Original Article

The Protective Effect of Powdered Tart Cherry Supplements or Eating Local Iraqi Tart Cherry Fruit on Moderate to Border Level of Uric Acid and Lipid Profile in Human Serum

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ABSTRACT

We aimed to compare the protective effect of short-term supplementation of a powdered tart cherry supplement (Prunus cerasus the sour cherry) and eating local tart cherry fruit in the north of Iraq, in managing hyperlipidemia and relieving the pain of gout. Four groups were recruited: Two for moderate to border level of uric acid and two for moderate to high hyperlipidemia patients; a group once dealing with 300g local fresh tart cherry and the other group with 500mg of freeze dryer powder capsule of tart cherry fruits daily for six weeks. The participants were randomly assigned into a placebo or treatment group. 24-hr dietary records were required to be filled by the volunteers before taking blood, which allowed the assessment of any dietary changes. A fasting blood sampling occurred on the first day of the study (baseline). After a 6-week intervention period, the whole blood was collected for lipid profile analysis. Total cholesterol, low-density lipoprotein (LDL), and triglyceride were significantly lower in participants receiving local tart cherry fruits compared with those receiving freeze-dried tart cherry powder capsule and in the control group, a significant increase in high-density lipoprotein (HDL), along with a significant lowering of serum uric acid, especially for border level of serum uric acid patients without medication was found. All participants had a high degree of interest, mainly as the fruits existing were cheap in northern Iraq instead of its capsule that was expensive and not present in this region. We recommend local freeze dryer powder capsule be available in Iraq.

GRAPHICAL ABSTRACT

The Protective Effect of Eating Local Iraqi Tart Cherry Fruit on Moderate to Border Level of Uric Acid and Lipid Profile
Introduction

Gout is a type of arthritis. This complication can occur as a sudden attack of pain and burning, stiffness, and swelling in the joint (usually in the big toe). These attacks can occur over and over again unless gout is treated. Over time, these attacks can damage joints, tendons, and other tissues. Gout is more common in men. Being overweight, drinking too much alcohol, or overeating meat and fish that contain large amounts of chemicals called purines will increase the risk of gout. Also, some medications, such as diuretics, can cause gout. As mentioned, the leading cause of gout is the accumulation of uric acid in the body. Uric acid is a waste of metabolism and protein burning in the body. Sometimes due to the high production of this substance in the body or the kidneys’ inability to excrete it, this substance accumulates abnormally in body fluids, and then the crystals of this substance deposit in the joints of the body. The deposition of uric acid crystals in the body stimulates immune cells, leading to inflammation and joint pain. The formation of uric acid crystals in the joint causes the immune cells to consider them invasive and eat them. These cells then secrete special chemicals called prostaglandins and leukotrienes, which cause symptoms of inflammation in the joint (pain and swelling). One of the common symptoms of this disease is sudden pain and swelling of the joints, especially the big toe's metatarsophalangeal joint. The sudden onset of joint pain and swelling is called a gout attack. Repeated blows to the big toe, for example, following a long walk, cause uric acid crystals that have previously been deposited in joint tissues to suddenly fall into the joint, causing the body to respond to inflammation. Anything that can suddenly raise blood uric acid levels, such as dehydration, alcohol consumption, and acidification of the blood, can also cause gout attacks.

One of the benefits of cherries is their nutritionally dense food rich in anthocyanins and contain total phenolics, carotenoids, and melatonin [1], which possess anti-inflammatory and antioxidant properties [2,3]. Numerous mechanisms clarify the positive impact of anthocyanins on serum fat parameters. It has been indicated that anthocyanins meaningfully decrease cholesteryl ester transfer protein (CETP) activity, which upsurges high-density lipoprotein (HDL) cholesterol and decreases low-density lipoprotein (LDL) cholesterol [4-7]. It has also been indicated that one of the most commonly observed anthocyanins in cherries is cyanidin-3-glucosidemia, which can have an adverse impact on adiposity [8]. Although three significant anthocyanins have shown little anti-inflammatory activity, part of anthocyanins' antioxidant capacity is thought to be attributed to their structure, which consists of several hydroxyl groups that can donate electrons to neutralize a radical [9,10]. It has been reported that the antioxidant activity of anthocyanins is reduced by glycosylation of anthocyanins [11]. The direct antioxidant activity of anthocyanins has also been attributed to their ability to chelate metals such as Fe (II), which catalyzes lipid oxidation [12]. Anthocyanin-derived Fe (II) complexes are ineffective and unable to perform lipid peroxidation but are said to retain their radical inhibitory abilities [13]. The occurrence of substantial levels of anthocyanins in tart cherries could show significant antioxidant activity. Anthocyanins' high antioxidant role in metabolic reactions, owing to their capacity to scavenge oxygen free radicals and other reactive species, is one of their most well-known properties (ROX). This property makes anthocyanins a promising method for research into oxidative stress and related diseases. Tart cherry-enriched diets, for example, have been shown in animal experiments to minimize oxidative stress and inflammation. Anthocyanins can inhibit Cyclooxygenase (COX-enzyme) activity [14,15]. Furthermore, the COX-1 and two enzymes, Tumor necrosis factor (TNF-α), Interleukin 6 (IL-6), C-reactive protein (CRP), and monocyte chemoattractant protein-1 (MCP-1) are biomarkers that also stimulate the inflammatory response. The impacts of cherry consumption on markers of inflammation in humans has been
studied [16]. Still, it should be noted that they used Bing sweet cherries instead of tart cherries. They did not report the actual serum lipid concentrations in their paper but stated that lipid levels did not significantly decrease in response to cherry supplementation. Studies call for further research with longer duration and different processes of cherry intake [17,18]. Studies have indicated that these pro-inflammatory messenger molecules, chemokines, and cytokines are meaningfully decreased following anthocyanin consumption [16,19–21], thereby providing additional mechanisms anti-inflammatory effects associated with anthocyanins. Many scholars have recognized the antioxidant features of sour cherries in vivo and in vitro, but some studies have investigated the impacts of sour cherries on blood lipids and inflammation. Cherries are nutritious fruit containing various chemical compounds beneficial for patients with insomnia, muscle aches, and gout. Several studies have reported that cherry extract and/or cherry juice can be used as a supplement to reduce acid levels. Uric is used in the body of patients suffering from gout [22-25]. The relation between cherry consumption and the risk of recurrent gout attacks has been examined [2], and it has been reported that after swallowing cherries for two days, there was a significant reduction in the risk of gout attacks. Also, combining allopurinol with cherries could increase the risk. It has also been helpful for patients with chronic diseases, such as cancer, diabetes, and cardiovascular disease [26]. Another study showed a reduction in inflammation of sour cherry soup in women with osteoarthritis (OA) [27]. The combination of allopurinol with cherries can reduce the risk of a gout attack. There is a need for further research in this area and further study to discover the responsiveness of a large sample size of such participants to a tart cherry fruit. Hence, this study aimed at using fresh tart cherry and tart cherry powder capsules to investigate the effect of local tart cherries on serum lipid profile mainly (s. Cholesterol, s. triglycerides, s. HDL, s. LDL) and s. uric acid and comparing the protective effect of short-term supplementation of a powdered tart cherry supplement (Prunus cerasus the sour cherry) and eating local tart cherry fruit in the north of Iraq on these pathological conditions. Hence, the question of to what extent cherries affect lipids profile and moderate to border level of uric acid on the human body was addressed by this study.

Participants
Between March 2018 and April 2020, participants aged 45-70 with border levels of uric acid (2,3 group), high cholesterol and triglyceride most likely to be at risk of dyslipidemia (4,5 group) without using lipid or uric acid-lowering medication), were invited to participate in the study. Trials were conducted on healthy participant without gout and multi diseases. Most of the donors were members of the teaching staff at the University of the AL-KITAB Kirkuk, Iraq and the others were Kirkuk and Irbil participants. We collected information on age, gender, race, gout diagnosis, duration, number of gout flares, and lipid profile test. Diabetes Mellitus (DM), viral hepatitis, and non-smokers with no unresolved infections and another disease were excluded from the study [28,29].

Material and methods
The same methods of the powder Capsules of Montmorency tart cherry and local tart cherry fruits were used with four groups, each group consisting of 25 participants [30,31]: Two for moderate to border level of uric acid and two for moderate to high hyperlipidemia patients; once they were treated with local fresh tart cherry fruits for a group and again with frieze dryer powder capsule of tart cherry fruits for the second set and one for formal study, for all tests.

Estimation of serum Uric acid
Manual procedure (Uricase method) was as follows:
Table 1: Estimation of serum Uric acid

| Pipette into well-identified test tubes | Blank   | Standard | Assay |
|----------------------------------------|---------|----------|-------|
| Working reagent                        | one ml  | one ml   | one ml|
| Specimen (note1)                       |         |          |       |
| Standard                               |         |          | 25 µl |
| Demineralized water                    |         | 25 µl    |       |

Mix. Let stands for five minutes at 25 °C.
Record absorbance was recorded at 520 nm against reagent blank.
Color is stable for thirty minutes.

Calculation of uric acid as follows in Serum:

\[
\text{Uric acid conc. (mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{standard concentration}
\] (1)

Where,
Uric acid conc. = uric acid concentration
Abs = Absorbance

Lipid-profile tests:

Cholesterol estimation

Table 2: Cholesterol estimation

| Pipette into well-identified test tubes | Blank   | Standard | Assay |
|----------------------------------------|---------|----------|-------|
| Reagent                                | one ml  | one ml   | one ml|
| Demineralized water                    | 10 µl   |          |       |
| Standard                               |         | ten µl   |       |
| Specimen                               |         | ten µl   |       |

Mix. Let stands for five minutes at 37 °C or ten minutes at room temperature; absorbance was recorded at 500 nm against reagent blank.
Color is stable for one hour.

Calculation of cholesterol in serum as follows

\[
\text{cholesterol conc. (mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{standard concentration}
\] (2)

HDL-Cholesterol estimation

Table 3: HDL-Cholesterol estimation

| Pipette in centrifuge tube | Macro-method | Micro-method |
|----------------------------|--------------|--------------|
| Specimen (*)              | one ml       | 0.5 ml       |
| Precipitant                | 100 µl       | 50 µl        |

Mix vigorously, let stand for ten minutes at room temperature.
Centrifuge 15 minutes at 3500-4000 RPM (1500 g)
Then the following procedure was applied.

Table 4: Assay

| Pipette into well-identified test tubes | Blank   | Standard | Assay |
|----------------------------------------|---------|----------|-------|
| Reagent                                | one ml  | one ml   | one ml|
| Demineralized water                    | 25 µl   |          |       |
| Standard 100 mg/dl                     |         | 25 µl    |       |
| Supernatant (*)                        |         |          | 25 µl |

Mix. Let stands for five minutes at 37°C or ten minutes at room temperature, the concentration of HDL-Cholesterol was calculated according to the absorbance at 500 nm against reagent blank.
Calculation of HDL as follows:

$$\text{HDL conc. (mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{standard concentration} \times 1.1$$  \hspace{1cm} (3)

Where,

HDL conc. = High density lipoprotein concentration

Abs = Absorbance

Standard remaining undiluted, 1.1 factor takes into account dilution of the specimen during the precipitation step.

**Table 5: LDL-Cholesterol estimation**

| Set up the instrument to read micro-volumes  | Blank | Calibrator | Assay |
|--------------------------------------------|-------|------------|-------|
| Reagent R1                                  | 300µl | 300µl      | 300µl |
| Calibrator                                 | three µl |            |       |
| Specimen                                   | three µl |            |       |

Mix vigorously, stands for five minutes at 37 °C. And the absorbance recorded A1 at 546 nm against reagent blank.

Add:

| Add:            | Blank | Calibrator | Assay |
|-----------------|-------|------------|-------|
| Reagent R2      | 100 µl| 100 µl     | 100 µl|

Mix vigorously. Let stands for five minutes at 37°C.

Calculation of LDL as follows:

$$\text{LDL conc. (mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Calibrator)}} \times \text{Calibration concentration}$$  \hspace{1cm} (4)

Where,

LDL conc. = Low density lipoprotein concentration

ΔAbs. = Change in absorbance

**Table 6: Estimation of Triglyceride**

| Pipette into well-identified test tubes | Blank | Standard | Assay |
|----------------------------------------|-------|----------|-------|
| Reagent                                | one ml| one ml   | one ml|
| Demineralized water                    | ten µl|          |       |
| Standard 100                           | ten µl|          |       |
| Specimen                               |       | ten µl   |

Calculation of serum triglycerides (mg/dl):

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{standard concentration}$$  \hspace{1cm} (5)

Where,

Abs. = Absorbance

**Result and Dissection**

Table 1 shows the effects of fresh local tart cherry fruit and supplement on human serum Uric acid receiving 300mg local tart cherry fruit and the other 500mg freeze-dried tart cherry powder capsule daily. And both of them were significantly lowering serum Uric acid, but the participants receiving a diet supplemented with 300g daily local tart cherry fruits for six weeks were
significantly lowering moderate to border level of uric acid compared with those receiving freeze-dried tart cherry powder capsule. In the control group, both were significantly lowering serum Uric acid.

Table 2 shows the effects of serum lipid profile receiving 300mg local tart cherry fruit and 500mg freeze-dried tart cherry powder capsule daily. Both of them were significantly lowering serum cholesterol, LDL, triglyceride, and a significant increase in serum HDL, but the participants receiving a diet supplemented with 300g daily local tart cherry fruits for six weeks were significantly increasing HDL and lowering serum triglyceride, cholesterol, and especially LDL, compared with those receiving freeze-dried tart cherry powder capsule in the control group.

Consumption of tart cherry fruit has proven effective in lowering urate levels. Previous studies have supported the suppression of gout-related inflammation to the anti-inflammatory properties of cherry [18,26,32–34]. Anthocyanin from cherry fruit inhibited cyclooxygenase 1 and 2, which are the key enzymes involved in inflammation [25]. In Sour, red, raw (Prunus cerasus), the Mean cyanidin content (mg/100 g edible portion) was 32.57 while in sour, dry, unsweetened was 6.83 [35]. In the present study that the fruit has more effect than the fruit powder capsule. The cherry extract has been indicated to reduce cytokines' levels in affected joints.

A diet containing 300 g daily for six weeks of entirely local cherry fruits for lipid analysis in participants receiving local sour cherry fruits compared with receiving local sour cherry powder. There was significant lowering in the control group. One explanation includes the inhibition of CETP, a cholesterol ester transporter protein that facilitates the transport of cholesterol ester and triglycerides between lipoproteins, in reverse cholesterol transfer. Anthocyanins have been shown to significantly reduce CETP activity, thereby increasing HDL cholesterol and decreasing LDL cholesterol [8]. Besides, anthocyanin supplementation has been shown to induce cholesterol flow from macrophages and foam cells in mice, which may act as a potential mechanism in humans to further aid reverse cholesterol transfer [22]. Anthocyanins may also work to improve serum lipid homeostasis by stimulating LDL receptor upregulation (LDLr). LDLr regulates cholesterol homeostasis by removing LDL cholesterol from the bloodstream [23]. Higher levels of functional LDLr are generally associated with higher secretion of LDL cholesterol from the blood. Anthocyanin supplementation has been shown to increase LDLr genes' expression, leading to an increase in the number of LDLRs in cell surface membranes [23]. We recommend researching a wider range of populations for more comprehensive and generalizable findings.

**Conclusion**

Inflammation and dyslipidemia are closely related to the cause of many chronic diseases, which is the leading cause of death in our studies on evaluating the effect of anthocyanin-rich interventions on serum lipid and lipid profiles. Because sour cherries have complete anthocyanin and phenolic, these studies suggest that daily consumption of 300 mg of cherry tart fruit significantly improves the biomarkers of dyslipidemia and inflammation more than its powdered capsule, daily consumption of sour cherry fruit may be a preventive measure necessary against the development of hyperlipidemia, inflammation, and chronic diseases associated with such conditions as gout.

A fundamental limitation of our study was patient self-selection. The study findings may not be generalizable to all gout patients, only moderate to border level of serum uric acid patients. Most of the donors were members of the teaching staff at the University of the Al-Kitab University, Kirkuk, Iraq.

In summary, this study highlights that if gout patients use natural, local tart cherry fruit, they will have a high degree of interest in non-pharmacological therapies, especially the fruit that exists cheap in northern Iraq instead of its capsule that is expensive and not present in this
region, especially for border level of serum uric acid patients without medication. Lipid profile analysis was significantly lower in serum (total cholesterol, LDL, Triglyceride) and a significant increase in serum HDL was observed in participants receiving 300g tart cherry fruits daily for six weeks compared with those receiving freeze-dried whole local tart cherry powder so that it will be an essential preventative measure against the development of dyslipidemia.

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Authors' contributions
All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest
We have no conflicts of interest to disclose.

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