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Original paper

Effects of Black Raspberry Extract on the Oxidant-Antioxidant System Balance in an Animal Model of Diabetes

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Abstract

Background and aim: The aim of this study was to Evaluation of the effects of hydroalcoholic extract of raspberry fruit (*Rubus fruticosus* L.) on the balance of the oxidant-antioxidant system and serum levels of total oxidant status (TOS) in male diabetic rats induced with streptozotocin.

Materials and methods: In this laboratory experimental study Diabetic rats were induced by diabetes induction by IP injection of streptozotocin. Raspberry fruit was extracted with the usual method. Rats were randomly divided into five control, diabetic and diabetic + doses 50, 100, 200 mg / kg. After intraperitoneal injection of raspberry fruit extract for 28 days, the Serum levels of total oxidant status was determined and the data were statically analyzed using ANOVA.

Results: Serum levels of TOS was significantly increased in the diabetic group ($P<0.05$) and significantly decreased after treatment with the extract ($P<0.05$).

Conclusion: Raspberry fruit extract with increased antioxidant, and inhibiting the production of Destructive oxidants in the serum level of diabetic rat, increase antioxidant defenses and improve nitric oxide performance.

Keywords: *Raspberry extract, Antioxidant, Oxidant, TOS, Diabetes*

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Introduction

Diabetes is a complex metabolic disease characterized by elevated blood glucose levels and disruptions in the metabolism of carbohydrates, lipids, and proteins. It is associated with various complications such as nephropathy, retinopathy, neuropathy, cardiovascular diseases, and male infertility. Two major factors contributing to the development of diabetes and its complications are oxidative stress and lipid disorders [1].

Oxidative stress arises from the presence of free radicals in different cells and tissues of the body. Free radicals can be classified into two categories: reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS includes important radicals such as superoxide anion, hydrogen peroxide, and hydroxyl, while RNS includes significant radicals like nitric oxide. Superoxide anion, derived from oxygen reduction in the electron transport chain or metabolic reactions, is one of the most critical radicals in cellular processes [2].

The chronic or excessive accumulation of free radicals exerts detrimental effects on cellular function and leads to irreversible structural changes in biological macromolecules [3]. These damages include DNA strand breaks, lipid peroxidation of cell membranes, impairment of carrier proteins in membranes, and disruption of intracellular enzymes [4]. Nucleic acids are particularly susceptible to free radical-induced damage, which can result in dangerous and irreversible alterations to their structure. Recent studies have indicated that oxidative damage, by inducing permanent changes in genetic material, is an initial step in mutation, cancer development, and aging [5].

Medicinal plants have been widely used worldwide for their antioxidant properties in the prevention and treatment of diabetes and its complications [6]. One such plant is raspberry, which possesses beneficial effects such as antibacterial, anti-inflammatory, antioxidant, anti-diarrheal, anti-tumor, wound-healing properties, and natural sugar content [7]. Numerous studies have demonstrated that raspberry, due to its bioactive compounds, possesses analgesic and neurosedative properties and is effective in improving memory and cognitive disorders [8]. Raspberries are rich in antioxidants and anti-inflammatory compounds, including neoxanthin, violaxanthin, β-carotene, lutein, anthocyanins, vitamin C, flavonoids, phenolic compounds, and minerals such as calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, manganese, and selenium [7].

Considering the limited research available on the potential effects of raspberry fruit in reducing diabetes complications within the antioxidant system, this study aims to investigate the impact of black raspberry (*Rubus fruticosus* L.) extract on the balance of the oxidant-antioxidant system in male diabetic rats. By assessing the antioxidant properties of raspberry extract, we seek to gain insights into its potential therapeutic benefits in managing diabetes-related complications.

Material and Methods

In this experimental study, a total of 50 male Wistar rats weighing between 220 and 250 grams were obtained from the Pasteur Institute of Iran. The rats were housed in an animal room maintained at a temperature of 22±2 degrees Celsius. A 12-hour light and 12-hour dark cycle was established, starting at 8 am, and the rats had ad libitum access to fresh water and standard rat food obtained from Pars Animal Feed Factory. At the beginning of the study, the rats were weighed, and they were then housed in cages based on their weights to ensure an equal weight distribution among the cages.

To prepare the raspberry extract, fresh raspberry fruits were sourced from orchards in northern Iran and authenticated by the Agricultural Research Center. The fruits were dried in the shade

under suitable conditions and subsequently ground into powder using a mixer. A total of 500 grams of dry fruit powder was mixed with a solution consisting of 80% ethyl alcohol and 20% distilled water. The mixture was then placed in a bain-marie at a temperature range of 50-55 degrees Celsius for 72 hours. Afterward, the supernatant was filtered using filter paper, and the filtered solution underwent evaporation under vacuum using a rotary device. The resulting extract was concentrated under a hood for 24 hours, and finally, based on the desired doses, the extract was dissolved in double distilled water and made ready for injection [9], [10].

Streptozotocin (STZ) obtained from Sigma-Aldrich, USA, was used as the diabetes-inducing drug. It was dissolved in citrate buffer at a dose of 55 mg per kilogram of body weight and administered via intraperitoneal injection to induce diabetes [11]. Blood glucose levels were measured 72 hours after STZ injection by collecting blood from the tail vein and using a glucometer. Blood sugar levels above 250 mg/dL were considered indicative of the development of diabetes [12], [13].

The animals were divided into five groups, each comprising 10 rats: a control group that did not receive any medication [14], a diabetic group that received STZ at a dose of 55 mg/kg, and three diabetic groups that received raspberry fruit extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively [15].

The extracts were administered via intraperitoneal injection once daily at 12-1 pm for a duration of 28 days. After this period, blood samples were taken to measure the desired factor. Open-heart blood sampling was performed directly from the left ventricle. To anesthetize the rats, each animal was placed inside a container containing cotton soaked in ether, ensuring mild anesthesia. Thirty minutes after blood collection, the samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was then used to measure the desired factor. The measurement of the serum level of Total Oxidant Status (TOS) was performed using a concentration measurement kit from ZellBio GmbH, Germany. All experimental procedures were conducted in accordance with the guidelines set by the International Committee on the Ethics of Working with Laboratory Animals.

For statistical analysis, the collected data was assessed for normal distribution and analyzed using SPSS25 software. The Kolmogorov-Smirnov statistical test and ANOVA were employed, followed by Tukey's post hoc test to determine differences between the groups.

Results

Based on the findings presented in Table 1 and Figure 1, it was observed that the serum level of Total Oxidant Status (TOS) exhibited a significant increase when compared to the control group ($P<0.001$). However, in the diabetic groups receiving doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg of raspberry fruit extract, there was a significant decrease in the serum level of TOS ($P<0.001$). Conversely, compared to the control group, the diabetic groups receiving doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg of raspberry fruit extract displayed a significant increase in TOS serum level ($P<0.001$, $P<0.05$).

Table 1. Comparison of the mean serum level of TOS in the experimental groups.

Groups	Control	DM	DM+Ext.(50mg/kg)	DM+Ext.(100mg/kg)	DM+Ext.(200mg/kg)
Mean±SEM	0.73±0.043	13.60±0.505	8.63±0.535	5.82±0.445	2.72±0.258
P_1	-	***	***	***	*
P_2	-	-	###	###	###

P_1 indicates significance difference compared to the control group

P_2 indicates significance difference compared to the DM (diabetes mellitus) group

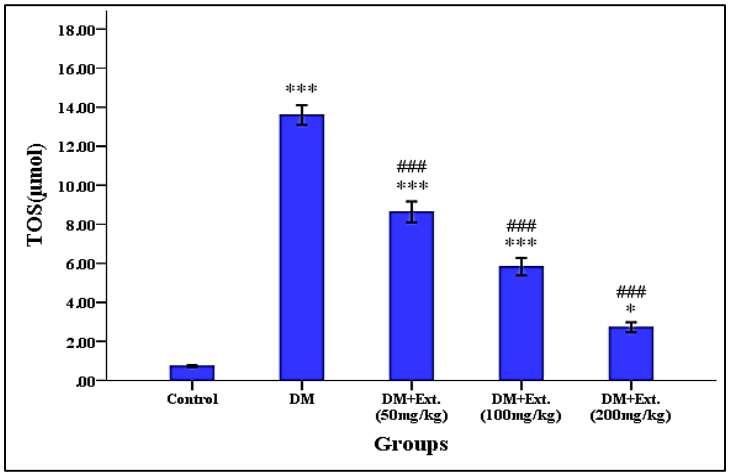

Figure 1. A comparison was made among the mean serum levels of Total Oxidant Status (TOS) in the control group, diabetic group, and diabetic groups treated with different doses of raspberry extract. Statistical significance is denoted by asterisks (*) when compared to the control group and hashtags (#) when compared to the diabetic group. The level of significance is indicated as follows: "***" denotes P<0.001, "*" denotes P<0.05, and "###" denotes P<0.001.

Discussion

In this study, we investigated the effects of hydroalcoholic extract of black raspberry fruit on the balance of the total oxidant-antioxidant system in male diabetic rats. Our findings, in conjunction with our previous research in this field [16], demonstrated that streptozotocin (STZ) injection during the current study reduced the serum level of total antioxidant capacity (TAC) and increased the serum level of total oxidant status (TOS). These results are consistent with previous studies indicating that individuals with type 1 diabetes have lower total antioxidant status compared to non-diabetic individuals [17]. Other studies have also reported a significant increase in TOS levels in diabetic patients compared to healthy subjects, with a greater increase observed with the progression of diabetes complications [18]. Thus, the disruption of the oxidative balance in favor of oxidative stress appears to play a crucial role in the pathogenesis of diabetes [19].

Considering the results from previous research and our current study on changes in antioxidant activity in diabetic rats, it is likely that increased production of free radicals and reduced levels of non-enzymatic antioxidants such as vitamin C, vitamin E, and carotenoids contribute to the decrease in total serum antioxidant capacity in diabetic patients [20]. The elevated levels of free radicals in diabetic patients may result from increased production of reactive oxygen species through processes such as glycosylation, peroxidation, autoxidation of glucose, and the conversion of glucose into acidic glucose, leading to a reduction in the body's antioxidants [21]. Consequently, with heightened oxidative stress in diabetic rats, the balance between TAC and TOS shifts towards decreased TAC and increased serum TOS.

Another significant finding from this study is that the administration of different doses of raspberry fruit extract to diabetic rats resulted in a significant decrease in the serum level of TOS compared to untreated diabetic rats. This is in line with a study showing that treatment with an aqueous extract of artichoke led to a significant increase in antioxidant capacity (TAC) compared to the untreated diabetic group [22]. Moreover, previous research has demonstrated that flavonoid compounds, known for their antioxidant properties, can scavenge free radicals such as xanthine oxidase and superoxide, thereby reducing oxidative stress in diabetic animals [23], [24].

Based on the results from previous studies and our current research, it can be postulated that the

phenolic and antioxidant compounds present in raspberry fruit [7], with their lipid-lowering effects, help reduce fat accumulation, stimulate cholesterol secretion through bile, and enhance cholesterol excretion through feces. The antioxidant compounds in raspberries, coupled with their ability to inhibit lipid peroxidation, have the potential to mitigate the adverse effects of diabetes and shift the balance towards increased total antioxidant capacity in diabetic rats. Furthermore, antioxidant compounds have been shown to improve hyperglycemia, hypertriglyceridemia, and reduce free fatty acid levels [25], which can contribute to a decrease in the serum level of TOS in diabetic rats.

Conclusion

The presence of antioxidant compounds in raspberry fruit extract helps shift the balance of the total oxidant-antioxidant system towards increased antioxidant production, thus reducing the complications caused by free radicals in diabetes. Therefore, it is anticipated that the use of this fruit extract may contribute to the management of diabetes complications.

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Conflict of interests

The authors declare that there are no competing interests.

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