

In vivo study of cherry stick effect on concentration of serum total cholesterol, triglyceride and total protein in white albino male mice

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Summary:

Background: Cherry extract has a wide range of biological activity. In the present investigation, two oral doses 0.04&0.08 mg/kg/day of cherry stick (ethanol extract) in albino male mice resulted in a significant decrease in concentration of serum total cholesterol concentration, triglyceride and total protein, albumin and globulin.

Objective: we investigated the impact of biological activities of cherry stick extract on serum cholesterol, triglyceride, total protein, albumin and globulin in white albino male mice.

Design: This study include three groups of white albino male mice. The first group (G1) was a control group comprised of 5 animals. The second group (G2) consisted of 5 animals treated with 1ml of cherry stick extract at concentration of 0.04 mg/kg/day for two weeks after treatment, while the third group (G3) consists of 5 animals treated with 1ml of cherry stick extract at concentration of 0.08 mg/kg/day for two weeks with treated animals.

Results: there was a statistically significant reducing effect on serum total cholesterol, triglyceride, total protein and albumin $P < 0.001$ at 0.04 mg/kg/day and 0.08 mg/kg/day for all biochemical parameters. Globulin was markedly decreased $P < 0.01$ in comparison with control but no significant change in ratio of albumin to globulin.

Conclusion: Extract of cherry stick exerts at low dose a remarkable effect on serum cholesterol, triglyceride and total protein.

Key words: Cherry, Cholesterol, Triglyceride, Albumin and Globulin.

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Introduction:

Plants and plant products have been used extensively throughout history to treat medicinal problems (1). There are various medicinal plants in the world, which are the potential sources of the drugs. It is believed that herbal medicine has little side effects as well as it requires no cost in few cases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (2). Fruits contain a wide variety of phytochemical that are suspected to provide health benefits, yet most phytochemicals have not been studied for their effect on human health in fruit (3). Major risk factors for cardiovascular disease (CVD) include hyperlipidemia; elevated total and LDL-cholesterol, elevated triglyceride, low HDL- cholesterol, as well as increased plasma homocysteine, thrombotic activity and hypertension. Atherogenesis is among multiple risk factors, causally related to elevated serum lipid levels (4,5). Additionally, a relation to the oxidation of low density lipoprotein (LDL) in the arterial wall is

assumed by Esterbauer et al (5). Numerous epidemiological studies indicate an inverse association between fruit and vegetable intake and the risk for CVD and ischemic stroke (6, 7). In addition to providing essential vitamins, minerals and dietary fibers, fruits contain polyphenols and flavonoids that exhibit antioxidants, anti-inflammatory, anticarcinogenic and lipid lowering properties action, which may reduce the risk of heart disease and other chronic diseases (8,9).Cherry is a sweet fruits with a nice taste and healthy effects on the human. Both sweet and tart cherries are rich in phenolic compounds, including anthocyanins with highest concentration responsible for red skins and flesh color, catechins, chlorogenic acid, flavonal glycosides, melatonin and other compounds which have been shown to exert antioxidant action (10). Anthocyanins extracted from cherries exhibit anti-inflammatory properties, via inhibition of cyclooxygenase activities (11).Cherry contained the oxidized form of vitamin C, dehydroascorbic acid but the reduced form ascorbic acid is unusual among fruits (12). The anti-inflammatory effects of cheery may be beneficial for the management and prevention of inflammatory

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disease. In a recent study (2006), the effect of consuming sweet cherries on circulating concentrations of inflammatory markers and lipid profile was examined. The study showed a reduction in the concentration of some markers of inflammation, but none of the lipid variables were altered by the intake of cherries (13). To our knowledge there is no published data on the effect of cherry stick extract on concentrations of cholesterol, triglyceride and total protein; therefore the aim of the present study is to investigate the effect of stick cherries extract on the concentration of blood cholesterol, triglyceride, total protein, albumin and globulin in serum of white albino male mice.

Materials and methods:

The cherries stick were collected from cherries (fruit summer) which was obtained during months (may-July) and rinsed with water and air dried for evaporation of water, 100 gm of crushed cherry stick were weighted and added to 500 ml ethanol (70%) in soxhlet at boiling degree for six hour and left it to be cooled with continuous slow mixing and then filtrate the solution in the rotary evaporator at 60 degree until getting a thick solution. After that drying the solution in the incubator at 37 C through 2-3 days until it becomes a crushed dried and then taken it and stored it in the refrigerator at 4 C. The resulted deposit was dissolved in distilled water to prepare the dose at day of the measurement.

Laboratory animals and sample collection:

A total of 15 white albino male mice weighting 25-30 gm aged of 2-3 months were used in this study. The mice were classified into three groups; the first group (G1) was the control group of five animals which have not received the extract of cherry stick; the second group (G2) comprised five animals which have had received orally 1ml of 0.04 mg / kg /day of cherry stick extract, and the third group (G3) comprised five animals which have had received orally 1 ml of 0.08 mg / kg / day of cherry stick extract. The mice were killed in the morning by decapitation after two week of receiving of the extract. The samples of collected whole blood were left for 15 minutes at room temperature for clotting, then centrifugation and separation of the serum. The serum measurements at the same day of collection of total cholesterol, triglyceride, total protein, albumin and globulin were done in control and treated animal groups.

Assay methods:

Total cholesterol was determined in serum by enzymatic colorimetric method using kit cholesterol MR CE (linear chemicals S.L.). The method for the measurement of serum total cholesterol involved three enzymes, cholesterol esterase, oxidase and peroxidase.

In the presence of the former the mixture of phenol and 4 aminoantipyrine (a-AA) are condensed by hydrogen peroxide to form quinonimine dye proportional to the concentration of cholesterol in the sample (14). Triglyceride was measured by using kit CE triglyceride GPO-POD Enzymatic colorimetric method (spinreact S.A.U.). The intensity of the color formed is proportional to the triglyceride concentration in the sample (15). Total serum protein was measured by Biuret reaction using kit method CE colorimetric method for total protein (linear chemicals S.L.) (16). Serum albumin was measured by kit CE colorimetric method of albumin (linear chemicals S.L.) (17). Serum globulin concentration was estimated from subtraction of albumin from total protein. The ratio of albumin to globulin was also calculated.

Statistical Analysis

Statistical analyses selected were mean±SD, coefficient of Variation (CV) and unpaired student's test (18).

Results

Table 1 showed the mean±SD values of serum total cholesterol and triglyceride. Table 2 demonstrated the result of total protein, albumin, globulin and albumin: globulin ratio in groups of treated and untreated mice. The mean ±SD of serum total cholesterol, triglyceride significantly decrease in animal treated with extract of 0.04 mg/kg and 0.08 mg/kg when compared to control animal group (P< 0.001, P< 0.001) respectively. The mean ±SD of serum total protein and albumin were significantly decrease in animal treated with extract 0.04 mg/kg and 0.08 mg/kg when compared to control animal group (P< 0.001, P< 0.001) respectively. There was statistically a remarkable change in the mean ±SD value of the globulin (P<0.01, P< 0.01) at both concentration of the extract 0.04 mg/kg and 0.08 mg/kg respectively. The ratio of albumin: globulin in treated animals with 0.04 and 0.08 mg/kg/day shows no significant change in comparison with untreated group.

Table (1): The mean±SD values of Serum Total Cholesterol and Triglyceride concentration in groups of treated and untreated mice.

Parameter	G1	G2	G3
Cholesterol (mg/dl)	233.90 ± 4.31 CV % = 1.84	144.20 ± 3.48 CV % = 2.41 t* = 36.20	111.70 ± 1.27 CV % = 1.13 t* = 60.85
Triglyceride (mg/dl)	185.68 ± 3.24 CV % = 1.74	110.86 ± 1.87 CV % = 1.68 t* = 44.80	103.08 ± 3.23 CV % = 3.13 t* = 40.4

t* = (P < 0.001) very highly significant difference.

Table (2): Mean ± SD values of Serum Total Protein, albumin, globulin and albumin: globulin ratio in groups treated and untreated mice.

Parameter	G1	G2	G3
Total Protein (g/dl)	7.32 ± 0.41 CV % = 5.60	5.51 ± 0.50 CV % = 9.07 t* = 6.26	4.23 ± 0.68 CV % = 16.07 t* = 8.7
Albumin (g/dl)	4.45 ± 0.43 CV % = 9.66	3.22 ± 0.28 CV % = 8.7 t* = 5.36	2.45 ± 0.48 CV % = 19.6 t* = 6.95
Globulin (g/dl)	2.87 ± 0.40 CV % = 13.93	2.29 ± 0.38 CV % = 16.6 t** = 2.50	1.78 ± 0.47 CV % = 26.40 t** = 4.00
Albumin : Globulin ratio	1.55 ± 0.50 CV % = 35.48	1.41 ± 0.42 CV % = 29.70 t*** = 0.48	1.37 ± 0.39 CV % = 28.46 t*** = 0.63

t* = (P < 0.001) very highly significant decrease.

t** = (P < 0.01) highly significant decrease.

t*** = No significant difference.

Discussion:

Dietary products with a lipid lowering properties and antioxidant capacity may be important in the reduction of risk of coronary heart disease (19,20). Cherries are loaded with diseases fighting antioxidant. High concentration of antioxidant compound called anthocyanins which is found in cherries. The anthocyanins provide the distinctive red color that is though to hold the key to the health benefits locked inside (20). Cherry are rich in phenolic compound including anthocyanins, flavonoids, vitamin A, vitamin C, soluble solid and other compounds which have been shown to exert antioxidant, anti-inflammatory and anti-aging properties. Of 150 different flavonoids found in plants, anthocyanins appear to have the greatest antioxidant capacity. Reduction of serum lipid levels and enhancement of resistance to oxidation of LDL cholesterol would contribute to reduction of cardiovascular disease (3,5). Pervious studies suggested a beneficial effect of anthocynins on lipid peroxidation (21). Deeply colored cherries contain large amount of antioxidant phenolic, compounds nine times the amount of vitamin C and water soluble compounds (22). The result showed a significant reduction in the concentration of serum total cholesterol and triglyceride table 1. The observed decreased in the cholesterol concentrations were 1.622 and 2.09 times lower than normal at 0.04 and 0.08 mg/kg/day respectively after treatment. Triglyceride show a remarkable changes by 1.67 and 1.80 times lower than normal at 0.04 and 0.08 mg/kg/day respectively, this is due to presence of phenolic compounds. The anthocyanins in cherry is natural compound which helps stop cholesterol clogging up arteries and also hope for relieving the pain of arthritis (5,10). Studies have identified the active antioxidant polyphenolic compounds, including anthocyanine, chlorogenic acid and gallic acid (22). These

phytochemicals suppress destructive oxygen free radical. An over abundance of free radicals can damage all compounds of the cell including protein, fat and DNA contributing to the development of several pathological condition (23,24). Low serum cholesterol concentration are achieved with the doses used in this study 0.04 and 0.08 mg/kg/day could be due to presence of high concentration of anthocyanin in the cherry stick which are water soluble compound. The concentration of anthocyanes present in the digestive tract is expected to be high, which could interfere with the intestinal absorption of lipids. In addition, anthocyanes may exert antioxidant properties within the intestinal tract (25,26). However, reports that cherry phenolic show strong antioxidant activity in phospholipids liposome (10) indicate that these compounds are active in lipophilic as well as hydrophilic systems support for this includes finding that less polar anthocyanin, has stronger antioxidant activity than its glycosides (10) and that flavonoids alter membrane fluidity by partitioning into the lipophilic core of model membranes (27,28). Serum total protein and albumin were show very highly significant decrease in comparison with control group table 3,4. The observed decreased in concentrations were 1.32 and 1.73 times lower than normal at 0.04 and 0.08 mg/kg/day respectively for total protein and 1.38 and 1.81 times lower than normal at 0.04 and 0.08 mg/kg/day respectively for albumin. A decrease in globulin concentrations were 1.25 and 1.61 times lower than normal at 0.04 and 0.08 mg/kg/day respectively. The ratio of albumin to globulin shows no significant change in comparison with control group, table 2. Pervious studies have shown that the compound in cherry may offer protection against heart disease and metabolic syndrome due to enhancement in blood vessel health, by lowering total cholesterol levels and reducing triglycerides and increasing blood antioxidant capacity. Other studies suggest that cherries have anti-inflammatory benefits that may relieve the pain of arthritis and gout (19,20). A suggestion that animals which have been received the cherry stick extract had lower total cholesterol, less fat storage in the liver, lower oxidative stress and might increase production of molecule that help the body handle fat and sugar in comparison with mice that did not received the cherry stick extract. In conclusion, the cheery stick extract reducing the lipids by decreasing total cholesterol, triglyceride concentration and enhancing total antioxidant capacity and anti-inflammatory this is due to anthocyanins as antioxidant, therefore the cheery stick considers as nature source for antioxidant products. It need further studies which comprise a larger group of animal and long term experiment using such high doses have to be evaluated for efficacy and safety.

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