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EFFECTS OF Highbush CRANBERRY (*VIBURNUM OPULUS*) ON HYPERGLYCEMIA AND OXIDATIVE DAMAGE IN THE KIDNEY OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

In this study, it was aimed to investigate the effects of highbush cranberry (*Viburnum opulus*, GILA) fruit extract on blood glucose levels and kidney tissue homogenates of diabetes-modeled rats by injection of Streptozotocin (STZ). Blood glucose levels of rats were measured in tail blood using a glucose meter. Glucose determination was done 48 hours after STZ injection. For this, 32 Wistar albino rats were employed. The rats were divided into 4 groups; Control, STZ, STZ+GILA, and GILA with containing 8 animals each. After 12 weeks, they were sacrificed. During the study periods, blood glucose levels were measured periodically and kidney tissue parameters were measured in their homogenates after sacrificing the rats. The measured parameters were Glutathion (GSH), Malondialdehyde (MDA), and Total Antioxidant Status (TAS). The results obtained from the study were analyzed by using SPSS for Windows software, and $p < 0.05$ was assigned as statistically significant value. The findings of the study showed that GILA consumption could help diabetic people to prevent possible nephropathy due to its potent antioxidant features.

Keywords: *viburnum opulus*, diabetes, kidney, oxidative damage, rat

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1. Introduction

Diabetes mellitus (DM) is one of the common chronic metabolic diseases and develops a lack of or nonfunctional insulin secretion from β cells of the pancreas. Its characterization can be exemplified as a disorder of both glucose and lipid metabolism. The occurrence of Hyperglycemia is the most special indication (Asmat et al., 2016).

The complications of DM could be expressed as macro and microvascular problems. Macro-vascular problems occur due to atherosclerosis and can be exemplified as myocardial infarcts, peripheral vascular disease, and cerebrovascular problems. General microvascular problems are retinopathy, nephropathy, and neuropathy. Microvascular complications cause irreversible alteration in the tissue and organs when occurred. Nephropathy is an example of that develop kidney failure (Atasoy et al., 2015, Chawla et al., 2016, Domingueti et al., 2016).

It is well-known that the reactive oxygen species (ROS) formation increases under diabetes conditions. The ROS products attack biomolecules (lipids, carbohydrates, proteins, and DNA) and damage them. This event is called cellular oxidative damage (Moussa et al., 2008, Çoban et al., 2015, Newsholme et al., 2016, Volpe et al., 2018). A literature survey indicates that STZ is the most used chemical agent to form a diabetes model in experimental animals. The target of STZ is the DNA molecules of the pancreatic β cells. STZ causes to the death of beta cells by alkylating their DNA molecules (Bedoya et al., 1996, Dandona et al., 1996)

Dietary recommendations are essential in the treatment of diabetes. However, the best dietary treatment for diabetic patients is still not entirely clear. Dietary research to prevent disease may include evaluating foods containing specific nutrients or non-nutrient chemicals, or a combination of both (Emekli-Alturfan et al., 2008). The purpose of the diet is to normalize changes in the metabolism of patients with diabetes, such as hyperglycemia and hyperlipidemia. Another perspective to diet therapy is to minimize long-term complications of diabetes mellitus, such as neuropathy, retinopathy, nephropathy and atherosclerotic vascular diseases (Iso et al., 2006, Emekli-Alturfan et al., 2007, Emekli-Alturfan et al., 2008). Antioxidants protect the cells via stopping or delaying the oxidative effect of the ROS. Some studies reported that flavonoids have an anti-diabetic effect (Emekli-Alturfan et al., 2008, Ghasemzadeh et al., 2010). It was earlier reported that Highbush cranberry (*Viburnum opulus*) (GILA) has an anticancer, antimicrobial and antioxidant effect due to its phenolic contents (Yunusova et al., 2004, Kraujalyte et al., 2013, Boyacı et al., 2016, Yilmaz et al., 2019).

In this study, it was aimed to study the effect of GILA in diabetic animal models formed by administrating STZ. For this, the study was designed to evaluate glutathione (GSH), Malonyldialdehyde (MDA) and total antioxidant capacity (TAS) levels in kidney tissue homogenate of the animals tested.

2. Materials and Methods

The ethics committee approval has been obtained from

T. C. Üsküdar University Animal Experiments Local Ethics Committee (Ü.Ü HAYDEK) report number of 2018-18 (02 Oct 2018).

2.1. Chemicals

STZ and other chemicals were obtained from Sigma-Aldrich, USA.

2.2. *Viburnum opulus* Extract

Viburnum opulus extract was obtained from IMMUNAT® (Muğla, Turkey, PSN: 011018-125-008). Ingredients of *Viburnum opulus*: highbush cranberry liquid extract, thickening agent-glycerol (plant product), preservative agent-potassium sorbate, antioxidant-ascorbic acid.

2.3. Animals

Wistar albino rats aged 12 weeks and weighing 200-240 g were used in this study. The rats were housed in a temperature and light controlled rooms (12 h dark-light cycles, 22 ° C \pm 2, and humidity 60 % \pm 5). All animals were free to access water and pellet food and experiments were performed according to the national laws and guidelines. The protocol used in this study was approved by the Committee on the Ethics of Animal Experiments of Üsküdar University (Istanbul, Turkey) (Ethic Number 2018-18).

2.4. Induction of Experimental Diabetes

Rats were given a single dose of 60 mg/kg intraperitoneally (i.p.) dissolved STZ injection in freshly prepared citrate buffer (0.05 M, pH: 4.5). Only citrate buffer was applied to the Control group. Diabetes formation was determined 2 days after STZ injection by measuring serum glucose concentrations and rats with a glucose level higher than 400 mg/dL were included in the diabetic group. Blood glucose levels of rats were measured in tail blood using a glucose meter. Glucose determination was done 48 hours after STZ injection.

2.5 Experimental Design

In this study, 32 rats were divided into four groups, with eight in each group as:

Control: Rats were subjected to once daily with citrate buffer

STZ: Rats were subjected to once-daily STZ (60 mg/kg) (i.p.)

GILA: Rats were subjected to once-daily GILA 1 ml/day (300 mg/kg) by oral gavage once a day

STZ + GILA: Rats were subjected to once-daily STZ (60 mg/kg) by i.p. and GILA (300 mg/kg) by oral gavage once a day

2.6. Blood and kidney tissue collection

At the end of the 20 days, the rats were killed under anesthesia by i.p. of ketamine HCl (80 mg/kg) and xylazine (10 mg/kg) and were then sacrificed by the withdrawal of blood via a cardiac puncture.

Fasting blood glucose levels were measured by using a glucose meter (Clever Chek-TD-4231).

Kidney tissue samples were gently washed saline solu-

tion (0.9 %) and homogenized in chilled phosphate buffer (pH:7.4). The homogenates were then centrifuged at $10.000 \times g$ for 5 min at 4°C. The supernatants were used for determining the GSH, MDA, and TAS levels.

2.7. Determination of GSH Levels

The level of GSH in homogenized kidney tissue samples was measured by using 5,5'-dithiobis (2-nitrobenzoic acid) on a spectrophotometer at 412 nm wavelength by following the methods of Beutler et al (1963).

2.8. Determination of MDA Levels

MDA levels in kidney homogenates were estimated based on the analysis of lipid peroxidation (LPO) levels. LPO was estimated by measuring the level of MDA by the thiobarbituric acid test as explained by Coban et al (2015).

2.9. Determination of TAS Levels

The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog. TAS in the kidney tissue was measured using the TAS Assay Kit (Rel Assay Diagnostics) by following the method of Erel (2003).

2.10. Statistical Analysis

Experimental results are presented as mean \pm SD. Data were analyzed using the SPSS software package (20th version, IBM, New York, USA). One-way ANOVAs followed by post-hoc Bonferroni tests were used for statistical analysis and $P < 0.05$ values were considered as significantly different.

3. Results

In this study, we aimed to evaluate the effect of high-bush cranberry (*Viburnum opulus*, GILA) fruit extract on blood glucose levels and kidney tissue of the rats that they became diabetics after administrating Streptozotocin (STZ).

As seen in Fig 1, the glucose levels were significantly higher in the STZ group when compared to Control ($P < 0.001$). Similarly, STZ+GILA group's glucose levels were found to be significantly high when compared to GILA group's data ($P < 0.001$). However, there was a non-significant decrease in serum glucose levels in the STZ + GILA group compared to the STZ group statistically (Fig 1).

When we look at the kidney tissue parameters: The GSH levels increased significantly in GILA when compared to control ($P < 0.001$). The same parameter in both STZ+GILA and GILA also increased significantly in comparison to STZ as $P = 0.030$ ve $P < 0.001$, respectively (Fig. 2-a).

While the MDA levels increased significantly in the STZ group compared to the Control group ($P = 0.015$), the same parameter decreased significantly ($P < 0.001$) in both STZ+GILA and GILA when compared to STZ (Fig 2-b).

The GILA group's TAS levels significantly increased in comparison to Control ($P = 0.006$). When we compared the STZ group with both STZ+GILA and GILA groups, TAS level increased remarkably in both groups with $P = 0.040$

and $P < 0.001$, respectively (Fig 2-c).

4. Discussion

Both oxidative stress and ROS formation are the spoilage of the balance between oxidants and the antioxidant system of the living organisms. This was shown by some studies in diabetes modeled rats by using STZ and the characteristics were a decrease of enzymic/non-enzymic antioxidants and an increase of lipid peroxidation (Vincent et al., 2004, Emekli-Alturfan et al., 2008, Kashihara et al., 2010). In this study, we observed that the fasting glucose levels of the STZ group were significantly high compared to the Control group ($p < 0.001$). Similarly, this finding, the same parameter was also significantly higher in STZ+GILA in comparison to GILA group.

Halliwel (2012) reported that polyphenol, carotenoid, and tocopherol rich diet could not be reducing the systemic damage due to the pro-oxidant activity of ascorbic acid. Because transition metals in the catalyzing activities such as iron and copper are already present in gastric and intestinal contents. Therefore, the statistically low level of serum glucose in STZ+GILA comparing to STZ could be because of the presence of ascorbic acid and its pro-oxidant activity in the *Viburnum opulus* extract.

In a study by Zaklos-Szyda et al. *Viburnum opulus* has been shown to be a phenolic compound consisting of important antioxidants such as chlorogenic acid, procyanidins, cyanidin glycosides, quercetin. In the study, *V. opulus* in CD36 knockout mice has been shown to reduce free fatty acid and glucose uptake by Caco-2 cells. Hence, it has been stated that *V. opulus* phenolics may have effects on the regulation and delay mechanisms of the absorption rate of glucose and fatty acids by intestinal cells (Zaklos-Szyda et al., 2019).

Either exogenic or endogenic ROS production causes some important defects in a number of body systems. The most effective strategies to overcome the ROS productions are either direct (scavenging the free radicals) or indirect (increasing the antioxidant enzymes) or using the natural or synthetic antioxidant compounds (Gonzalez-Burgos & Gomez-Serranillos 2017) In this manner, GILA flowers and/or juices are widely used in traditional medicine due to its rich phenolics and vitamins. The GC-MS analysis showed roughly the chemical contents of GILA as having potent antioxidants such as monoterpenes: α -pinene 27.2%, β -pinene 27.3%, terpineol 11.1%, dl-limonene 5.5%; and sesquiterpenes: germacrene D 10.1% (Rop et al., 2010, Kraujalytė et al., 2013, Sariözkan et al., 2017).

Recently, some natural antioxidants are used as well as the chemotherapeutics agents in cancer treatment. There are a number of studied shreds of evidence showing the anticancer properties of Phenolics as they have both antioxidant and anti-inflammatory effects. Ceylan et al. (2018) studied the effect of GILA juice on colon cancer. They reported that GILA juice increased both catalase and superoxide dismutase activity in both in vivo and in vitro, and reduced the MDA levels. As a result, they conclude that it had an anti-tumoral effect.

Our findings are in concordance with the findings of Sarıözkan et al. (2017) regarding the levels of GSH, TAS and MDA levels. In this way, in the GILA group's kidney homogenate, while the GSH and TAS levels are increasing the MDA levels decreased significantly, $P < 0.001$, $P = 0.006$, $P = 0.015$, respectively when compared to control. Both GSH and TAS levels in STZ+GILA and GILA groups increased significantly when they compared to STZ group with the p values were $P = 0.030$, $P < 0.001$, and $P = 0.040$, $P < 0.001$, respectively. When we looked at the MDA levels, it decreased importantly as having the $P < 0.001$ in both compared groups.

For the interpretation of these results, it thought that GILA fruit extract has a potent antioxidant activity. This could be because of its phenolic contents. As it is well known, the phenolics can convert the free radicals to the more stable compounds or increase the antioxidant enzyme activities. Whatever the reason is, they have good and positive effects on diabetes-modeled rats.

5. Conclusion

In our best knowledge, we did not come across any literature on GILA's effect on diabetes-modeled animals. Therefore, it is thought that GILA consumption would be very helpful in reducing the nephropathy in diabetics.

Patient informed consent : There is no need for patient informed consent.

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