ether anesthesia, using usually a trace of heparin and the plasma was separated. Total cholesterol (TC), HDL-C, LDL-C and triglycerides were determined in the plasma of the rats by adopting the protocol outlined in the manufacturer's assay kit from Randox Laboratories Ltd, Ardmore, Co. Antrim, UK. AST and ALT were determined by the enzymatic colorimetric methods.

All data were expressed as Mean ± SEM (Standard Error of the Mean). For comparing the mean of different parameters in different groups, ANOVA and the Tukey post hoc tests were used. For comparing the percentages, the independent samples t-test was used. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. Differences were considered significant at $p < 0.05$.

RESULTS

The results of the experiment are shown in Table 1. Our results indicated that garlic reduced plasma total cholesterol and LDL both in rats on normal (14.01% and 10.25%) ($p>0.05$) and those receiving fat rich diet (31.87%, 19.04%) ($p<0.05$). Although the garlic increased the level of HDL more in rats on fat rich diet (4.04%), the difference was not significant when it was compared to the level of increase in those on normal diet (2.67%). Garlic decreased the level of triglycerides in both rats on normal and fat rich diet 2.75% and 5% respectively ($p>0.05$). Simvastatin reduced total cholesterol and LDL both in rats on normal (19.01% and 16.6%) and those receiving fat rich diet (40.64% and 25%) ($p<0.05$). Simvastatin increased the level of HDL in both rats on normal 3.57% ($P>0.05$) and fat rich diet 6.06% ($p<0.05$). Simvastatin decreased the level of triglycerides in both rats on normal ($p>0.05$) and fat rich diet ($p<0.05$), 10.09% and 48.8% respectively. Simvastatin in rats on garlic + fat rich diet and on garlic + normal diet reduced total cholesterol ($p<0.05$) when compared to respective controls. Simvastatin in rats on garlic + fat rich diet reduced triglycerides and LDL significantly ($p<0.05$).

Table 1: Mean and standard error of different parameters in various groups of rats fed normal and fat rich diet (n=40)

Groups	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	Triglycerides (mmol/L)	ALT (IU/L)	AST (IU/L)
G1 (N)	1.57 ± 0.05^{a}	1.12 ± 0.02	0.78± 0.02	1.09 ± 0.05a	32 ± 5.63	102 ± 10.78
G2 (F)	3.42 ± 0.12^{ab}	0.99 ± 0.01^{a}	1.68 ± 0.07^{abd}	1.8 ± 0.29^{acd}	35 ± 5.83	108 ± 10.53
G3 (NG)	1.35 ± 0.04	1.15 ± 0.06	o.7 ± 0.02	1.06 ± 0.06	33 ± 6.64	107 ± 9.12
G4 (FG)	2.33 ± 0.09^{bc}	1.03 ± 0.04	1.36 ± 0.08^{abd}	1.71 ± 0.06^{bcd}	34 ± 4.18	96 ± 7.19
G5 (NS)	1.27 ± 0.04^{d}	1.16 ± 0.06	0.65 ± 0.01^{cd}	0.98 ± 0.03	35 ± 4.08	105 ± 7.59
G6 (FS)	2.03 ± 0.05^{bd}	1.05 ± 0.01	1.26 ± 0.03^{acd}	0.92 ± 0.04^{abc}	33 ± 5.24	104 ± 7.39
G7 (FGS)	1.94 ± 0.05^{bc}	1.21 ± 0.03^{a}	1.15 ± 0.01^{abc}	0.82 ± 0.02^{abd}	29 ± 3.49	106 ± 7.73
G8 (NGS)	1.18 ± 0.04^{ad}	1.19 ± 0.03	0.6 ± 0.01	0.81 ± 0.01	32 ± 4.46	110 ± 10.89

In each column, similar letters show significant difference ($p<0.05$). G1: rats on normal diet, G2: rats on normal diet + fat, G3: rats on normal diet + garlic, G4: rats on normal diet + fat + garlic, G5: rats on normal diet + oral simvastatin, G6: rats on normal diet + Fat + oral simvastatin, G7: rats on normal diet + fat + oral simvastatin + garlic, G8: rats on normal diet + oral simvastatin + garlic.

HDL in rats on simvastatin in rats on garlic + fat rich diet increased significantly versus nonsignificant increase in simvastatin + garlic + normal diet group. Simvastatin in rats on garlic + fat rich diet reduced more cholesterol, triglyceride and LDL levels (43.27%, 54.44% and 31.54%) when compared to the cholesterol, triglyceride and LDL levels in rat on garlic + fat on normal diet (31.87%, 5% and 19.04%) ($p<0.05$). No significant changes were detected in ALT and AST levels in different groups.

DISCUSSION

Statins are first-line pharmacotherapeutic agents used to lower cholesterol levels in humans. Increased cholesterol levels have been associated with cardiovascular diseases [13]. Statin therapy is associated with a reduced relative risk of all cause mortality, cardiovascular mortality, CHD mortality and fatal myocardial infarction [14]. It is also associated with a reduced relative risk of morbidity [14]. Simvastatin is one of a new class of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors and is used to lower total and LDL cholesterol, triglycerides and to increase HDL cholesterol [15, 16]. Garlic (*Allium sativum* L.) is cultivated worldwide and its potential medical properties have been recognized for thousands of years and have acquired a reputation in many cultures as a formidable prophylactic and therapeutic medical agent [17]. The antilipidemic effects of garlic have also been previously reported [18, 19]. It is also well established that garlic extracts, in particular the powders can show a significant anti-cholesterol activity [20]. Allyl sulfur compounds are the major active constituents found in crushed garlic. Oxygenated sulfur molecule, termed allicin [21] has a variety of biological effects including antimicrobial, hypolipidemic, antithrombotic, anticancer and antioxidant activities [22-24]. The antiatherogenic effects of these organosulphur compounds were attributed to such reactions that inhibit HMG-CoA reductase and other lipogenic enzymes [20, 25]. Our results indicated that garlic powder reduced plasma total cholesterol and LDL and triglycerides both in rats on normal and those receiving fat rich diet. Lee *et al.* [26] reported that Dietary High hydrostatic pressure extract of garlic lowered the hepatic TG and total cholesterol (TC) levels compared to the high-fat control diet group. He also reported that the plasma triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels significantly decreased in comparison with the high-fat control diet group [26]. It was stated that the hypocholesterolemic effects of the garlic extract stem occurs in part from the inhibition of hepatic cholesterol synthesis by water-soluble sulfur compounds, such as Sallylcysteine and ajoene [27].

Human [28, 29] and animal [18] studies have shown an inverse association between dietary garlic and the concentration of plasma TG. Also, studies using cell lines and animal models have shown that garlic has beneficial effects on reducing the accumulation of TG in blood. These effects were suggested with activation of liver phosphatidate phosphohydrolase (PAP) and fatty acid synthease (FAS), which play a critical role in controlling the biosynthesis of TG [30-32]. Our study indicated that garlic increased the level of HDL in rats on normal and fat rich diet and it was significantly increased when a combination of garlic and simvastatin were fed to rats on fat rich diet. The primary function of HDL is to remove cholesterol from peripheral cells and transport cholesterol to the liver for biliary excretion by reverse cholesterol transport. In addition, HDL has been shown to prevent foam cell formation, thus retarding the progress of atherosclerosis [33]. Our finding was in agreement with other studies, which reported that garlic consumption increased HDLC level in hyperlipidemic individuals [34], animals [35] and patients with coronary artery disease [36]. Kwon *et al.* [37] reported that when garlic powder was added into the diet at a concentration of 1%, plasma TC and LDL-C levels were decreased and HDL-C level were increased in rabbits. Lee *et al.* [26] reported that High hydrostatic pressure extract of garlic ameliorates plasma lipid profiles and attenuates hepatic lipid accumulation in the high-fat fed rats and the increase of the plasma HDL-C level was at least partially mediated by up-regulation of hepatic genes expression such as apoA-I, ABCA1 and LCAT in rats fed a high-fat diet. At the doses given for simvastatin and garlic powder, no significant rise in plasma AST and ALT activities were seen when compared with those of respective controls. These data indicate that the simvastatin and garlic powder (alone and in combination) were well tolerated by the rats. Our study showed that a combination of simvastatin and garlic in rats on fat rich diet caused a greater effect (in comparison to rats on normal diet) on lowering the cholesterol, triglyceride and LDL levels when either simvastatin or garlic (alone) was used. A greater effect on lipid profiles was exhibited when both simvastatin and garlic powder were simultaneously given to rats on fat rich diet. It was also indicated that the effect of combined garlic and simvastatin had a greater effect on HDL increase in rats on fat rich diet. Moreover, there may be a suggestion for more careful monitoring of lipid profile in those patients who have a history of regular consumption of garlic while they are on simvastatin.

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