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Therapeutic potentials of *Hibiscus trionum*: Antioxidant, anti-lipid peroxidative, hypoglycemic, and hepatoprotective effects in type 1 diabetic rats

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ABSTRACT

Recent advances in diabetes treatment have primarily focused on insulin and hypoglycemic agents; however, there is growing interest in exploring herbal and synthetic alternatives. Numerous studies have highlighted the preventive effectiveness of regular plant consumption in managing chronic conditions, particularly diabetes. Hibiscus, a medicinal plant recognized in various cultures, is known for its diverse health benefits. This study investigated the impact of *Hibiscus trionum* on glycemic control and assessed its influence on glucose and insulin levels in diabetes-induced rats. The concentrations of antioxidant enzymes, particularly superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), were scrutinized across multiple body tissues (plasma, heart, muscle, liver, and kidney). The malondialdehyde (MDA) concentration, an indicator of lipid peroxidation, was examined in both plasma and tissue samples. Serum total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were evaluated. Diabetic Group (D) exhibited a significant decrease in body weight, increased fluid and food consumption, elevated blood glucose levels, and increased antioxidant enzyme activity. Moreover, the diabetic group also showed increased levels of MDA, TC, TG, AST, and ALT, along with reduced insulin levels, compared to the control group. A substantial improvement in all parameters impaired by diabetes was observed following the application of *Hibiscus trionum* (HTT) in the Diabetes+HTT group. The antioxidative stress-reducing, lipid peroxidation-improving, and hepatoprotective potential of *Hibiscus trionum* in mitigating diabetes-induced oxidative stress is noteworthy. These findings indicate that HTT supplementation has valuable beneficial effects in protecting against the harmful impacts of diabetes.

1. Introduction

Diabetes mellitus (DM) is a persistent metabolic anomaly characterized by heightened glycemic concentration due to inadequate pancreatic insulin secretion and/or compromised cellular receptivity to insulin. This leads to imbalances in protein, fat, and carbohydrate metabolism, which can result in complications in nearly all body systems [1]. The etiology of DM is diverse, encompassing genetic/physiological determinants, inactive living patterns, excessive weight, suboptimal dietary behaviors, elevated intake of sugars and saturated fats, and disturbances in lipid metabolism arising from oxidative stress [2]. It is widely acknowledged that numerous plants possess antidiabetic properties and are utilized in traditional medicine. Given that DM is a chronic condition, improvement in quality of life requires lifestyle changes, adherence to treatment, and complementary methods in addition to medical treatment. Patients often resort to herbal and synthetic treatment methods, and traditional plant therapies are widely sought as supplements for diabetes treatment in various regions of the world [3].

There is significant scientific research on the hypoglycemic effects of medicinal plants, which may be due to the side effects of therapeutic agents, cost-effectiveness of herbal products, their accessibility, high antioxidant content, and their contribution to the drug treatment adherence process [4].

Hibiscus spp. belongs to the Malvaceae family and comprises more than 220 species. Although previously classified together, hibiscus and okra have now been categorized into separate subgroups within the Malvaceae family. As a result, hibiscus is commonly referred to as "black okra" or "African okra" in Turkey. The calyx, which encloses the petals, is hairy, has a thick fleshy structure, and can be a light or dark cherry-

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green color. Eight to twelve smaller, slender external sepals surround the calyx [5–7]. Only the calyx and external sepals were used in the preparation of tea and natural medicines, whereas the other parts were discarded.

Hibiscus's primary constituents include organic acids, anthocyanin glycosides such as cyanidin-3-xyloglucoside, flavon glycosides such as gossypetin and gossypetin-3-glucoside, vitamin C, and mucilage [7]. Research has demonstrated antibacterial, antifungal, antihypertensive, antiatherosclerotic, antioxidant, antihypercholesterolemic, antipyretic, and antimutagenic properties of the Hibiscus plant [7,8]. Furthermore, Hibiscus tea has remarkable antioxidant properties and has been found to reduce Low-Density Lipoprotein (LDL) levels, thus offering protective effects against cardiovascular diseases. Previous studies have indicated that Hibiscus spp. possess hypoglycemic and hypolipidemic properties, which can be beneficial in supporting the diabetic profile [9]. Various species of Hibiscus are cultivated worldwide, with the naturally occurring species in Turkey being *Hibiscus trionum*. *H. trionum* L., which is the sole species endemic to Turkey and commonly referred to as Venice mallow, bladder ketmia, flower-of-an-hour, or rosemallow, is notorious for invading soy and corn fields, thus regarded as a harmful weed [10]. It also serves as a host plant for Potato Virus Y (PVY) and Tomato Spotted Wilt Virus (TSWV) [11]. Furthermore, it has been reported that in China, the fruits and seeds of this plant are traditionally used in herbal medicine for the treatment of colds, burns, and other inflammatory conditions [12]. However, research on this species is limited, and no studies have been conducted to demonstrate its effects on diabetes.

In this study, the effects of *Hibiscus trionum* L. tea on glucose and insulin levels in rats with induced type 1 diabetes were investigated. The hepatoprotective, hypolipidemic, and antioxidant properties of the control and diabetic groups were examined. And was explored the potential protective effects of *H. trionum* against diabetes-induced oxidative damage.

2. Materials and methods

2.1. Preparation of Hibiscus trionum tea

H. trionum were collected from the Orhaneli locality of Bursa Uludağ and was identified by Prof. Dr. Hulusi Malyer. The voucher specimen was deposited in the Herbal Products Laboratory at Bursa Uludağ University with a specific code: 45707. The necrotic parts were removed. Plant samples were treated with 30% ethanol for 10 minutes to purify them from possible contaminants. "Then the leaf samples were rinsed with tap water and distilled water respectively. It was then dried in the shade and ground into fine powder."

The preparation and administration of *Hibiscus trionum* tea in this study involved steeping dried *Hibiscus trionum* leaves in boiling water for 10 minutes, followed by filtration to remove solid particles. The tea was then cooled to room temperature (38°C) before administration to animals.

In the experiment, each animal was housed individually in separate cages (36 cages), and 33 mL of the prepared 10% *H. trionum* tea was administered in bottles. The daily dose consumed per animal per kg was 8.25 g. The animals were ensured to consume the intended amount of tea. Subsequently, drinking water was supplemented throughout the day. Care was taken to replace the tea daily to maintain freshness and potency.

2.2. Analysis of chemical composition of Hibiscus trionum tea

Hibiscus trionum tea administered to animals was prepared fresh and analyzed by using twice HPLC-DAD (1200) system to determine phenolic compounds described by Aybastier et al. [13]. The mobile phase gradient consisted of 1% formic acid in water (component A) and acetonitrile (component B), with gradient levels of 10% B at the start (0 minute), increasing to 13% B at 10 min, 41.5% B at 20 minutes peaking at 70% B at 25 minutes, and returning to 10% B from 35 to 38 minutes. To separate the phenolic compounds, an XBridge C18 column (4.6 × 250 mm, 3.5 μm) manufactured by Waters in Ireland was employed. The volume injected was 10 μL, and the flow rate was maintained at 0.5 mL/min. Peaks were determined by comparing the UV spectra and retention times with those of standard references of phenolic compounds.

2.3. Animals

In this study, 36 adults male Wistar rats (400 g each) were used. The animals used were provided by the Bursa Uludag University Animal Care and Usage Center and the research was conducted in accordance with the ethical guidelines established by the Committee for Animal Care and Usage (Ethical Number: 2018–06/07). The rats were housed at room temperature (25 ± 2 ∘C, humidity 55% ± 5%) under a 12 h light-dark cycle. They were placed in laboratory controlled and uniform dietary conditions and given *ad libitum* access to water [14]. We were careful not to violate both national and international ethical rules. In each experimental group, control (n=9) and diabetic animals (n=9) were placed individually in separate cages. Observations and controls were conducted until the animals consumed the specified amount of *Hibiscus trionum* tea (HTT), ensuring equal consumption in both diabetic and healthy animals. Regular drinking water was provided to the cages after the animals consumed the desired quantity.

2.4. Induction of diabetes

Type 1 diabetes was performed by one intraperitoneally injection of streptozotocin (STZ), at dose 55 mg/kg freshly dissolved in sodium citrate buffer (pH of 4.5) body weight in overnight fasted rats [14,15], procured from Sigma-Aldrich, St. Louis, USA.

Rats in the control group (C) were injected with citrate buffer alone. Two days post-streptozotocin injection, we assessed the blood glucose levels. For the purposes of our study, rats with a blood glucose level of ≥ 200 mg/dL were classified as diabetic and included in subsequent tests.

2.5. Grouping of animals

Rats were randomized and divided into four groups as the healthy rats (control group, untreatment) "C", the healthy rats administered with *Hibiscus trionum* tea "C+HTT", the diabetic rats (positive control) "D", and the diabetic rats administered with *Hibiscus trionum* tea "D+HTT". After the induction period (one week) rats in the "C+HTT" and "D+HTT" groups were given HTT (at a dose of 40 (g/kg) (tea/body weight) for four weeks.

Throughout the four-week experimental process, daily intake of food and liquids, along with weekly body weight measurements, were assessed with in all experimental groups.

2.6. Sample preparation and biochemical analyses

At the end of the experimental period, the animals were starved for 12 hours, and they were euthanized by the internationally laboratory approved method of them puncturing of blood from the heart was completely under anesthesia. Blood samples collected in tubes specially manufactured for serum and plasma analyses were centrifuged at 1500 rpm for 10 minutes to separate serum and plasma. Separated samples were stored −20 ∘ C. Heart, kidney, liver, and skeletal muscle tissues were removed immediately after blood collection and stored at −20 °C until they were washed with saline and studied.

Before euthanasia, during the experiment, before the lifespan of the rats. Blood glucose (mg/dL) will be measured with a drop of blood from the rat tail once a week with a blood glucose meter (Optima, Taiwan). Insulin, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were measured in the plasma and heart, skeletal muscle, liver

and kidney using ELISA kits method (Sunlong Biotech, China), a standard set of reagents adapted from commercially available rat diagnostic kits, according to the manufacturer's instructions.

For the lipid peroxidation test, blood was collected into tubes containing EDTA, after which the plasma was separated and examined. Plasma and tissue (heart, skeletal muscle, liver and kidney) malondialdehyde (MDA) content was determined by using spectrophotometric method [16]. The concentration of lipid peroxide in the tissue was denoted as nmol of MDA per mg of tissue. Total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in the serum using commercial test kits (Fujifilm FDC, Japan) in the autoanalyzer (Fuji Dri-Chem, Japan).

2.7. Determination of antioxidant capacity

The radical-scavenging activity of *Hibiscus trionum* leaves was determined using 2,20 -azino-bis-(3-ethylbenzothiazolin6-sulfonic acid) diammonium salt (ABTS) according to a previously described method with minor modification. Absorbance was measured using a microplate reader (BioTek, USA) at 735 nm. From a Trolox standard curve, the Trolox equivalent antioxidant capacity (TEAC) was calculated and expressed in mg of Trolox equivalent (TE) per gram of dry sample.

2.8. Statistical analysis

All statistical analyses were performed using SPSS 28.0 for Windows. Results are expressed as Mean ± Standard Error. The type of analysis used non-parametric Kruskal-Wallis test was based on the results of the normality test. Post Hoc Testing was conducted using Tamhane's T2 test to determine intergroup differences. The level of significance was set at $p \leq 0.05$.

3. Results

3.1. Chemical composition of Hibiscus trionum Tea

The chemical composition of *Hibiscus trionum* was analyzed using HPLC-DAD (Fig. 1). Four peaks were observed with retention times of 22.284, 22.907, 23.785 and 27.912 minutes in the chromatogram, which were identified as rutin, quercetin-3-B-D- glucoside, kaempferol 3-B-D-glucoside and quercetin, respectively.

The contents of rutin as 6.4 ± 0.1 mg/L tea, Quercetin 3-B-D glucoside 22.7 ± 0.3 mg/L tea, Kaempferol 3-B-D glucoside 6.5 ± 0.2 mg/L tea, and Quercetin 0.5 ± 0.0 in HTT were determined, respectively.

3.2. Effect of HTT on the body weight, food intake and fluid consumption of normal and induced diabetic rats

Table 1 represents the body weight loss-gain effect of HTT in normal and type 1 diabetic rats.

The body weight of diabetic animals (D) reduced with statistically significant compared with control group (C, $p < 0.05$). As shown in Table 1, daily oral treatment with HTT caused weight gaining effect on

Table 1
Food and fluid intake, final body weight in control and experimental groups of rats. Values are expressed as mean ± SEM for rats in each group (n=9).

	C	C + HTT	D	D + HTT
Food intake (g/24 h)	15± 1	23 ± 1^{a*}	41 ± 1^{a*}	30 ± 2^{b*}
Fluid intake (mL/24 h)	30 ± 2	37 ± 4^{a*}	127 ± 1^{a*}	117 ± 1
Final body weight (g)	413± 5	424 ± 2	385 ± 8^{a*}	408 ± 5^{b*}

a: Compared with control. b: Compared with diabetes group. Statistical significance: *p<0.05.
C; Control, C +HTT; Control + *Hibiscus trionum* tea, D; Diabetes, D+HTT; Diabetes + *Hibiscus trionum* tea.

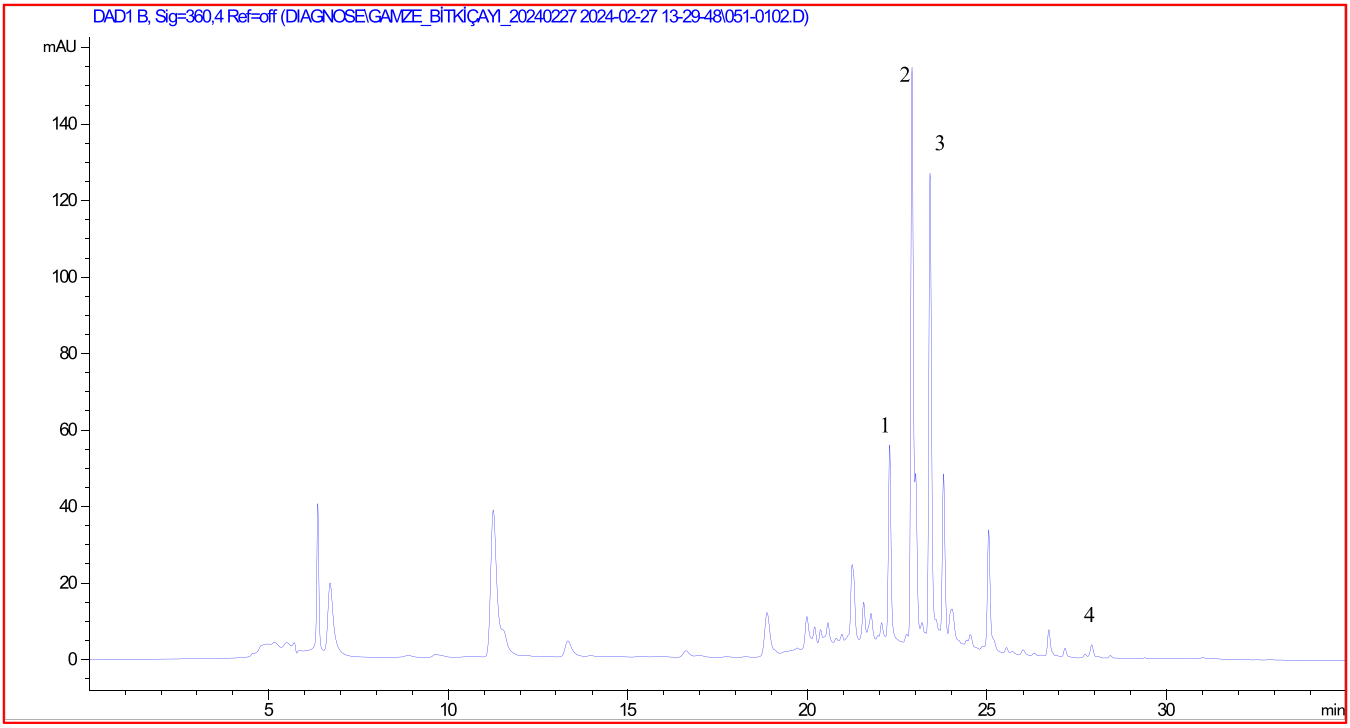

Fig. 1. HPLC-DAD chromatograms of components in standards recorded at 360 nm. Quantification showed that *Hibiscus trionum* contained (Liter of tea).Peak 1: 6.4 ± 0.1 mg/L tea Rutin (22.284 min)Peak 2: 22.7 ± 0.3 mg/L tea Quercetin 3-B-D glucoside (22.907 min)Peak 3: 6.5 ± 0.2 mg/L tea Kaempferol 3-B-D glucoside (23.785 min) Peak 4: 0.5 ± 0.0 mg/L tea Quercetin (27.912 min).

the treated rats with the most pronounced and significant ($p < 0.05$) effect seen in the group treated with HTT (D + HTT) when compared with the diabetic group (D).

In the D group, food and fluid consumption were significantly increased compared with the C group ($p < 0.05$). In the C + HTT group, food consumption and fluid consumption were statistically higher than those in the C group ($p < 0.05$). Food and fluid intake were significantly decreased in D + HTT group compared with the D group ($p < 0.05$).

3.3. Biochemical analyses

Table 2 and Fig. 2 depict the effect of daily oral administration of HTT on the blood glucose, plasma insulin, SOD and GSH-Px levels. SOD ve GSH-Px levels of heart, skeletal muscle, liver and kidney tissues are also shown in Table 2.

In the D group, blood glucose significantly increased, insulin levels significantly decreased compared with the C group ($p < 0.05$). Blood glucose significantly decreased in D + HTT group compared with the D group; also, serum insulin level significantly increased D + HTT group compared with D group ($p < 0.05$). Both plasma and tissue (heart, muscle, liver, and kidney) GSH-Px and SOD activity were significantly higher in the diabetic group than in the control group ($p < 0.05$, 0.01). *H.trionum* caused significantly increases in these parameters in the C + HTT and D + HTT groups compared to the C and D groups, respectively ($p < 0.05$, 0.01, Table 2).

MDA levels of plasma, heart, muscle, liver, and kidney tissues are shown in Fig. 3. MDA levels of different tissues were significantly higher in group D than in the C ($p < 0.05$, 0.01). However, *H. trionum* significantly decreased plasma and tissue MDA levels in the D + HT group compared to those in the D group ($p < 0.05$, 0.01, Fig. 3).

TC, TG, AST and ALT levels of serum are given in Table 3. In the D group, TC, TG, AST and ALT levels significantly increased compared with the C group ($p < 0.05$, 0.01). Serum TC, TG and AST levels significantly decreased in D + HTT group compared with the D group ($p < 0.05$, 0.01).

3.4. Antioxidant capacity

"The in vitro antioxidant ability of *Hibiscus trionum* was evaluated using ABTS. It demonstrated a noticeable ABTS+ radical neutralizing activity, as measured by its Trolox-equivalent antioxidant potential." (TEAC) values of 8.28 ± 0.21 mg. Trolox was used as a positive control.

Table 2
Blood glucose, serum insulin, plasma glutathione peroxidase (GSH-PX) and plasma superoxide dismutase (SOD) in control and experimental groups of rats. Values are expressed as mean ± SEM for rats each group (n=9).

	C	C + HTT	D	D + HTT
Glucose(mg/dL)	110 ± 7	103 ± 2	472 ± 3^{a*}	324 ± 3^{b*}
Insulin (mIU/L)	5.6 ± 0.1	6.1 ± 0.3	3.7 ± 0.5^{a*}	5.1 ± 0.3^{b*}
GSH-Px (ng/mL)	8.9 ± 1	28.8 ± 2.4^{a*}	14.4 ± 2^{a*}	34.6 ± 3.9^{b*}
Heart GSH-Px (ng/mL)	13.2 ± 1	20.7 ± 1.4^{a*}	32.3 ± 2^{a*}	34.4 ± 1.8
Muscle GSH-Px (ng/mL)	14.2 ± 1.1	18.1 ± 1.1^{a*}	33.0 ± 0.3^{a*}	36.9 ± 0.8^{b*}
Kidney GSH-Px (ng/mL)	12.7 ± 1.1	18.0 ± 0.4^{a*}	27.0 ± 0.9^{a*}	35.1 ± 0.8^{b*}
Liver GSH-Px (ng/mL)	11.8 ± 0.2	13.9 ± 1.1	23.3 ± 1.5^{a*}	24.2 ± 1.2
SOD (ng/mL)	0.6 ± 0.1	1.6 ± 0.1^{a**}	1.3 ± 0.7^{a*}	1.7 ± 0.1^{b**}
Heart SOD (ng/mL)	1.4 ± 0.2	1.9 ± 0.1^{a*}	2.9 ± 0.3^{a*}	3.5 ± 0.5
Muscle SOD (ng/mL)	1.1 ± 0.1	1.8 ± 0.1^{a*}	2.3 ± 0.1^{a*}	2.8 ± 0.1^{b*}
Liver SOD (ng/mL)	1.1 ± 0.1	2.0 ± 0.3^{a*}	1.7 ± 0.3^{a*}	3.2 ± 0.1^{b*}
Kidney SOD (ng/mL)	1.3 ± 0.2	1.6 ± 0.1^{a*}	2.1 ± 0.1^{a*}	2.6 ± 0.1^{b*}

a: Compared with control. b: Compared with diabetes group. Statistical significance: *p<0.05, **p< 0.01.
GSH-Px;Glutathione peroxidase, SOD; superoxide dismutase. C; Control, C +HTT; Control + *Hibiscus trionum* tea, D; Diabetes, D+HTT; Diabetes + *Hibiscus trionum* tea.

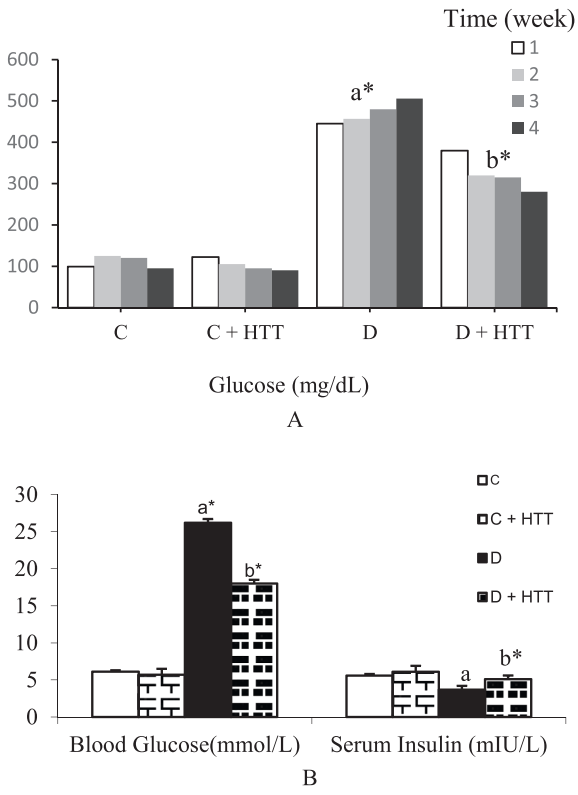

Fig. 2. A. Blood glucose levels in rats during the 4 weeks of *Hibiscus trionum* tea treatment periods (n=9). **B**. Blood glucose, plasma insulin in control and experimental groups of rats.Values are expressed as mean ± SEM for rats each group (n=9). a: Compared with control b: Compared with diabetes group. Statistical significance: *p<0.05, C; Control, C +HTT; Control + *Hibiscus trionum* tea, D; Diabetes, D+HTT; Diabetes + *Hibiscus trionum* tea.

4. Discussion

Plants have been used in folk medicine for many years due to its high content of biological active compounds and excellent healing properties like antioxidant, antidiabetic, anti-cancer, antimicrobial, antiviral, antiulcer and anti-bacterial activities. Herbal infusions are often employed as healing agents in various traditional medicinal systems. They have gained which are consumed have gained widespread global popularity as drinks, are consumed worldwide, and serve as therapeutic agents. One of the benefits of these teas is the rapid absorption of water-soluble or uniformly dispersed phytochemicals starting from the oral cavity and continuing seamlessly throughout the digestive system [17–20]. The aim of this study was to investigate the antidiabetic, antilipidemic, and antioxidant effects of *Hibiscus trionum* tea in experimental type 1 diabetes.

Studies have shown that hibiscus plants exhibit antibacterial, antifungal, antihypertensive, anti-atherosclerotic, antioxidant, anti-hypercholesterolemic, antipyretic, and antimutagenic properties [19, 21]. It has also been observed that this plant has beneficial effects on disrupted liver enzymes in diabetes. Research conducted on various species of Hibiscus spp. has demonstrated the hypoglycemic and hypolipidemic characteristics of this plant [9,22–28]. Although there are very few studies on *H. trionum*, the only species that grows naturally in our country, there are no studies showing the effect of these plants on diabetes.

Diabetes mellitus is characterized by elevated levels of blood glucose

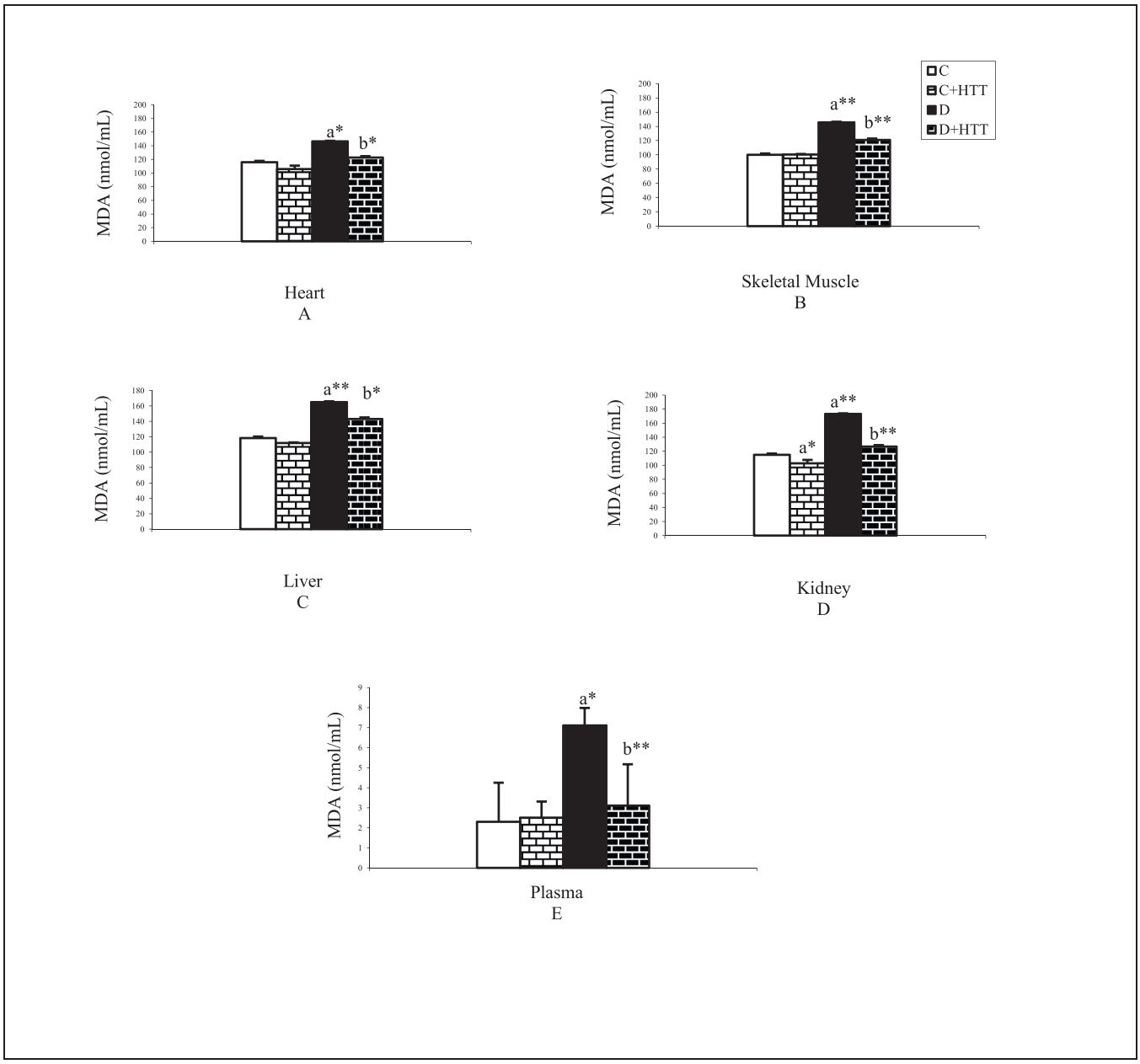

Fig. 3. Malondialdehyde (MDA) control and experimental groups of rats. Values are expressed as mean ± SEM for rats each group (n=9).a: Compared with control. b: Compared with diabetes group. Statistical significance: *p<0.05, **p< 0.01 C; Control, C +HTT; Control + *Hibiscus trionum* tea, D; Diabetes, D+HTT; Diabetes + *Hibiscus tri*onum tea.

Table 3
Serum triglyceride (TG), serum total cholesterol (TC), serum aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) levels control and experimental groups of rats. Values are expressed as mean ± SEM for rats each group (n=9).

	C	C + HTT	D	D + HTT
TG (mg/dL)	89.5 ± 0.5	60.8 ± 1.8	138.3 ± 1.4^{a*}	101.5 ± 1.6^{b*}
TC (mg/dL)	67.5 ± 1.5	61.6 ± 4.3	159.7 ± 3.3^{a*}	111.8 ± 5.7^{b*}
AST (U/L)	103.0 ± 1.1	108.6 ± 4.6	431.0 ± 3.5^{a**}	280.9 ± 4.8^{b*}
ALT (U/L)	62.0 ± 1.5	63.0 ± 2.5	190.0 ± 4.4^{a**}	175.4 ± 4.4

a: Compared with control. b: Compared with diabetes group. Statistical significance: *p<0.05, **p< 0.01
TG; Triglyceride, TC; Total Cholesterol, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase,
C; Control, C +HTT; Control + *Hibiscus trionum* tea, D; Diabetes, D+HTT; Diabetes + *Hibiscus tri*onum tea.

and lipids, leading to oxidative stress, which plays a central role in the onset of diabetes-associated complications. Oxidative stress is primarily caused by the stimulation of reactive oxygen species (ROS) emanating from the mitochondria. Numerous studies have reported that an increase in intracellular and extracellular glucose concentrations leads to oxidative stress [29,30]. We observed a decline in blood glucose levels concomitant with an elevation in serum insulin levels in the D + HTT group (Table 2). The findings were consistent with those of *Hibiscus sabdariffa* [27]. It can be concluded that *H. trionum* exerts similar effects to *Hibiscus sabdariffa by* inhibiting the pancreatic α-amylase enzyme and slowing down the digestion of carbohydrates into more absorbable monosaccharide forms [28]. This effect can be attributed to the bioactive compounds (such as Quercetin-3 glucoside, quercetin, and kaempferol) found in *Hibiscus trionum*. The components contained in *Hibiscus trionum* may potentially aid in the restoration of pancreatic β-cells or act synergistically and independently by stimulating insulin release,

potentially aiding the restoration of pancreatic β-cells, or inhibiting the intestinal absorption of glucose. The potential mechanism responsible for the hypoglycemic action of *H. trionum* may involve several factors, including the inhibition of glucose absorption in the intestines, suppression of polysaccharide digestion by inhibition of α-amylase and glucosidase enzymes, inhibition of hepatic gluconeogenesis, and activation of glucokinase and glycogen synthesis enzymes. Quercetin-3-Glucoside has been found to be equally effective as quercetin in improving insulin resistance through the modulation of insulin receptor substrate 1 (IRS-1) functionality in the endothelium [31]. Wang et al. reported that *Hibiscus taiwanensis* enhanced insulin sensitivity to improve diabetic hyperglycemia by regulating peripheral glucose utilization and hepatic glucose output in STZ-diabetic rats [32]. Furthermore, metabolites derived from plants have demonstrated advantageous effects on GLUT4 and cellular glucose uptake, whether in adipocytes or myocytes. Certain of these metabolites, whether through acute increases in cell surface GLUT4 levels or by exhibiting insulin-mimicking activity, have been shown to have a positive impact [33,34]. Notably, quercetin and kaempferol extracted from diverse botanical sources have been shown to markedly facilitate glucose absorption in preadipocyte cells [35]. In accordance with the literature, stimulation of glucose uptake in peripheral tissues (adipocytes, muscle, etc.) by *H. trionum* can be considered. Although many of these effects may be direct, the increase in glucose uptake and suppression of hepatic glucose production could also be mediated through the stimulation of insulin secretion.

As shown in Fig. 1, *H. trionum* tea contains rutin (kuersetin-3-O-rutinosid), isoquercitrin (Quercetin 3-B-D glucoside), kaempferol 3-B-D glucoside and quercetin, respectively. Quercetin 3-O-glucoside, identified from plants, has shown antidiabetic properties through the promotion of insulin synthesis and secretion as well as by blocking the sodium-glucose co-transporter (SGLT) in rats [36]. Quercetin and kaempferol derivatives are the most predominant flavonoids in many plant leaf extracts and have been reported to have an excellent inhibitory effect on α-glucosidase [37,38]. Quercetin reduces serum lipid levels and has positive effects on complications related to dyslipidemia, including atherosclerosis, myocardial infarction, and coronary diseases, making it beneficial for these conditions. Its antioxidant and radical-scavenging properties offer protection against pancreatic β-cell damage caused by oxidative stress associated with diabetes. In addition, it increases GPX4 activity in the pancreas, reduces oxidative stress, and enhances β-cell production and insulin secretion [39]. Kaempferol, on the other hand, can slow the progression of diabetic complications, particularly diabetic nephropathy, by increasing GLP-1 and insulin secretion, and inhibiting the RhoA/Rho Kinase (Ras Homology Family Member A/ Ras Homology Family Kinase) pathway. Both compounds and their derivatives have emerged as potential therapeutic agents for the treatment of diabetes and its associated complications [39]. Alternatively, they may reduce glucose absorption in the intestine [40,41]. These plant components can affect multiple targets simultaneously. For instance, flavonols, such as kaempferol and quercetin, act as protein tyrosine phosphatase 1B (PTP1B) inhibitors, while quercetin has been reported to possess the ability to regenerate pancreatic beta cells. Polyphenols, increase glucose uptake and glucose transporter type 4 (GLUT4) expression in adipose tissue and skeletal muscle via AMP-activated protein kinase (AMPK) and phosphoinositide 3-kinase (PI3K-Akt) dependent pathways. They activate AMPK and modulate genes involved in insulin-glucose signaling pathways, peroxisome proliferator-activated receptor alpha (PPARγ) expression, and adipocyte-specific genes (lipoprotein lipase) and can upregulate adiponectin gene expression while downregulating plasminogen activator inhibitor-1 and interleukin-6 (IL-6) genes [42]. Kaempferol enhanced glucose uptake and antioxidant production, inhibited apoptosis, improved cyclic adenosine monophosphate (cAMP) signaling, enhanced insulin synthesis and secretion, mitigated PPARγ agonist activity, and reduced caspase-3 activity in β-cells [26,43]. Quercetin blunts free radicals and oxidative stress, stimulates GLUT4 translocation in the skeletal muscle, inhibits glucose-6-phosphatase (G6Pase) in hepatocytes, and reduces intestinal glucose absorption by inhibiting glucose transporter type 2 (GLUT2) [44–46].

Previous research has shown that a 50% zingiber extract is effective in preventing insulin resistance in rats and regulating blood glucose levels. This effect was attributed to the antioxidant properties of the extract [47]. Multiple studies have demonstrated that different species of hibiscus reduce blood glucose and increase serum insulin levels induced by STZ or alloxan in diabetic rats [5,6,9].

Multiple pathways, including non-enzymatic glycation, metabolic stress, and sorbitol pathway activity, to factors such as hypoxia and ischemia-reperfusion, are responsible for the surge in free radical generation and variations in antioxidant defense in diabetes [48,49]. Numerous studies have reported variable antioxidant activities in diabetes, with some indicating an elevation and others suggesting a decline [14,50–53]. In the current study, there was a statistically significant increase in the activities of antioxidant enzymes, namely SOD and GSH-Px, in diabetic rats compared to those in the control group (Table 2). Such an increase in enzyme function can be interpreted as an enhanced counteraction of the heightened lipid peroxidation observed under diabetic conditions. In this study, the increase observed in the activities of selected antioxidant enzymes could potentially be attributed to an excessive compensatory response of HTT to the production of reactive oxygen species (ROS) in diabetes. Notably, *Hibiscus trionum* tea exhibited the capacity to elevate the levels of erythrocyte superoxide dismutase (SOD) and serum/tissue glutathione peroxidase (GSH-Px) in both the C + HTT and D + HTT groups compared to their respective counterparts' groups (Table 2). Based on these findings, it can be concluded that *Hibiscus trionum* tea can be attributed to have a direct stimulatory effect on the activity of antioxidant enzymes is respective of whether the individual is affected by diabetes or not. These findings are likely due regardless of whether the individual is affected by diabetes. This is likely due to the presence of phytochemicals, such as phenolic compounds like quercetin, kaempferol and its derivatives, which have been recognized as potent bioactive components that can enhance antioxidant potential in vivo.

MDA, the product of lipid peroxidation, serves as a pivotal marker that reflects the extent of oxidative stress damage induced by a free radical assault. Experimental evidence suggests that lipid peroxidation byproduct, MDA, when bound to proteins exacerbates glycoxidation-induced damage, further intensifying oxidative stress in diabetes mellitus [54]. The findings also suggest that *H. trionum* tea possesses potential antihyperlipidemic properties in rats with diabetes. This attribute could crucially prove in the prevention or treatment of complications associated with diabetes mellitus (DM), particularly atherosclerotic heart disease. As demonstrated in the study, hyperglycemia and hyperlipidemia may be closely linked to oxidative stress, which can lead to cellular damage and induce lipid peroxidation, ultimately resulting in the generation of malondialdehyde (MDA). In the study, we observed a significant decrease in malondialdehyde levels in all tissues of hibiscus tea-treated diabetic rats. ($p<0,05$–$0,01$, Fig. 3). Furthermore, there was a discernible trend towards diminished tissue MDA levels in the C + HTT group (Fig. 3). Kidney MDA levels were significantly lower in the C + HTT group. Conversely, while the plasma MDA levels remained unaltered in the C + HTT group, a statistically significant reduction was evident in the D + HTT group ($p< 0,01$, Fig. 3). It is crucial to acknowledge that the glycation of enzymes can result in oxidative stress. The reduction in tissue MDA levels observed in the D + HTT group may partly be ascribed to the ROS scavenging effect, stimulation of antioxidant enzymes, and the reduction of oxidative stress by *Hibiscus trionum*. In comparative studies, the significant reduction in MDA levels observed in induced diabetic rats administered *Hibiscus sabdariffa* has been attributed to its antioxidant properties [25,55]. Mustafa et al. [56] reported that the methanolic extract of *Euphorbia helioscopia* exhibits antioxidant and antidiabetic properties, as evidenced by its ability to normalize lipid profiles, and reduce malondialdehyde levels. In another

study, supplementation with Egyptian Anna apple extract reduced MDA levels in diabetic rats. This suggests that the polyphenolic components of apples may possess antioxidant properties that counteract the increase in MDA, offering protection to the pancreas from diabetes-induced oxidative stress [57]. Hyperlipidemia observed in individuals with diabetes facilitates the use of lipids as substrates for lipid peroxidation, thus supporting the increase in MDA levels.

The body possesses antioxidant defense mechanisms to combat oxidative stress. These include enzymes such as Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx). These enzymes neutralize reactive oxygen species (ROS), thereby protecting cells from oxidative damage. The activities of SOD and GPx play a crucial role in preventing the formation of free radicals and constitute the core of the cellular defense system against oxidative damage [58]. The results are consistent with those of previous studies, which attributed the MDA depletion and oxidative stress rescue effects of diabetes to the presence of dietary polyphenols. This is further supported by evidence revealing the use of natural extracts from plant sources to increase the risk of oxidative stress owing to their rich source of phytochemicals.

Diabetes mellitus is associated with significant changes in plasma lipids and lipoproteins, which increase the risk of accelerated atherosclerotic and cardiac diseases. Various plant-derived products have demonstrated hypoglycemic, hyperlipidemic, and antioxidant properties [59]. However, information regarding the hypolipidemic effects of *Hibiscus trionum* under diabetic conditions is deficient research data. *Hibiscus sabdariffa* extract at both 100 and 200 mg/kg dose-dependently decreased alloxan-induced increases in total cholesterol and triglycerides both in the liver and kidneys [60]. In a study conducted with the methanolic leaf extract of *Hibiscus articulatus*, a species native to Nigeria, it was reported that the low-density lipoprotein (LDL) levels in diabetic Wistar rats significantly decreased compared to those in control rats following the application of *H. articulatus* extract (125 mg/kg, $p < 0.05$) [23]. Additionally, experimental diabetes studies involving different Hibiscus species and extracts obtained using various solvents, such as *Hibiscus sabdarifa* [9,22], *Hibiscus rosasinensis* [60], *Hibiscus taiwanensis, Hibiscus cannabinus, Hibiscus. platanifolius, Hibiscus surratensis, and Hibiscus tiliaceous* have been reported that applications of Hibiscus spp. significantly reduced TC (total cholesterol), TG (triglycerides), LDL (Low Density Lipoprotein) levels, and increased HDL-C (High Density Lipoprotein) levels compared to diabetes control [24]. *Hibiscus sabdariffa* leaf polyphenolic extract inhibits LDL oxidation and foam cell formation involving up-regulation of Liver x receptor α / ATP-binding cassette transporter A1 (LXRα/ABCA1) pathway [61]. The findings on the lipid profile of *Hibiscus trionum* are consistent with these reports. In this study, it was observed that a significant reduction in the plasma levels of total cholesterol (TC) and triglycerides (TG) in the D + HTT group compared to those in the D group ($p<0,05$, Table 3). This decrease may be attributed to the presence of polyphenols in *H. trionum*, which have been shown to regulate the activity of enzymes involved in lipoprotein metabolism. It is possible that elevated insulin levels in groups receiving *Hibiscus trionum* could inhibit hormone-sensitive lipase and may also reduce cholesterol absorption from the intestine by inhibiting the activity of enzymes like 3-hydroxy-3 methyl glutaryl (HMG) CoA reductase and acetyl CoA cholesterol acyltransferase (ACAT), a transmembrane enzyme. Alternatively, there may be an increase in the activity of lecithin cholesterol acyltransferase, which catalyzes the formation of cholesterol esters in lipoproteins [62]. As documented, the presence of phytosterols in *H. trionum* may contribute to this effect by inhibiting the intestinal absorption of cholesterol, as documented previously.

The liver plays a pivotal role in the management of glucose, insulin, and lipid metabolism, and its function is significantly altered in diabetes. In the diabetic state, significant alterations are observed in liver toxicity markers [63]. This change is often attributed to hepatotoxic effects, as evidenced by the leakage of enzymes from the liver into the bloodstream in STZ-induced diabetic groups. Increases in plasma activities of AST, ALT, and ALP suggest the induction of hepatic dysfunction in diabetes mellitus (DM) [64]. AST and ALT, enzymes that convert amino acids to keto acids, are elevated in diabetic states. AST, which is found in several tissues throughout the body, is particularly concentrated in hepatocytes. When these tissues undergo necrosis, there is an associated increase in serum AST levels, primarily due to its release from damaged cells. It is important to note that a more severe disruption in mitochondrial membrane permeability is required for the release of mitochondrial AST, making it a sensitive marker of hepatocellular damage [5,65].

ALT, on the other hand, is primarily a cytoplasmic enzyme. Elevated serum ALT levels are indicative of substantial hepatocellular damage, as they are released into the extracellular space when hepatocellular membrane permeability is compromised. ALT, categorized within the transferase group, collaborates with aspartate aminotransferase in albumin metabolism. In patients with diabetes, an increase in serum aminotransferases is frequently observed, which is often attributed to hepatic fat infiltration [5]. As a result, due to increased ketogenesis and gluconeogenesis in diabetes, damage occurs to the structural integrity of hepatocytes, as evidenced by the increase in AST and ALT levels. In the study, we observed a significant decrease in the levels of AST and ALT was observed which are known to increase markedly with diabetes, following hibiscus treatment ($p<0.05$–0.01, see Table 3). The results align with those of Adeyemi et al. [27] who reported a reduction in the activities of these liver marker enzymes upon treatment of STZ-induced diabetic rats with *H. sabdariffa* extract. The potential therapeutic effect of hibiscus tea in ameliorating liver damage may be attributed to its antioxidant constituents such as quercetin and kaempferol. Quercetin has the potential to prevent oxidative damage in diabetic mice by suppressing cytochrome P450 2E1 (CYP2E1) liver enzymes [66]. Quercetin reduces gluconeogenesis by inhibiting glycogen phosphorylation in the liver and decreases the levels of the liver enzymes AST and ALT, which are complications of diabetes, through the inhibition of antitumor Necrosis Factor alpha (TNF-α) and interleukin 4 (IL-4) activity [39]. Treatment with *Hibiscus trionum* could potentially prevent the accumulation of increased central fat, diacylglycerols, and triacylglycerols in cells outside adipose tissue, such as hepatocytes and pancreatic β-cells. his may contribute under conditions, enhance hepatic insulin sensitivity, and exhibit restorative and/or protective effects on pancreatic β cell functionality, it may enable the liver to continue gluconeogenesis without exacerbation, thereby improving insulin resistance in both adipose and muscle tissues. Furthermore, a reduction in liver fat accumulation could prevent the onset of inflammation and decrease oxidative stress levels caused by diabetes, thus aiding in the maintenance of metabolic balance.

The Pancreatic and Duodenal Homeobox 1 gene (PDX1), also known as insulin promoter factor 1, is a transcription factor specific to pancreatic β-cells, particularly found in *Xenopus leavis*. PDX1 regulates the expression of various critical genes such as insulin, glucokinase, and GLUT2, aiding in the maintenance of mature β-cell function and glucose metabolism in mice and humans [67]. The number of studies investigating PDX-1 in experimental diabetic animals treated with herbal extracts is limited. However, these studies demonstrated that herbal treatments can positively affect PDX-1 expression [68–73]. Based on these findings, it can be considered that *Hibiscus trionum* may also increase PDX-1 expression. Further research is required to explore the effects of hibiscus on this gene.

5. Conclusion

In summary, our research emphasizes the therapeutic potential of *Hibiscus trionum* tea in the management and prevention of diabetes mellitus and its related complications, including cardiovascular and liver disorders, as well as nephropathy. This study demonstrated that *H. trionum* tea effectively reduced blood glucose and lipid levels, alleviated oxidative stress, and boosted antioxidant enzyme activity under in both diabetic and non-diabetic conditions.

The regulation of liver enzymes and the observed improvements in diabetic nephropathy underline its potential as a natural intervention. Diverse bioactive phytochemicals, particularly phenolic compounds found in Hibiscus, play a critical role in these health benefits, making the inclusion of bioactive *Hibiscus trionum* in the diet a significant therapeutic approach to the management of diabetes. This also raises the significance of Hibiscus tea being accepted as a socially raking nice beverage into days modern society, with the potential for the development of derivative products such as coffee, biscuits, and mineral water. Given the increasing global prevalence of diabetes and the perpetual quest for alternative and complementary therapeutic options, our findings pave the way for the use of *H. trionum* as an accepted beneficial, nutritional supplement.

The potential clinical application of natural products in diabetes care necessitates comprehensive research on their bioavailability, specificity, and the fundamental mechanisms underlying their actions, which include a nutrigenomic perspective and a thorough exploration of the interactions between phytochemicals and genes. Unraveling these mechanisms could lay the groundwork for creating highly selective antioxidant agents tailored for diabetes management. Concurrently, these mechanisms enable plant-based compounds to function as preventive agents, reducing the risk of diabetes onset.

Future research should focus on gene interactions and molecular pathways, optimizing the efficacy and bioavailability of *Hibiscus trionum* through suitable formulations, such as enteric encapsulation for daily use. This is crucial for the development of highly selective antioxidant agents specifically designed for diabetes management.

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Ethical Statement

The animals used were provided by the Bursa Uludag University Animal Care and Usage Center and the research was conducted in accordance with the ethical guidelines established by the Committee for Animal Care and Usage (Ethical Number: 2018–06/07).

Declaration of Competing Interest

The authors declare no conflict of interest.

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