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An evaluation of the effects of Pistacia atlantica gum hydro-alcoholic extract on the phagocytosis ability of macrophages and atherosclerosis development in hypercholesteremic rats

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Original Article

Abstract

BACKGROUND: Atherosclerosis is an inflammatory disease that various factors affect the onset and its progression, including free radicals, hypertension, diabetes, genetic changes, hypercholesterolemia, and even some microorganisms such as herpes viruses and chlamydia. Therefore, compounds that can be effective in any of the above cases may be considered as a useful therapeutic agent in the process of atherosclerosis. The aim of the present study was to evaluate the effects of Pistacia atlantica gum hydro-alcoholic extract on macrophage phagocytosis ability and development of atherosclerosis in hypercholesterolemic rats.

METHODS: The statistical population of the present study consisted of 25 rats that were randomly divided into 5 groups (one control group under standard diet, 4 treatment groups under high-fat diet). After consumption of high-fat food for 45 days, the treatment groups orally received 100, 200, and 400 mg/kg of Pistacia atlantica gum hydro-alcoholic extract for 30 days. Then, peritoneal macrophages were isolated and blood samples were collected to measure the level of nitroblue tetrazolium (NBT), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG). $P < 0.05$ was considered significant in all evaluations.

RESULTS: The level of cholesterol (503.66 ± 17.15), TG (436.66 ± 16.80), LDL-C (343.66 ± 11.59), HDL-C (54.33 ± 7.02), and NBT (0.64 ± 0.02) decreased in the treatment groups. Besides, exactly in a concentration-dependent manner, plant extract significantly reduced the level of respiratory potential level in macrophages.

CONCLUSION: Hydro-alcoholic extract of Pistacia atlantica gum could effectively decrease hypercholesterolemia and increase phagocytic ability of macrophages. Therefore, it can be suggested for more investigation as a blockage of atherosclerosis.

Keywords: Pistacia Atlantica Gum; Atherosclerosis; Macrophage Ability; Hypercholesterolemic Rats

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Introduction

Herbal medicine is one of the most important types of complementary medicine. The term complementary and alternative medicine (CAM) is widely used to refer to activities that are not fundamental elements of contemporary or conventional medicine and, as a result, would not be included in traditional medical school curricula.[1]

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Traditional medicine practices and procedures include the use of herbal medicines into their practice; therefore, herbal medicines play a significant part in traditional medicine.[2] Herbal medicines are plant components that are frequently used as natural resources for self-administered medicinal treatments and as supplements in the general public. Fruits, seeds, berries, four roots, rhizomes, leaves, bark, and flowers are among the plant components used.[3] Pistacia atlantica is an Anacardiaceae plant which grows in nature in several parts of Iran. The Anacardiaceae class has traditionally been known to have antibacterial, fungicidal, and cytotoxic characteristics.[4] In folk medicine, Pistacia atlantica, which is called Baneh, has been used to treat dermatitis, oral infections, kidney stones, and asthma. Baneh has been shown to have a pro-apoptotic and anti-proliferative impact on malignant cells.[5] It has also been shown to have antioxidant,[6] antimutagenic,[7] antibacterial and antifungal,[8] anti-inflammatory,[4] antidiabetics,[9] anticancer,[10] anti-hepatitis,[11] anti-atherosclerosis,[12] and anti-cholinesterase[13] properties. Across the last several years, extreme changes in the habits of people all over the world have shifted human civilizations away from farmed goods and active lifestyles and toward fast meals and inactive lifestyles.[14] The combination of such a diet and rising cigarette consumption has increased the risk of heart disease. Atherosclerosis is the primary cause of coronary heart disease and stroke, which are globally the major causes of mortality.[15] Atherosclerosis is a chronic inflammatory disease of the arteries that produces serious clinical issues such as severe coronary heart disease and stroke. Atherosclerotic plaques are defined by an accumulation of macrophages, lipids, smooth muscle cells, fibroblasts, and, in late stages, necrotic material inside the artery wall. In the absence of additional risk factors, high serum cholesterol can increase the occurrence of atherosclerosis.[16] In rat models of atherosclerosis, lipid-enriched diets are frequently used to promote or expedite the formation of atherosclerotic lesions.[17] A number of diets have been employed, with varying levels of cholesterol, fatty acid levels and types, and the lack or existence of cholate.[18] The aim of this research was to examine the effect of a hydro-alcoholic extract of Pistacia atlantica gum in hypercholesterolemic rats.

Materials and Methods

Study design: The experimental sample consisted of 25 mature Wistar male rats weighing 150-180 g, purchased from Baqiyatallah University of Medical Sciences, Tehran, Iran, and transported to the Animal Home of the Applied Virology Research Center. Rats were placed in the usual light regime for 12 weeks (12 hours of day, 12 hours of night), relative humidity of 55%, and at 25 °C to acclimatize to the setting. This study was approved under the supervision of Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran (ethics code: IR.SBMU.RETECH.REC.1398.671).

Treatment groups: The standard diet group consisted of five healthy rats fed a regular diet for 75 days. Hypercholesterolemia diet group included 20 rats which were fed a high-cholesterol diet (1% cholesterol by weight of food) for 75 days.

To demonstrate the emergence of atherosclerosis on the 45th day after the spread of the disease beginning, blood samples were collected from all rats in the following sequence, and the biochemical profile of the blood was analyzed. Rats with hypercholesterolemia were divided into four equal and random groups as follows:

The disease group comprised 5 rats fed a hypercholesterolemia diet (1% cholesterol by food weight) for additional 45 days. These rats received the same volume of normal saline buffer as the treatment groups for 30 days.

Treatment groups of 100, 200, and 400 each had five rats that were fed a high-cholesterol diet for additional 45 days, much like the prior group. For 30 days, these rats were given oral doses of 100, 200, and 400 mg/kg of Pistacia atlantica extract, respectively.

Extracting Pistacia atlantica gum: Khorramabad grocery stores in Lorestan Province, Iran, provided fresh Pistacia atlantica gum. The gum was purchased and identified by a herbarium collection manager at the University of Khorramabad (Herbarium No. HLu27061397). Using an electric mill, the gum was powdered and vacuum-dried after rinsing/drying. The powder was then steeped in filtered water for 48 hours before being filtered. The extract was incubated at 50° C before being re-distilled.[19]

Biochemical assays: The animals were fasted for 12 hours before the start of the trial. On day 45, and at the completion of the investigation, the blood was drawn from the middle ear vein to test for biochemical variables. Total cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were tested in Virology Research Center Laboratory by using biochemical enzyme kits.

Macrophage isolation: Phosphate-buffered saline (PBS) injection was used to collect resident macrophages from the peritoneal cavity of rats. Cells were washed twice in PBS before being suspended in Roswell Park Memorial Institute **(**RPMI) 1640 medium with 10% heat-inactivated fetal bovine serum (FBS), 10000 U/ml penicillin G sodium, 10 mg/ml streptomycin sulfate, and 2 mM glutamine. Neubauer's chamber was used to count the cells, and trypan blue dye exclusion was used to assess cell viability. Then, for 40 minutes at 37 °C in a damp environment of 5% carbon dioxide (CO_2), 100 µl of live cell suspension (2×10^6 cell/ml) were preincubated in 96 well microplates. This procedure increased macrophage adhesion to the plate. The plate was washed twice with PBS to eliminate non-adherent cells.[20]

Respiratory burst: Respiratory burst of phagocytic cells in macrophage population was checked using nitroblue tetrazolium (NBT) dye reduction as described previously with some modification. In brief, 100 µl of suspension of macrophage with 0.1 ml of Staphylococcus aureus suspension (10^8 cell/ml) and 0.1 ml of 0.1% NBT in PBS (pH = 7.4) was mixed. The mixture was incubated at room temperature for 15 minutes and subsequently kept at 37° C for additional 15 minutes. The reduced dye was extracted in dioxane and quantitated at 520 nm.[20]

Statistical analysis: Continuous data were reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test, was used to evaluate the differences between groups. P-values less than 0.05 were regarded as statistically significant. SPSS (version 19.0, SPSS Inc., Chicago, IL, USA) was used as a statistical software.

Results

Lipid profile changes in the studied rat: The results of our study showed that extract in a concentration-dependent manner significantly reduced the level of cholesterol, TG, and LDL-C and significantly increased the level of HDL-C compared to the positive control group (Figure 1).

Macrophage respiratory burst: The results of our study showed that extract in a concentration-dependent manner significantly reduced the level of respiratory potential level in macrophages (Figure 2).

Discussion

Blood cholesterol is transported in the form of various lipoproteins, among which LDL has the greatest role in the development of atherosclerosis. Various studies have shown that elevated cholesterol initiates and accelerates the progression of atherosclerosis.[21,22]

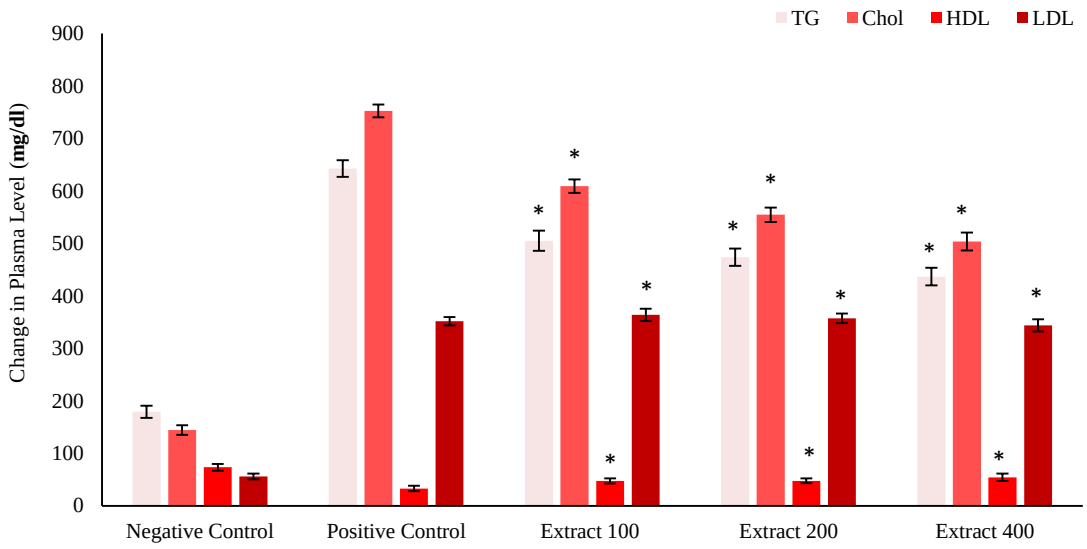

Figure 1. Evaluation of fat profile changes in the studied rats after being treated with extract (Dunnett's post-hoc test)

*A significant P-value level (< 0.05) compared with positive control group

Negative control: Healthy rats; Positive control: Rats received hypercholesterolemia diet + normal saline; Extract 100: Rats received hypercholesterolemia diet + 100 mg/kg of Pistacia atlantica extract; Extract 200: Rats received hypercholesterolemia diet + 200 mg/kg of Pistacia atlantica extract; Extract 400: Rats received hypercholesterolemia diet + 400 mg/kg of Pistacia atlantica extract

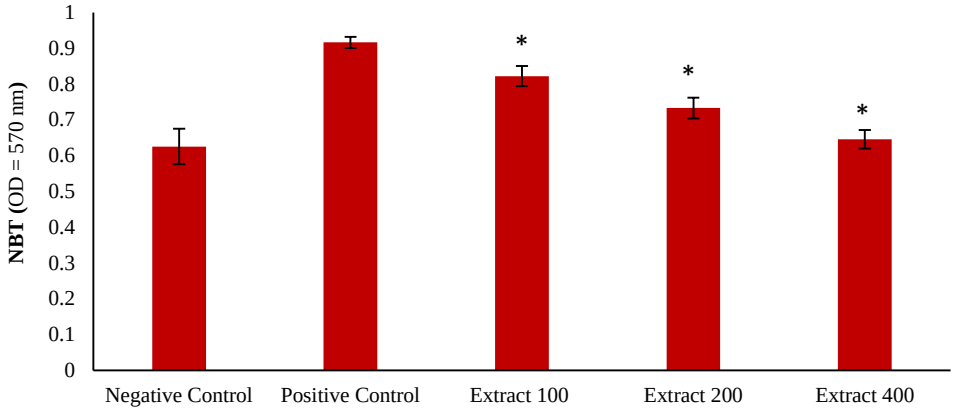

Figure 2. Evaluation of fat profile changes in the studied rats after being treated with extract (Dunnett's post-hoc test)

*A significant P-value level (< 0.05) compared with positive control group

Negative control: Healthy rats; Positive control: Rats received hypercholesterolemia diet + normal saline; Extract 100: Rats received hypercholesterolemia diet + 100 mg/kg of Pistacia atlantica extract; Extract 200: Rats received hypercholesterolemia diet + 200 mg/kg of Pistacia atlantica extract; Extract 400: Rats received hypercholesterolemia diet + 400 mg/kg of Pistacia atlantica extract

In this study, we also looked at increasing cholesterol as a biomarker associated with disease progression. Based on the findings of this study, it appears that consuming a hydro-alcoholic extract of the plant has a positive effect on blood lipid profile and inflammatory markers in hypercholesterolemic rats. It appears that ingesting a high-cholesterol diet for 45 days produced hypercholesterolemia in rats due to a rise in cholesterol, TG, and atherogenic index levels. Likewise, comparable findings have been observed in earlier trials with a high-cholesterol diet (1%).[23]

The findings of this study also demonstrated that the hydro-alcoholic extract of Pistacia atlantica gum, at all dosages, was effective in lowering cholesterol, TG, and the atherogenic index levels. Atherosclerosis is a chronic inflammatory condition in which macrophages play many significant roles. Phagocytic proinflammatory cells proliferate in atherosclerotic lesions, where they actively contribute to cholesterol build up. Furthermore, by maintaining a proinflammatory environment, macrophages encourage the development of complex and unstable plaques. Simultaneously, anti-inflammatory macrophages aid in wound healing and remodeling, as well as plaque stability. Macrophages are thus appealing candidates for antiatherosclerotic treatment development, which can attempt to decrease monocyte recruitment to the lesion site, inhibit proinflammatory macrophages, or induce anti-inflammatory responses and cholesterol efflux. The respiratory burst potential of macrophages taken from atherosclerotic rats was reduced after treatment with Pistacia atlantica extract, according to our findings. So far, no research has been conducted on the characteristics and effects of Pistacia atlantica gum in the treatment of atherosclerosis.

Hosseini et al. discovered that administering Pistacia atlantica extract for three weeks in a row enhanced the lipid profile, oxidative stress, and inflammatory process by lowering lipid peroxidation and boosting antioxidant capacity.[1] Omidi et al. discovered that feeding rats with Pistacia atlantica nut powder for 15 days raised cholesterol in all lipoprotein fractions while decreasing TG levels in the liver. Although liver phosphatidate phosphohydrolase PAP activity was reduced by around 11%, it was not statistically different from the control group. In contrast, rats fed Pistacia atlantica nut powder for 60 days showed no significant change in any lipoprotein fraction when compared to the control group; however, the TG level in the liver fell substantially. Furthermore, as compared to the control group, liver PAP activity reduced by approximately 16%. It may be concluded that Pistacia atlantica nut powder has the capacity to lower TG levels in the liver of rats.[2] Sarir et al. discovered that the Diabetes + exercise + mastic extract (DEM) group had significantly reduced TG levels than the diabetic group ($P < 0.05$). The mean cholesterol and HDL-C levels were not substantially different across groups. T3 and T4 plasma concentrations were considerably lower in diabetic control than in normal control. Pistacia atlantica extract, alone or in combination with exercising, significantly increased T4 levels in

diabetic rats.[3] According to Jamshidi et al., several types of wild pistachio (WP) (Pistacia atlantica mutica) oil produced hypotriglyceridemia, while only the mixed and kernel oil groups induced hypocholesterolemia. Kernel oil also decreased LDL-C and HDL-C levels considerably ($P < 0.05$). Furthermore, as opposed to the fructose group, mixed and kernel oils significantly reduced glycemic indices (fasting blood glucose and insulin resistance). Serum insulin levels in the kernel oil group were substantially higher ($P < 0.05$). All WP oils also reduced inflammation substantially [interleukin 6 (IL-6)].[4] Abtahi Froushani et al. demonstrated that utilizing herbal medication had favorable benefits on hypercholesterolemia reduction in an animal model.[24] Behmanesh et al. discovered that while blood glucose, malondialdehyde (MDA) levels, and the number of atretic follicles were raised, catalase (CAT), superoxide dismutase (SOD), and the number of corpora lutea were dramatically lowered in diabetic rats that were not treated. In the treated rats, these alterations reverted to normal or were reduced by Pistacia atlantica extract and glibenclamide. Furthermore, the results demonstrated that the extract of Pistacia atlantica possessed antihyperglycemic and antioxidative characteristics, as well as the ability to reduce ovarian problems in experimental diabetic rats.[5] This study shows that extract of Pistacia atlantica has antihypercholesterolemic and hypotriglycemic properties. It lowers LDL-C and TG, and raises HDL-C in rats that were fed a cholesterol-enriched diet. To assure the safety of this plant extract, however, toxicological tests must be conducted.

Conclusion

It was discovered that the hydro-alcoholic extract of Pistacia atlantica gum had excellent benefits on lowering the biochemical profile of hypercholesterolemia in the animal model. This is merely a preliminary study, and additional research will be conducted.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contribution

The conceptualization was done by MG and HEGG. The formal analysis and interpretation were done by MG and HEGG. The resource and original draft preparation were carried out by MG, HEGG, MMN, SV and SMY. The supervision was done by SMY. The whole manuscript was read and approved by all the authors.

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