Baneh Fruits (Pistacia Atlantica) improved Some Biochemical Parameters in Wistar Male Rats

Mohsen Jafarian Dehkordi(PhD)[1], Reza Azadbakht(MD)[2], Reza Fathi Hafshejani(MD)[2], Soudeh Khanamani Falahati-pour (PhD)[3], Sakineh Khanamani Falahati-pour (PhD)[4*]

[1] *Assistant Professor, Department of Clinical pathology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*

[2] *Graduate of Veterinary, Faculty of Veterinary Medicine, University of Islamic Azad of Shahrekord, Shahrekord, Iran*

[3] *Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

[4] *Department of Pharmacology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

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Corresponding Author:

Sakineh Khanamani Falahatipour

Email: Falahatipour@yahoo.com

Tel: +98-34-33221211

Abstract

Introduction: In traditional medicines, almost all Asian countries, particularly Iran, consider plants for therputical purposes. This study assessed the impact of the Baneh Fruits (*Pistacia Atlantica*) hydroethanolic extract on certain enzymes and biochemical elements in rats.

Materials and Methods: 200 and 400 mg/kg of the extract were given orally once a day for 28 days to two rat groups, each with eight animals. The control group was given only distilled water. Under chloroform anesthesia, the rats fasted overnight were dissected on the 29th day. Then, their blood was directly taken from their hearts. In the next step, some liver biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin, biochemical parameters of kidney including blood urea nitrogen (BUN), creatinine, urea, and metabolic parameters including fasting plasma glucose (FPG), cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were measured in the collected blood serums.

Results: The plant fruit extract decreased significantly the serum concentrations of triglycerides, cholesterol, AST, creatinine, and total bilirubin in the two doses ($p<0.05$). The 200 mg/kg dose decreased BUN and urea concentrations and increased HDL-C concentration significantly ($p<0.05$). While only a dose of 400mg/kg of the extract decreased the GGT level significantly ($p<0.05$).

Conclusions: The hypocholesterolemic, hypotriglyceridemic, and hypo bilirubinemic impact of the extract coupled with the decreased AST activity indicated that the Baneh fruit may be beneficial in controlling hyper-triglyceridemia and hyper-cholesterolemia.

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1. Introduction

From prehistoric and ancient times to modern times, almost all Asian countries, particularly Iran, have been considering plants in traditional medication. Herbal medication still has an important role in various therapeutic procedures despite recent advancements in contemporary medication using synthetic drugs. In fact, therapeutic properties of various plants have raised the interest in herbal medication [1]. Various plant parts (root, stems, leaves, fruit, and seeds) are used for remedy purposesbecause they contain biologically active ingredients that can be used for preventing and managing mild or chronic diseases. Tannins, alkaloids, flavonoids, and phenolics are the major bioactive compounds present in plants. The use of of medicinal plants to treat various diseases is getting a new interest [2]. Over 7,000 species of plants grow in Iran of which almost 1,000 are estimated to have remedial effects. The presence of diverse ecological factors and climatic conditions provide an environment in which abundant varieties of plants grow in different regions of the country of Iran [3].

The high nutritious, protein level, and considerable fiber nuts with diatry contributions are factors that are anticipated to increase the demand for them. Nuts have well-known effects in the human diet [4]. Pistachio is a nutrient-dense nut commonly but much less recognized than others. Different pistachio species are a rich energy source and contain several health-benefiting minerals, nutrients, vitamins, and antioxidants necessary for human health [4].

P. atlantica genus has a wide distribution in Iran [2]. *P. atlantica* is one of the most extensive wildlife species mostly found in moderate forests, tropical hardwoods, and boreal conifers [5]. Their fruit 0.5-0.7 cm in diameter is round to oval and flat to some extent. Their hard wooden shell is enclosed by a relatively dry hull that can be removed easily by pressing between fingers [6].

Widespread pharmacological properties of *Pistacia* species have interested researchers to further investigate different parts of this plant such as leaves, kernels, and hulls. Pistachios have various biological properties mainly due to the presence of various phenolic components [2]. The *Pistacia atlantica* fruits are called "*Baneh*" in Iran. Once ground and mixed with other constituents, the fruits of this plant are used by the natives as food [6]. It has a rich history in prehistoric medicine [7]. Various parts of the plant, such as the leaf, resin, hull, and fruit have been extensively applied as traditional drugs in treating different diseases, including gastrointestinal (e.g. upper abdominal discomfort, dyspepsia, peptic ulcer, and antidiarrheal effect) [5], respiratory, cutaneous, renal, and infectious diseases [8]. *P. atlantica* species also have restorative and sedative impacts, as well as various pharmacological characteristics, including anti-inflammatory, anti-microbial, anti-bacterial, anti-fungal, anti-hyperlipidemia [1], anti-asthmatic [8], antioxidant, antidiabetic, anticancer, anticholinesterase activity [9], and anxiolytic-like effects [10].

The fruit is rich in oil (39.80%) and protein (10.39%). Chemical studies on *P.* atlantica have shown the presence of fatty acids, flavonoids, triglycerides, the chemical composition of the oleoresin, and the chemical composition of essential oils [11]. Tocopherols, carotenes, alcohols, and unsaponifiable matter with

substantial anti-oxidative activity equal to vitamin E are the major elements of *P. atlantica* essential oil. According to several studies, the anti-oxidative activity of *P. atlantica* essential oil is considerable. Further, the nutritional index of the food is improved by its anti-oxidative activity [7].

The present study aims to assess the impact of various concentrations of hydro-ethanolic extract of *P. atlantica* fruits on biochemical factors.

2. Material and methods

2.1. Plant material and extract preparation

The hulls of *P. atlantica* were manually collected and picked up from the central region of Lordegan, Chaharmahal and Bakhtiari province, Iran in July. Then, the plant's genus and species were recognized and approved in the Herbarium of Medical Plants Research Center of Shahrekord University of Medical Sciences (Iran). The hulls were separated from the fruits. At room temperature (23-24 ℃) naturally in shadow on laboratory benches, the fruits were dried and powdered by an electric blender. The powder was then suspended in 1000 ml of ethanol 96% and distilled water solution (7:3, v/v) for 72 h at oven temperature (37 ℃). Using a fine muslin cloth, the mixture was filtered, followed by filter paper (Whatman No 1). The filtrate was centrifuged at 4500 rpm for 8 min. Then, the supernatant fraction was saved. The obtained clear residue was placed in a rotary evaporator for solvent evaporation. Then, the resulting extract was placed at oven temperature (37 ℃) to evaporate the remaining solvent for 48 hours. Afterward, the required amounts of dried extracts were dissolved in distilled water, based on the weight of the rats, and the intended doses were prepared and homogenized by a shaker. Finally, the extracts were stored at 4 ℃ to be used in later experiments [1].

2.2. Animals

Matured Wistar strain male albino rats (180-240 g) were used to conduct experiments. They were kept in controlled standard laboratory settings (22 ± 3 ℃, 12 h light/dark photo-cycle, and 45% humidity) with ad libitum access to water and food. The research was performed following the ethical principles of laboratory animal guidelines. The Institutional Ethics Committee of Shahrekord University of Medical Sciences approved the experimental protocol [9].

2.3. Experimental design

This study was conducted using an experimental design with control and test groups. Twenty-four male Wistar rats were placed into two test groups and one control group, each with eight animals. *Pistacia atlantica* hydro-alcoholic extract in a single dose of 200 and 400 mg/kg body weight was given to the rats in two test groups orally for 28 days daily at a specified time.Then, blood samples were taken from their heart, and biochemical parameters were measured. Most serum biochemical parameters including blood urea nitrogen (BUN), urea, creatinine, fasting plasma glucose (FPG), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), serum total cholesterol, high-density lipoprotein (HDL-C), triglyceride, and total bilirubin levels were assessed in the samples collected using commercial kits (Parsazmoon, Iran) and an autoanalyzer (BT 3000 Pluse, Italy).

2.4. Statistical analysis

SPSS software version 20 was used to analyze the data. The outputs were expressed as means ± SD. Further, a one-way analysis of variance (ANOVA) and posthoc Tukey tests were performed. Values with $p < 0.05$ were considered significant statistically.

3. Results

As shown in Table 1, the plant fruit extract significantly decreased the serum concentrations of triglycerides, cholesterol, AST, creatinine, and total bilirubin in the two doses ($p < 0.05$). The 200mg/kg dose significantly raised the HDL-C level ($p < 0.05$). Besides, 200 mg/kg dose decreased BUN and urea concentrations significantly ($p < 0.05$) and the 400 mg/kg dose decreased the concentrations of BUN and urea, but they were not significant. While only 400mg/kg dose of the extract significantly decreased the GGT level (Table 1). However, the 200mg/kg dose of the extract decreased the GGT level, but it was not significant. *P. atlantica* extract decreased the ALT level, but it was not significant.

Table 1: Effect of hydroethanolic extract of *P. atlantica* on biochemical parameters in Wistar Rats.

Parameters	Control	B200	B400
Glu (mg/dl)	259.62±35.66	253.75±95.1	291.12±74.49
Cho (mg/dl)	55.37±6.67	41.62±7.43*	40.5±2.67*
Tg (mg/dl)	126.12±35.69	66.5±8.26*	59.25±16.78*
HDL-C (mg/dl)	39.75±6.75	63.62±31.16*	43.37±1.30
SGOT (AST) (u/l)	191.75±41.4	146.12±17.3*	154.25±23.28*
SGPT (ALT) (u/l)	47.87±6.83	46±6.41	46.25±4.83
T Bil (mg/dl)	0.375±0.09	0.262±0.05*	0.212±0.03*
BUN (mg/dl)	22.5±3.34	18±2.93*	19.62±3.07
Cr (mg/dl)	1.45±0.81	0.45±0.05*	0.425±0.05*
Urea (mg/dl)	28.19±7.14	18.55±6.27*	22.037±6.57
GGT (u/l)	4.75±1.49	3.66±2.12	2.125±1.13*

*: Significantly different from the control group at $P < 0.05$. B200: Test group1, B400: Test group2, Glu: Fasting plasma glucose (FPG), Cho: Cholesterol, Tg: triglyceride, HDL-C: High-density lipoproteins, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TBil: Total bilirubin, BUN: Blood Urea Nitrogen, Cr: Creatinine, GGT: Gamma Glutamyl Transferase

4. Discussion

Regarding the unique availability of chemical diversity, natural products, including plant extracts, as either pure or standardized mixtures, offer many prospects to discover new drugs. According to the World Health Organization (WHO), above 80% of the world's population count on traditional medications for their initial healthcare needs [8].

Over recent years, interest in *P. atlantica* and its extracts has dramatically increased owing to positive clinical impacts reported [12].

The findings of this study indicated the *P. atlantica* extract can decrease cholesterol and triglyceride levels and increase HDL-C levels. In fact, *P. atlantica* contains principal components such as phytosterol and terpene [10]. Phytosterols are effective in reducing both total and LDL cholesterol concentrations and producing some positive effects on the HDL-C cholesterol concentration [13]. Recent evidence indicates that phytosterols prevent cholesterol absorption due to their hydrophobicity, which is higher than cholesterol due to a bulkier hydrocarbon molecule and includes a superior affinity for micelles than has cholesterol. Cholesterol is, therefore, displaced from micelles by limiting the available amount for absorption [14]. Thus, the greater intake of pistachio-derived phytosterols may be due to a hypocholesterolemic effect [13]. The L-arginine content of nuts as rich sources of proteins often is high. Typically, L-arginine is prescribed in people with high cholesterol as a supplement [14]. The low content of saturated fatty acids may also contribute to the blood-lipid-lowering effect of pistachio nuts since it is now clearly documented that replacing polyunsaturated fat with saturated fat is effective in reducing LDL-C and the total cholesterol to HDL-C-C ratio [13]. Furthermore, cholesteryl ester transfer protein (CETP), a plasma protein with a vital role in transporting cholesteryl esters from HDL-C particles to those of LDL and VLDL, and Stearoyl-CoA desaturase (SCD), a rate-limiting enzyme that catalyzes the synthesis of monounsaturated fatty acids from saturated fatty acids and has a key role in triacylglycerol, cholesterol, and lipoprotein metabolism, are regulated through fatty acids and are significant potential mechanisms to clarify how *P. atlantica* affect lipoproteins and lipids [15]. In agreement with these findings, Li et al. found that pistachio consumption reduced body weight and plasma triacylglycerols compared to a carbohydrate snack in overweight people [16]. Moreover, Sari et al. showed the pistachio diet for 4 weeks, by substituting the monounsaturated fat content constituting about 20% of daily caloric consumption, reduced significantly total triacylglycerol (P=0.008, -13.8+/-33.8%) and cholesterol (P<0.001, -21.2+/-9.9%) [17]. Anyway, animal and epidemiologic studies indicated that increasing HDL-C levels can delay atherosclerosis development [18] and prevent coronary artery disease [19]. Based on the data in the present study, *P. atlantica* fruit extract can be beneficial for dyslipidemia. Even though it is soon to suggest a treatment modality for humans and animals based on the present animal study, increaing HDL-C cholesterol with the consumption of *P. atlantica* might suggest a reduction in coronary artery disease risk.

The indicators of concentration of hepatic intracellular enzymes are serum aminotransferases including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) leaked into the circulation. These are the signs of hepatocellular injury. Aminotransferases are sensitive indicators of liver cell damage and beneficial in diagnosing acute hepatocellular diseases

including hepatitis [20]. The obtained results showed that oral administration of hydro-alcoholic extract of *P. atlantica* to rats improves liver functional factors, including serum ALT and AST levels. Since the fruits of P. atlantica has significant antioxidant activity and free radical scavenging activity [21], *P. atlantica* extract can increase antioxidant enzymes including the glutathione peroxidase, superoxide dismutase, and catalase activities in the liver and reduce the malondialdehyde level and nitric oxide synthase activity, hence improving the innate antioxidant defense system in the liver. Therefore, *P. atlantica* extract can protect liver tissue from reactive oxygen species (ROS) damage by decreasing oxidative stress and inhibiting liver lipid peroxidation, thus decreasing the ALT and AST activities in serum [22]. In factthe ingestion of this plant can help the body to better combat continuously produced free radicals and further potentiate the scavenging capacity of the liver [23]. In agreement with our results, Iranmanesh et al. evaluated the impact of hydro-alcoholic extract of *pistacia vera* on carbon tetrachloride-induced hepatotoxicity in male rats. They reported that gavage of hydro-alcoholic extract of *P. vera* (10, 50, 100 mg/kg) significantly decreased ALT and AST indices ($p<0.05$).

Urea is an organic compound with a key role in the metabolism of compounds containing nitrogen by animals. It is also the major nitrogen-containing ingredient in the urine of mammals. Tissue breakdown e.g. hemorrhage can result in increasing urea levels in the blood [24]. The data in the present study showed a decrease in urea and BUN concentrations of the treated rats. Though the decrease in test group 2 was not significant ($P>0.05$] as compared with the control. However, the decrease in test group 1 was significant ($P<0.05$] as compared with the control. The creatinine levels decreased in both test groups significantly ($P<0.05$]. Since the main nitrogen excretion pathway is urea synthesis in the liver released into the blood and cleaned by the kidney, the blood urea reduction in animals receiving *P. atlantica* extract can either be because of lower urea synthesis in the liver or higher urea excretion in the kidney. P. atlantica can stimulate liver performance and urea synthesis due to its antioxidants activity [21]. Furthermore, since in nephrons, urea will be passively reabsorbed, the BUN reduction in animals receiving *P. atlantica* extract suggests urea reabsorption inhibitory system in the nephrons [25].

Creatinine is a muscle metabolism product excreted unchanged by the kidney. Serum creatinine is a sign of kidney health and is easy to measure.. If the filtration ability of the kidney decreases significantly, such as with renal disease, the creatinine blood level rises. As the data in this study showed a decrease in creatinine level, the extract is presumed to be nephroprotective. However, this decrease in creatinine level may be attributed to the chemical constituent of the extract such as flavonoids. In general, the results indicated that the plant extract may be beneficial for the management of renal diseases [24].

Bilirubin [BR], a catabolic product of hemoglobin and other haem-containing compounds in mammals, is delivered to the liver by albumin for additional metabolism. *P. atlantica* can reduce total bilirubin probably by protecting from liver damage, enhancing its bilirubin conjugating feature, and assisting in clearing bilirubin from circulation. In fact, the following mechanisms can be proposed as the bilirubin-lowering potential of *P. atlantica*. *P. atlantica* extract might activate the Constitutive Andostane Receptor, a vital regulator in the

bilirubin clearance pathway, and increase the activity of glucuronyl transferases, the synthesis of ligandin, a transporter of bilirubin, and the conjugation of bilirubin in the liver. Besides, *P. atlantica* extract may inhibit the activity of haem oxygenase, the bilirubin pathway's rate-limiting enzyme [26]. Furthermore, glucosides in the extract [27] might be transformed into glucuronic acid to conjugate with bilirubin for excretion.

GGT is an enzyme embedded in the hepatocytes' plasma membrane. Any damage to the plasma membrane will result in release of GGT into serum. GGT is, therefore, regarded as the best sign of liver damage. *P. atlantica* extract lowered the GGT level significantly in test group 2 [400 mg/kg], showing that the extract prevents liver damage by preserving plasma membrane integrity [28].

5. Conclusion

Overall, this study suggested that balanced consumption of *P. atlantica* nuts may be beneficial for lowering the cardiovascular risk, especially that attributable to hypercholesterolemia. Moreover, the results provided scientific evidence of using *P. atlantica* in traditional medicine to prevent and treat hepatic, renal, and cardiovascular diseases.

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Declarations

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Conflicts of interest/Competing interests

All authors declare that they have no conflict of interest.

Availability of data and material

Not applicable

Code availability

Not applicable

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