Original Article

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Pharmacology Section

Influence of Grape Seed Extract and Zinc Containing Multivitamin-Mineral Nutritional Food Supplement on Lipid Profile in Normal and Diet-Induced Hypercholesterolemic Rats

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ABSTRACT

Background: Zincovit tablet is combination of grape seed extract and zinc containing multivitamin-mineral nutritional food supplement.

Aims: To investigate the influence of single combined formulation of grape seed extract and zinc containing multivitamin-mineral nutritional food supplement tablets (Zincovit) on lipid profile in normal and diet-induced hypercholesterolemic rats.

Materials and Methods: Anti-hyperlipidemic activity of combined formulation of grape seed extract and Zincovit tablets doses ranged from 40 to 160 mg/kg, p.o. was evaluated in normal and diet-induced hypercholesterolemic rats.

Results: Hypercholesterolemic animals treated with combined formulation of grape seed extract and Zincovit tablets (nutritional food supplement) at 40, 80 and 160 mg/kg exhibited drastic decrease in serum triglycerides, total cholesterol, LDL-C, VLDL-C and rise of HDL-C in comparison to hypercholesterolemic control group animals. The anti-hyperlipidemic effect of single combined formulation of grape seed extract and Zincovit tablet was comparable with the standard drug atorvastatin treated animals and the variations were statistically non-significant. There was no significant impact of combined formulation of grape seed extract and Zincovit tablets on lipid profile among normal animals in comparison with normal control group.

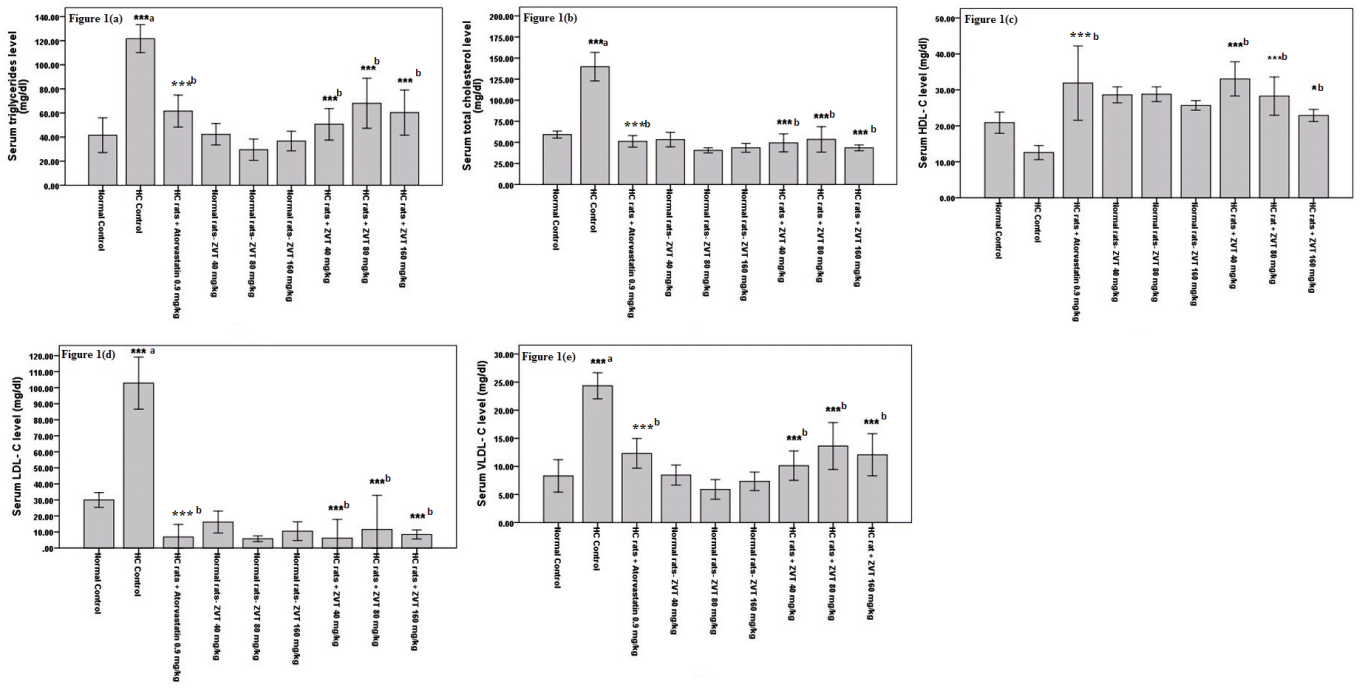
Conclusion: The present study demonstrated that the single combined formulation of grape seed extract and Zincovit tablet is the potential functional nutritional food supplements that could offer a novel therapeutic opportunity against diet-induced hypercholesterolemia in Wistar rats.

Keywords: Antioxidant, Atherosclerosis, Atorvastatin, Grape seed extract, Cardiovascular disease, Reactive oxygen species, Zincovit tablets

INTRODUCTION

According to 3rd report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults-hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases [1]. In 2002, World Health Organization reported that high blood cholesterol contributes to approximately 56% cases of cardiovascular disorders worldwide and causes about 4.4 million deaths each year [2]. Atherosclerosis is the most important manifestation of cardiovascular disease (CVD). Atherosclerosis is a chronic inflammatory disorder that occurs as a result of mononuclear lymphocyte infiltration to the arterial wall, smooth muscle cell proliferation and damage in the arterial wall caused by extracellular matrix accumulation. In this disease, fatty plaques develop on the inner arterial wall, which consequently obstructs blood flow. In addition to several genetic and environmental factors, increased serum cholesterol and oxidized low-density lipoproteins are considered to be major inducing factors of atherosclerosis. Oxidative damage to lipids is a primary cause of atherosclerosis [3]. There is a relationship between atherosclerotic risk factors and increased vascular production of reactive oxygen species (ROS). Oxidized low-density lipoproteins (LDL) and ROS may directly cause endothelial dysfunction by reducing endothelial nitric oxide (NO) bioavailability [4]. Reactive oxygen species induce cardiac dysfunction and cardiac apoptosis and/or necrosis in heart failure [5]. The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification and DNA breaks [6]. There are many choices for treatment, but no definite therapeutic strategies are designed to prevent occurrence of the atherosclerosis. Even after the prescription of dietetic, lifestyle and therapeutic interventions the incidence and prevalence of lipid abnormalities and resultant fatal complications are increasing drastically. Hence there is huge scope for the introduction of effective hypolipidemic and anti-hyperlipidemic drugs in to existing therapeutic armamentarium.

Zincovit tablet is an advanced combined formulation of vitamins, minerals and grape seed extract. Long-term daily administration of grape seed extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation [7]. The biologically active constituents of grape seed extracts are proanthocyanidins, which represent a variety of polymers of flavan-3-ol, such as catechin and epicatechin and have a strong antioxidative effect in aqueous systems [8]. One of the studies suggests that flavonoids possess antioxidant properties and have been shown to be potent inhibitors of LDL oxidation in vitro [9]. We had reported combined formulation of grape seed extract and Zincovit tablets for its strong in vitro, in vivo antioxidant, anti-hyperglycemic and anti-cataractogenic potential [10-14]. Consequently, the aim of the present study was to investigate the influence of single combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) on lipid profile in normal and diet-induced hypercholesterolemic rats.



[Table/Fig-1]: Effect of combined formulation of grape seed extract and Zincovit tablets among experimental animal groups on lipid profile: (a) triglyceride level (b) total cholesterol level; (c) High-density lipoprotein cholesterol level (d) Low-density lipoprotein cholesterol level; (e) Very low-density lipoprotein cholesterol level, *n = 6, number of rats in each group; HC, hypercholesterolemic; ZVT, zincovit tablets with grape seed extract. Values are mentioned as mean. Error bars, +/- 2 standard error of the mean ****p<0.001, *p<0.01, a and b- compared to normal control and hypercholesterolemic control group.*

MATERIALS AND METHODS

Drugs and Reagents

Zincovit tablets (grape seed extract and zinc containing multivitamin-mineral nutritional food supplement) were supplied by Apex Laboratories Private Ltd., Chennai, India. Atorvastatin (Atocor 10 mg tablets; Dr. Reddy's Laboratory Ltd., Hyderabad, India) was purchased from pharmacy of Kasturba hospital, Manipal, Karnataka (India). The diagnostic kits for estimation of triglyceride (TG), total cholesterol (T-CHO) and high-density lipoprotein cholesterol (HDL-C) were procured from Aspen Laboratories, New Delhi (India). The reagents were equilibrated at room temperature for 30 min before use, either at the start of analysis or when reagent containers were refilled.

Preparation of aqueous solution of Zincovit tablets for oral administration: Zincovit tablet is a single combined formulation of vitamins, minerals and grape seed extract. Each tablet of Zincovit weighs 850 mg. Ten tablets of Zincovit were crushed and fine powder form was dissolved in 100 ml of distilled water containing 2 g gum acacia (2% gum acacia). The aqueous solution of Zincovit tablets was stored in an amber coloured bottle at 4ºC in refrigerator.

Animals

Adult male Wistar rats aged 8-12 weeks weighing 150-250 g were used. They were housed in individual cages, maintained under the temperature (22–24°C), 12-h light/12-h dark cycle and relative humidity 40–60%. The animals were fed with a normal pellet diet obtained from Amrit Feeds Ltd., Pune, India and they had free access to water. Animals described as fasted were deprived of food for 12-h but had allowed free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/86/2012) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Diet-induced hypercholesterolemia animal model

The hypercholesterolemic diet included hydrogenated vegetable oil (Vanaspati Ghee) and cholesterol extra pure powder made in to 20% suspension in coconut oil. The suspension was administered at the dose of 5 ml/kg/day at evening hours for 30 consecutive days [15].

Experimental procedure: In the experiment, 54 adult male Wistar rats were divided into nine groups (n= 6). The corresponding doses of drugs were administered orally till 30 days as follow-

Group I: Normal control animals received 2% gum acacia (1 ml/kg/day)

Group II: Hypercholesterolemic control animals received cholesterol in oil (5 ml/kg/day) + 2% gum acacia (1 ml/kg/day)

Group III: Hypercholesterolemic animals received cholesterol in oil (5 ml/kg/day) + Atorvastatin (0.9 mg/kg/day)

Group IV: Normal animals received Zincovit tablets with grape seed extract (40 mg/kg/day)

Group V: Normal animals received Zincovit tablets with grape seed extract (80 mg/kg/day)

Group VI: Normal animals received Zincovit tablets with grape seed extract (160 mg/kg/day)

Group VII: Hypercholesterolemic animals received cholesterol in oil (5 ml/kg/day) + Zincovit tablets with grape seed extract (40 mg/kg/day)

Group VIII: Hypercholesterolemic animals received cholesterol in oil (5ml/kg/day) + Zincovit tablets with grape seed extract (80 mg/kg/day)

Group IX: Hypercholesterolemic animals received cholesterol in oil (5ml/kg/day) + Zincovit tablets with grape seed extract (160 mg/kg/day)

Blood samples

At the end of the experimental period, the animals were anesthetized with ketamine (80 mg/kg; i.p.) following a 12 h fast. Serum was obtained by centrifugation of blood at 3,000 rpm for 20 min at 4ºC using a refrigerated centrifuge (MIKRO 22R, Andreas Hettich GmbH & Co. KG, Germany). The resulting supernatant (serum) was stored at-20˚C.

Biochemical parameters

Serum was analysed further for assay of triglyceride (TG), total cholesterol (T-CHO) and high-density lipoprotein cholesterol (HDL-C) according to the standard protocols given along with respective kits (Aspen Laboratories, New Delhi, India). Low-density lipoprotein cholesterol (LDL-C) and Very low-density lipoprotein cholesterol (VLDL-C) was calculated by using Friedewald's equation:

VLDL-C = Triglycerides (TG)/5

LDL-C = Total cholesterol – (HDL-C+VLDL-C)

STATISTICAL ANALYSIS

Using Statistical Package for the Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, USA), data were expressed as mean ± standard error of mean and analysed by one-way analysis of variance (ANOVA) followed by post-hoc Tukey test. A level for $p \leq 0.05$ was considered to be statistically significant.

RESULTS

Effect on serum lipid profile

There was no significant impact of combined formulation of grape seed extract and Zincovit tablets on lipid profile among normal animals in comparison with normal control group.

In hypercholesterolemic control animals, there was significant increase in serum triglyceride, total cholesterol, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol level when compared with normal control animals.

Both atorvastatin and combined formulation of grape seed extract and Zincovit tablets significantly attenuated triglyceride, total cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol ($p < 0.001$) and improved high-density lipoprotein cholesterol level ($p < 0.001$) among hypercholesterolemic treated rats in comparison with hypercholesterolemic untreated animals [Table/Fig-1a-e].

DISCUSSION

In this study, we investigated the influence of combined formulation of grape seed extract and Zincovit tablets (nutritional food supplement) on lipid profile of Wistar rats. Increased levels of different types of lipids have been implicated in the production of atherosclerosis. Reactive oxygen species induced depletion of antioxidants is a key factor for the initiation of atherosclerosis and the development of cardiovascular disease [16]. Oxidized low-density lipoprotein (LDL) is present in atherosclerotic plaques and is involved in the transition of stable atherosclerotic lesions into active lesions [4]. The precise mechanisms involved have not been fully explained, but oxidation of LDL is believed to play a role in the initiation of atherosclerosis, leading to LDL uptake by macrophages and foam-cell formation, but becoming less important in the later stages of the condition [17,18]. Current knowledge includes effective percutaneous and/or medical management of coronary disease and heart failure [19]. The beneficial effects of medical treatments may be related to their known antioxidant properties [20]. This study demonstrated that, all the three different doses (40, 80 and 160 mg/kg) of combined formulation of grape seed extract and Zincovit tablets treated animals exhibited drastic decrease in serum triglycerides, total cholesterol, LDL-C, VLDL-C and rise of HDL-C in comparison to hypercholesterolemic control group animals. The anti-hyperlipidemic effect of single combined formulation of grape seed extract and Zincovit tablet was comparable with the standard drug atorvastatin (0.9 mg/kg) treated animals and the variations were statistically non-significant. All statins including atorvastatin are well known for the risk of myopathy, rhabdomyolysis and hepatotoxicity in addition to wide range of drug interactions in a dose dependent fashion. Thus, the combined formulation of grape seed extract and Zincovit tablets (nutritional food supplement) can be a better add-on/substitute drug for statins in hyperlipidemic conditions.

Earlier, we had reported combined formulation of grape seed extract and Zincovit tablets for its strong in vitro, in vivo antioxidant, anti-hyperglycemic and anti-cataractogenic potential [10-14]. A recent study has shown that a combination of IH636 grape seed proanthocyanidin extract and niacin-bound chromium can decrease total cholesterol, LDL and oxidized LDL level in hypercholesterolemic human subjects [9]. In one of the study, it has been suggested that proanthocyanidins, might trap reactive oxygen species in plasma and interstitial fluid of the arterial wall, thereby inhibit the oxidation of LDL and show an anti-atherosclerotic activity [21]. In the current study, the anti-hyperlipidemic property of combined formulation of grape seed extract and Zincovit tablets might be attributed to the synergistic interplay of constituents of Zincovit tablets, such as-grape seed extract proanthocyanidins which comprise only procyanidins [subunits constituted of (+) catechin (C) and (–)-epicatechin (EC)], Vitamins A, B, C, D, E, folic acid, biotin and minerals like zinc, copper, selenium, magnesium, manganese, chromium and molybdenum mainly, which are promoters of antioxidant activity and act against oxidative stress.

LIMITATIONS OF THE STUDY

Before moving from bench to bedside, it is essential to further investigate the influence of long term therapy of the combined formulation of grape seed extract and zinc containing multivitamin-mineral nutritional food supplement (Zincovit tablet) on lipid profile in other relevant models. The therapeutic effect seen in animal studies cannot always be entirely extrapolated to humans. Hence, clinical evaluation should be performed to precisely define the cardio-protective role of Zincovit tablets with grape seed extract in humans

CONCLUSION

Thus, the present study demonstrates that the single combined formulation of grape seed extract and Zincovit tablet is the potential functional nutritional food supplements that could offer a novel therapeutic opportunity against diet-induced hypercholesterolemia in Wistar rats. Our study opens the perspective to clinical studies which could improve the clinical outcome of patients subjected to percutaneous angioplasty/statin therapy, a novel view likely to give rise to the performance of clinical trials devised to demonstrate the validity of this paradigm as nutritional food supplement. "This information would eventually complement our findings, opening the way to sustain cardiovascular disease progression in human population".

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