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Hepatoprotective Effect and Antioxidant Activity of Aqueous Cherry Extract on Rats

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Abstract

Background: Fruits and vegetables rich in antioxidant compounds which scavenges free radicals. In this research, the hepatoprotective effect of cherry fruit aqueous extracts was studied. **Methods:** Fruits of the cherry were collected; the seeds were removed and extracted by maceration method. For *in vitro* antioxidant activity, Diphenylpicryl Hydrazyl (DPPH), Trolex Equivalent Antioxidants Capacity (TEAC) and Ferric Reducing Antioxidant Power (FRAP) was carried out. Total phenol and flavonoid contents were also determined. For in *vivo study,* 28 wistar male rats were prepared and divided into negative control, plant control, carbon tetrachloride control (toxin) and treatment groups. At the end of 14 days, for evaluation of hepatoprotective effects of extracts, animals were exsanguinated by diethyl ether anesthesia. Blood samples were collected by heart puncture and the serum was used for assay of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB) and albumin. All results are expressed as mean ± Standard Deviation (SD). Statistical analysis was calculated by one way analysis of variance (ANOVA). **Results:** Total phenol and Flavonoid contents were reported 930 mg/kg GAE and 380 mg Rutine in one Kg of fresh fruit respectively. *In vitro* antioxidant activity of extract in FRAP 4.8, DPPH 9.3 Mm trolox and TEAC 2.8 /kg fresh fruit were reported. There was a significant increase in hepatic enzymes activities and bilirubin level in carbon tetrachloride group compare to negative control. The extraction of cherry in 500 mg dose was able to alleviate the induced damages compare to toxic group ($p<0.05$). **Conclusion:** Cherry extract shows hepato-protective potential in selected dose.

Keywords: Antioxidant Activity, Carbon Tetrachloride, Cherry Extraction, Hepato-Protective

1. Introduction

The oxidation process in the human body induces damages in cell membranes, cellular proteins, lipids, and DNA. During oxygen metabolism in cells free radicals was produce. They can attack to biological membrane and induce damage cells and tissues. Human body to some extent is able to neutralize free radicals and it needs to the antioxidants derived from plant and human foods. The excess accumulation of free radicals may lead to induce much disease such as hepatic, heart diseases and cancers[1]. Researches show that free radicals-induced oxidation reactions may lead to the proteins, lipids oxidation, break of DNA strands and some changes in gene expression. The changes may induce pathologic conditions in many diseases including cardiovascular diseases, cataract, cancer, hepatic bleeding and aging also[2,3].

The liver cells have various and crucial roles in homeostasis and health maintaining. In short, these roles include blood filtration, storage of biochemical compounds, carbohydrates, proteins, fats, hormones, external chemicals metabolism, bile production, vitamins, iron storage and biosynthesis of coagulation factors[4]. Liver plays various roles in human body, so any damage imposed on liver may lead to the different dysfunction of body and

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put ones health in danger. The oxidative damages caused by free radicals are one of risk factors of liver disease. Because of very limited production rate of antioxidants in animal cells, there is a need for the external sources of antioxidants. Phenol acids, polyphenols and flavonoids show free radical scavenging activity which can inhibit the oxidative and aging processes[5].

The cherry was selected to being studied because it is abundant in Kohgiloye and Boyerahmad province. Cherry (*Laurocerasus officinalis* Roem.) belongs to the *Rosaceae* family and has a deep red color. In many years ago in Turkey, both cherry and its seed has been used as a dietary product as well as a traditional medicine for remedy of gastric ulcer, digestive system dysfunctions, bronchitis, eczema and hemorrhoid[6]. The aqueous extract of cherry prevent from accumulation of lipid peroxidation in plasma[1]. In several studies anthocyanin in black berry was useful for inflammation and degenerative diseases via blocking cyclooxygenase –1, 2 enzymes. The potential of cherry extracts associate with its antioxidant activity[7,8]. The present study was managed for hepatoprotective effect of cherry extract on hepatotoxic rat liver which induced by carbon tetrachloride.

2. Materials and Methods

Fruits of the Cherry (*Laurocerasus officinalis* Roem.) were collected in the Yasuj, Iran, in June 2013. Samples were identified and a voucher specimen (HMRC-J 11/09/2012) was deposited in the Herbal Medicinal Research Center, Yasuj University of Medical Sciences, Yasuj. Fruits were washed, and the seeds were removed.

2.1 Extraction

The fruit without seeds and seeds were extracted with distilled water by maceration method in room temperature. The extracts were collected and dried using a rotary evaporator (Hyedolph model 4000; Germany) and were kept in a refrigerator for further studies.

2.2 In-Vitro Studies

The antioxidant activity was assessed by 3 tests such as Diphenyl Picrylhydrazil Radical (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Antioxidant Power (FRAP) tests. To determine the antioxidant compounds, total phenol and flavonoid were determined.

2.3 Determination of Total Phenolic Compounds

Total phenol content was measured by Folin-ciocalten reagent. Total phenol was expressed as Gallic Acid Equivalent (GAE)/g extract[9]. The total flavonoid content was determined with Aluminum Chloride ($AlCl_3$) method. The total flavonoid value was determined in terms of Rutin equivalents/g[10].

2.4 Antioxidant Activity

The antioxidant activity of Dipheny-Picrylhydrazyl (DPPH) extract was assessed with little modifications. Percent of inhibition was calculated as follow: % Inhibition = $[(A0 - A1)/A0] \times 100$[11]. A0 is the absorbance of control and A1 is the absorbance of the plant extracts.

2.4.1 Trolox Equivalent Antioxidant Activity (TEAC)

The antioxidant activity was measured using TEAC based on Re method with some modifications. Percent of inhibition same DPPH method was calculated[12].

2.4.2 Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) test was carried out according to Benzie and Strain with little modifications[13].

2.5 In-Vivo Study

In the present experimental study 28 adult male Wistar rats (180 to 250 g) were selected and randomly divided into 4 groups (1 to 4). Animals were maintained in a controlled environment of 24 ± 2°C, 55 to 60% humidity, and 12 h light/dark cycle, and were fed a standard laboratory diet (Pars, Iran Ltd., Tehran, Iran).

Groups (1), served as negative control was given olive oil (1 ml/kg) intraperitoneally (i.p.) and Groups (2) or extract control received aqueous cherry extract (250 mg/kg) in single dose, daily p.o. for 14 days. Group (3), toxic group, received CCl_4 (1 ml/kg) mixed with an equal volume of olive oil as a single i.p. dose every other day for 14 days. Groups (4), the protective group received aqueous cherry extract (500 mg/kg,) p.o. for 14 days, 1 h after the injection of CCl_4 every day. At the end of the 14^{th} days, animals were exsanguinated by diethyl ether anesthesia.

2.6 Biochemical Analysis

Blood samples were collected by heart puncture and the serum was centrifuged at 2000 g for 10 min and used for Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB) and albumin. Analyses were done via local company kits Pars Azemon.

2.7 Statistical Analysis

All results are expressed as mean ± Standard Deviation (SD). Statistical analysis was calculated by one way analysis of variance ANOVA using Statistical Package for Social Sciences (SPSS) software version 13. Statistical significance was set at $P < 0.05$.

3. Results

3.1 In vitro Findings

Total Phenol Content (TPC) of cherry fruit extract was reported 930 mg/kg GAE of fresh fruit weight however; TPC in cherry seed extract was 230 mg/kg which is equal to 24.7 percent of the cherry fruit. Flavonoid content of cherry fruit and its seed extract was 380 and 65 mg Rutine in one Kg of fresh fruit respectively (Figure 1).

Ferric antioxidant power was measured based on the reducing property. Results were expressed as Mm trolox per kg of fresh sample. In this study the FRAP value for cherry fruit and seed extract were 4.8 and 1.1 mmol /kg fresh fruit respectively. DPPH activity in fruit and seed extract were 9.3 and 3.1 Mm trolox respectively. The antioxidant activity in terms of TEAC test was estimated 2.8 and 0.9 in cherry fruit and seed extract respectively (Figure 2).

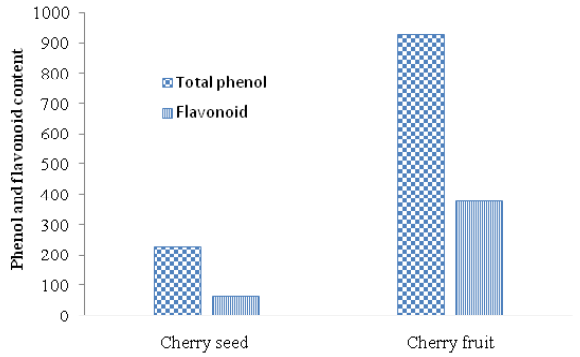

Figure 1. Total phenol and flavonoid content of aqueous extract of cherry seed and fruit.

Total phenol content mg/kg GAE of fresh fruit in one Kg of fresh fruit
Flavonoid content mg Rutine in one Kg of fresh fruit

3.2 *In-Vivo* Findings

No significant differences were observed in biochemical tests between control and extract groups. The effects of cherry on serum marker enzymes are shown in Table 1. The results of this study show that the administration of CCl_4 produced hepatic damage and liver dysfunction. Liver dysfunction was confirmed by significant increases ($P < 0.001$) in the marker enzymes ALT, AST, ALP, TB and albumin (ALB) ($P < 0.05$) level when compared with the control groups (Tables 1, 2).

The level of bilirubin was significantly ($P < 0.05$) increased however albumin was decreased in toxin group. The serum enzyme activity of AST, ALT and AlP

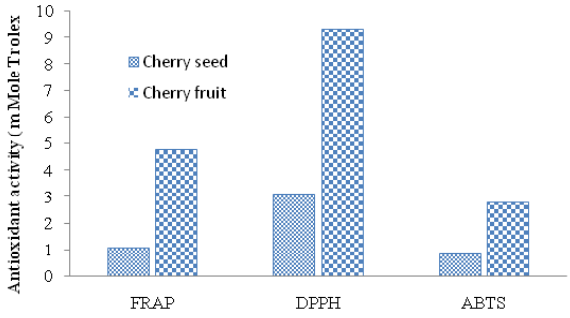

Figure 2. The average antioxidant activities in terms DPPH, ABTS and FRAP in aqueous seed extract and fruit of cherry.

Diphenyl Picryl Hydrazyl (DPPH), Trolex Equivalent Antioxidants Activity (TEAC), Ferric Reducing Antioxidant Power (FRAP).

Table 1. Effects of aqueous cherry extract on enzyme markers in CCl_4 induced hepatoxicity in rats

Group	Dose	ALP	ALT	AST
Negative Control	1ml/kg	150 ± 19.8	65 ± 9.2	111.3 ±5
Toxin (CCL4)	1ml/kg	530 ± 11**	232 ± 15.6**	270 ± 15.8**
Extract control (fruit)	250 mg/kg	141 ± 12.1	59 ±7.2	105 ± 12.5
Treatment + CCL_4	500 mg/kg	230 ± 17*	119 ± 12.8*	163±15.3*

Values are expressed as mean ± SD of seven animals per groups *statistically significant difference compare to toxin group ($P < 0.01$); **statistically significant difference compare to negative group ($P < 0.01$). Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Carbon Tetrachloride (CCL_4).

Table 2. Effects of aqueous cherry extract on albumin and total bilirubin in CCl_4 induced hepatoxicity in rats

Groups	Dose	ALB	TB
Negative Control	1ml/kg	3.2 ± 0.1	0.46 ± 0.08
Toxin (CCL_4)	1ml/kg	1.8 ± 0.12a*	0.83 ± 0.16b*
Extract control	250 mg/kg	2.9 ± 0.18	0.48 ± 0.08
Treatment + CCL_4	500 mg/kg + 1ml/kg	2.7 ± 0.2c*	0.42 ± 0.09d*

Values are expressed as mean ± SD of seven animals per groups. *statistically significant difference compare to toxin group ($P < 0.05$; a, b: compare to negative control; c, d: compare to toxin group.Total Bilirubin (TB), and Albumin (ALB), Carbon Tetrachloride (CCL_4).

in toxin group increased compared to control groups ($P < 0.001$). Cherry extracts at doses of 500 mg/kg remarkably prevented CCl_4-induced hepatotoxicity (Table 1).

4. Discussion

Aqueous extracts of cherry has antioxidant potentials revealed by different *in vit*ro antioxidant assays. The DPPH and TEAC tests are routinely used to estimate of antioxidant or scavenging potentials.

The FRAP test is a fast and routine procedure that measure antioxidant activity according to ferrous ion production[14]. The antioxidant potential of plant extracts is related with their phenolic content. Phenolic and flavonoid compounds, in plants, have potent antioxidant activity, due to the functional group[15]. Aqueous cherry extracts was selected for *in vivo* hepato-protective activity in rats.

The hepatotoxic effects of CCl_4 are mostly caused by peroxidation of lipids and the presence of the free radicals[16]. In the recent work, the hepatotoxicity was demonstrated by significant ($P < 0.001$) increases in the activity of AST, ALT, ALP enzyme markers and TB concentration in CCl_4-treated animals compared to control. Hepatocellular damages were estimated by AST and ALT activity in plasma.

The ALT activity is normally predominant in the cytoplasm of hepatocytes, and its concentration elevated in hepatocellular inflammation and necrosis[17]. In present study the reduced of serum transaminase activities in the treatment group designates maintenance of the hepatocyte protection against injury which induced by CCl_4[18].

The insignificant result of the cherry extract on all biochemical parameters indicates that cherry extract itself does not impose any toxic effects on liver functions.

The highest activity of ALP s normally present in liver, bone, and kidney. Determination of ALP activity in plasma is a sign of hepatocyte function[19]. In toxic group ALP was significantly increased due to damage to liver tissue. Furthermore, bilirubin is an end product of red blood cell in circulation that increases in hemolytic condition, hepatic disease and necrosis[20]. The reduction in rat serum bilirubin after treatment with cherry extract in the CCl_4-induced liver damages model designates the efficacy of the extract in normalizing the functional status of the liver.

A significant ($P < 0.001$) normalization of enzyme marker (ALT, AST, and ALP) was detected follow use of cherry extract. Plant derived- drugs which used for liver protection comprise different chemical substances including phenols, flavonoids, tannins, and alkaloids[21]. In this study, the cherry extract may due to antioxidant activity shown hepatoprotective effects by improved of the biochemical markers.

5. Conclusion

The aqueous cherry extract, due to antioxidant activity, exhibited important hepato-protective activity and may be safely used to treat liver disease.

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