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Hypolipidemic Effects of Amaranth Oil in Experimental Doxorubicine Cardiomyopathy.

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ABSTRACT

Amaranth oil is rich in unsaturated fatty acids, polyphenols, and active forms of tocopherol and contains up to 9% of squalene (depending on method of production). Squalene, quencher of singlet oxygen and toco-trienol tocopherol contribute most of beneficial effect of amaranth oil. Squalene, the important intermediate of human cholesterol synthesis, prevents peroxidation of membrane lipids during oxidative stress and maintains the balance between the hydrophilic and hydrophobic clusters inside the cell membrane. Experimental doxorubicine-induced cardiomyopathy attributed to peroxidation of endogenous lipid, excessive free radical formation, involves accumulation of fat, hypercholesterolemia and disturbance in lipoprotein metabolism. The effects of low dose amaranth oil on lipid profile in doxorubicine-induced experimental cardiomyopathy were investigated. The protective role of dietary amaranth oil against doxorubicine-induced cardiomyopathy was estimated. Low doses of amaranth oil did not return plasma lipid profile parameters in doxorubicine-induced cardiomyopathy groups to the normal levels but tend to decrease them. There were no significant differences in lipid profiles in groups received amaranth oil and squalene after 21 days of gavage.
Keywords: squalene, amaranth oil, cardioprotective, lipid, doxorubicine, experiment

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INTRODUCTION

Amaranth has current nutritional potential, being high in protein, fiber, lysine, magnesium, calcium, and squalene. Amaranth is the important grain in the Central Earth Region of Russia. It is a source of gluten-free flour [28]. Amaranth is oil that is rich in unsaturated fatty acids, polyphenols, active forms of tocopherol, etc [20]. The unique combination of natural antioxidants and important nutritive factors make use of amaranth oil very promising as a functional food and drug supplementation [2, 7, 15, 18-21]. Amaranth oil, prepared with the cold press method, contains up to 9% of squalene (depending on method of production). Squalene contains products derived from deep-sea shark (Squalus spp.) liver oil widely used in India and Japan folk medicine [6]. Squalene also can be found in some vegetable oils in relatively smaller amounts like amaranth oil, olive oil, brown rice oil. It is easy to hypothesize those squalene and toco-trienol tocopherols contribute most of beneficial effect of amaranth oil. Squalene, an isoprenoid molecule, the important intermediate of human cholesterol synthesis, demonstrates hypolipidemic, antiatherosclerotic, anticancer, radioprotector activities in animal models, in vitro environment and in humans. Effects of squalene are based on its antioxidant [13] membrane stabilizing [3, 11] and lipid-lowering properties. It is a quencher of singlet oxygen, it prevents peroxidation of membrane lipids during oxidative stress [14] and maintains the balance between the hydrophilic and hydrophobic clusters inside the cell membrane and suppresses the effect of hydrolyzed products that affects the membrane stability [8]. But squalene and toco-trienol tocopherol are not the only two important factors in beneficial action of amaranth products. Unsaturated fatty acids and polyphenols can also contribute some effects. To estimate the meaning of their presence in amaranth oil is the aim of our work. In spite of the fact that some data on possible cardioprotective action of cold press amaranth oil exist, the comparison of cardioprotective effects of amaranth oil and squalene in equal doses of squalene have not yet been explored.

In the present study we investigated the effects of amaranth oil and squalene on lipid profile in doxorubicine-induced experimental cardiomyopathy.

MATERIALS AND METHODS

Drugs

Doxorubicin was obtained from Teva Pharmaceutical Ind. LTD, squalene from Sigma-Aldrich Co, St. Louis, MO, USA (Specific gravity: 0.858). Amaranth oil with 6% of squalene was obtained from “RusOliva”, Voronezh, Russia.

Animals

Male albino rats, weighing 180–200 g, were randomly housed in groups of five in a temperature-controlled environment (22 ± 2°C) under a 12-h light/dark cycle, with *ad libitum* access to food and water except during experimental procedures. The experimental protocol was approved by the Voronezh State University Ethics Committee.

Drug treatment

Five days after acclimatization, the animals were randomly distributed into six groups (n = 7). All groups received daily gavage of 0.25 ml/kg of amaranth oil (groups 1 and 4), or squalene composition (6% of squalene in refined corn oil as a vehicle, groups 2 and 5) 3 weeks until the end of the experiments. Group 6 received vehicle only (refined corn oil). In 21 days group 4 and group 5 rats were intraperitoneally (i.p.) injected with 15 mg/kg doxorubicin (i.p.), in six injections over 2 weeks for the induction of cardiomyopathy [27]. Control animals group 1, 2 and Group 5) were i.p. injected with physiological saline alone. After the end of the experiment animals were killed, blood was collected using heparin as anticoagulant and the plasma separated was used for the determination of plasma lipid profile parameters. Levels of LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides, free fatty acids (FFA), and phospholipids in the plasma were estimated by enzymatic colorimetric methods.

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Statistical analysis

All results were expressed as the mean ± S.D. for seven animals in each group. All the grouped data were statistically evaluated with SPSS 10.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test; significance level at $p < 0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

In the present investigation, there was a significant ($p< 0.05$) elevation of cholesterol, LDL cholesterol and decreased level of HDL cholesterol in plasma of group 6 doxorubicin-administered rats as compared to groups 1, 2 and 3 control rats, that demonstrated presence of doxorubicin-induced hyperlipidemic condition (Table 1, 2). The amount of triglycerides, free fatty acids and phospholipids in the experimental group 6 reflected hyperlipidemic state according to doxorubicin toxicity too.

Table 1: Levels of LDL-cholesterol, HDL-cholesterol, total cholesterol, in the plasma of normal and experimental groups of rats

	Group №	Supplementation	LDL-Cholesterol	HDL-Cholesterol	Cholesterol
Control	group 1	Amaranth oil	34.3 ± 2.3	19.4 ± 1.28	49.2 ± 3.91
	group 2	Squalene + vehicle	34.9 ± 2.8	20.9 ± 2.40	47.5 ± 3.86
	group 3	Vehicle (refined corn oil)	33.1 ± 2.2	20.0 ±1.80	46.5 ± 4.31
Doxorubicine	group 4	Amaranth oil	42.8 ± 3.10 * ●	16.5 ± 1.10 * ●	84.00 ± 9.45*●
	group 5	Squalene +vehicle	41.9 ± 2.95 #●	17.7 ± 1.20 #●	81.40 ± 6.10# ●
	group 6	Vehicle (refined corn oil)	68.2 ± 6.80*#■	10.8 ± 1.12*#■	120.00 ± 8.60*#■

Results are mean ± SD for 6 animals; one way ANOVA.
Values that have a * differ significantly ($p < 0.05$) with control group 1
Values that have # differ significantly ($p < 0.05$) with control group 2
Values that have ■ differ significantly ($p < 0.05$) with control group 3
Values that have a ● differ significantly ($p < 0.05$) with group 6

Table 2: Levels of triglycerides, free fatty acids, and phospholipids in the plasma of normal and experimental groups of rats

	Group №	Supplementation	Triglycerides	Free fatty acids	Phospholipids
Control	group 1	Amaranth oil	23.6 ± 2.0	14.7 ± 1.2	71.3 ± 5.1
	group 2	Squalene + vehicle	22.1 ± 1.7	12.9 ± 1.0	70.2 ± 4.3
	group 3	Vehicle (refined corn oil)	22.9 ± 1.2	13.4 ± 1.8	72.1 ± 3.9
Doxorubicine	group 4	Amaranth oil	39.80 ± 3.6*●	21.30 ± 1.6 *●	113.25 ± 8.8 *●
	group 5	Squalene + vehicle	37.6 ± 2.9 #●	20.30 ± 2.1# ●	110.00 ± 3.9 #
	group 6	Vehicle (refined corn oil)	50.45 ± 5.0*#■	32.40 ± 2.6*#■	129.56 ± 5.1*#■

Results are mean ± SD for 6 animals; one way ANOVA.
Values that have a * differ significantly ($p < 0.05$) with control group 1
Values that have# differ significantly ($p < 0.05$) with control group 2
Values that have ■ differ significantly ($p < 0.05$) with control group 3
Values that have a ● differ significantly ($p < 0.05$) with group 6

Dietary squalene (group 4) and amaranth oil (group 5) significantly ($p< 0.05$) diminished the doxorubicin-induced elevation in total cholesterol, LDL cholesterols, triglycerides and free fatty acids in plasma as compared to that of group 6 rats (Table 1, 2). The level of HDL cholesterol was significantly ($p< 0.05$) higher in groups 4 and 5 compared to group 6 (Table 1). Squalene (group 4) and amaranth oil (group 5) significantly changed plasma lipid profile with tendency to normalization in doxorubicin-induced cardiomyopathy, but did not return them to the normal level.

It was estimated that the level of LDL-cholesterol and HDL-cholesterol in plasma in Amaranth oil and squalene supplemented groups were not comparable to that of group 1, 2 and 3 correspondingly but sufficiently change them towards the natural level (Table 1, 2). The comparison of lipid profile in healthy rats in three control groups after 21- day administration of amaranth oil and squalene was statistically insignificant.

Squalene has been previously reported to its ability to modulate levels of cholesterol in a dose dependent manner. The cardioprotective effect of squalene-containing food might be explained with its antioxidant property in counteraction of free radicals [4, 5]. Pallavi S et al., (2012) reported that the cardioprotective effect of squalene is probably related to its antilipidemic ability through inhibition of doxorubicin-induced lipid accumulation. Doxorubicin injections into experimental animals lead to different metabolic and morphological aberrations in the heart tissue similar to those observed in human cardiomyopathy. The pathogenesis of cardiac toxicity of doxorubicin is complex and generally attributed to peroxidation of endogenous lipid [24], excessive free radical formation [1], impaired adrenergic stimulation, altered calcium homeostasis [16, 17], concealed mitochondrial function, infiltration of inflammatory cells. It involves accumulation of fat, hypercholesterolemia and disturbance in lipoprotein metabolism [10, 26].

Earlier reported studies suggested several mechanisms of anticholesterolemic property of squalene. It is related to regulation of 3-hydroxy-methylglutaryl coenzyme A (HMG CoA reductase) activity through a feedback inhibition mechanism [25]. HMG CoA reductase is rate-limiting enzyme of mevalonate pathway in cholesterol biosynthesis. Khor and Chieng (1997) reported regulating role of squalene in cholesterol esterification process. The enhanced fecal excretion of cholesterol and its non-polar derivatives in experimental animals is the alternative way to regulate lipid profile in squalene supplementation [22].

High levels of atherogenic LDL-cholesterol and low-risk HDL-cholesterol demonstrate a positive and negative correlation with cardiomyopathy correspondingly [9].

It is necessary to mark that dosage regimens of oral supplementation in studies cited above are not uniform. The most widely used regimens are food supplementation with fixed squalene concentration (1.5-8%) or gavage of squalene, oils and their compositions with fixed dose of active substance. Moreover, dosage recommendations vary considerably depending upon the application. Pallavi S et al., (2012) reported in his papers about higher doses of squalene than its doses applied during the current study. Thus, the recent study had limitations on squalene dose due to maximum squalene content in amaranth oil (6%) and recommended volume of gavage per animal (about 1 ml).We aimed at comparison of amaranth and squalene effects in the same regimens of administration in low doses.

CONCLUSIONS

The low dose dietary supplementation of squalene and amaranth oil had a protective role against doxorubicin-induced cardiomyopathy in rats. There were no significant differences in lipid profiles in groups received amaranth oil and squalene in equal doses of squalene after 21 days of supplementation. The presence of tocopherol and polyunsaturated fatty acids in cold press amaranth oil with 6% squalene concentration did not significantly improve lipid lowering properties of squalene in the current protocol of our experiment. Low doses of amaranth oil with 6% of squalene and 6% squalene in refined corn oil as a vehicle significantly changed plasma lipid profile with tendency to normalization in doxorubicin-induced cardiomyopathy, but did not return them to the normal level.

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