Commagene Journal of Biology

Özmen & Taş, (2024) *Comm. J. Biol.* 8(2), 120-126.
DOI: 10.31594/commagene.1587511

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Therapeutic Potential of *Quercus ithaburensis* subsp. *macrolepis* Fruit Extract in Streptozotocin-Nicotinamide-İnduced Type 2 Diabetic Rats

Burcu ÖZMEN, Sibel TAŞ*

Bursa Uludağ University, Biology Department, Science Arts Faculty, Bursa, P.O. Box 16059 TÜRKİYE
ORCID ID: Burcu ÖZMEN: https://orcid.org/0009-0002-4253-1101; Sibel TAŞ: https://orcid.org/0000-0001-8880-0771

Received: 18.11.2024 **Accepted:** 23.12.2024 **Published online:** 27.12.2024 **Issue published:** 31.12.2024

Abstract: In this study; the effect of *Quercus ithaburensis* subsp. macrolepis fruit extract (QIFE) on blood glucose and oxidant-antioxidant systems in streptozotocin (STZ)-nicotinamide-induced type 2 diabetic rats was investigated. Type 2 diabetes was induced in rats by intraperitoneal injection of STZ (65mg/kg)-Nicotinamide (45 mg/kg). Rats were given 535mg/kg QIFE fruit extract in their drinking water for 21 days. Rats were divided into four groups; Control (C), Control+QIFE (C+QIFE), Diabetes (D), and Diabetes+QIFE (D+QIFE). Plasma and tissue malondialdehyde (MDA) levels were measured by spectrophotometry. Whole blood glutathione peroxidase (GSH-Px), serum superoxide dismutase (SOD) enzyme levels, serum paraoxonase (PON), and arylesterase (ARE) enzyme activities were determined using commercial kits. Serum insulin levels and blood glucose were evaluated using a Rat ELISA Kit and glucometer, respectively. Also, the autoanalyzer was used to assess the lipid profile. While blood sugar and serum total cholesterol (TC) levels showed a statistically significant decrease in the C+ QIFE and D+ QIFE groups (C and D groups, respectively), serum insulin levels showed a statistically significant increase in the D+QIFE group compared to the D group. In the D+QIFE group, a statistically significant increase was observed in PON and ARE enzyme activities compared to the D group, but in the C+QFE group, a significant increase was found in whole blood GSH-Px and serum SOD levels compared to the C group. A statistically significant decrease was detected in plasma, heart, muscle, and liver tissue MDA levels in the D+QIFE group compared to the C group. As a result, it was concluded that *Q. ithaburensis* fruit extract has anti-hyperglycemic, anti-hyperlipidemic effects, strengthens the antioxidant system, and is a good phytotherapeutic agent that prevents/improves metabolic processes and related complications related to diabetes mellitus.

Keywords: Antioxidant, lipid peroxidation, phytotherapeutic agents, oxidative stress.

Streptozotosin-Nikotinamid ile Oluşturulmuş Tip 2 Diyabetik Sıçanlarda *Quercus ithaburensis* subsp. *macrolepis* Meyve Ekstraktının Terapötik Potansiyeli

Öz: Bu çalışmada; *Quercus ithaburensis* subsp. macrolepis meyve ekstraktının streptozotosin (STZ)-nikotinamid ile oluşturulan tip 2 diyabetli sıçanlarda kan glikozu ve oksidan-antioksidan sistemler üzerindeki etkisi araştırıldı. Sıçanlarda tip 2 diyabet, STZ (65 mg/kg)-nikotinamidin (45 mg/kg) intraperitoneal enjeksiyonuyla oluşturuldu. Sıçanlara 21 gün boyunca içme sularına 535mg/kg *Q. ithaburensis* meyve ekstraktı verildi. Sıçanlar dört gruba ayrıldı; Kontrol (K), Kontrol+*Q. ithaburensis* meyve ekstraktı (K+QIFE), Diyabet (D), Diyabet+*Q.ithaburensis* meyve ekstraktı (D+QIFE). Plazma ve doku malondialdehit (MDA) düzeyleri spektrofotometre ile ölçüldü. Tam kan glutatyon peroksidaz (GSH-Px), serum süperoksit dismutaz (SOD) enzim düzeyleri, serum paraoksonaz (PON) ve arilesteraz (ARE) enzim aktiviteleri ticari kitlerde belirlendi. Serum insülin düzeyleri ve kan glikozu sırasıyla bir Rat ELISA Kiti ve glukometre ile değerlendirildi. Ayrıca, lipid profilini değerlendirmek için otoanalizör kullanıldı. Kan şekeri ve serum total kolesterol (TK) düzeyleri K+QIFE ve D+ QIFE gruplarında (sırasıyla K ve D grupları) istatistiksel olarak anlamlı bir azalma gösterirken, serum insülin düzeyleri D+QIFE grubunda D grubuna kıyasla istatistiksel olarak anlamlı bir artış gösterdi. D+QIFE grubunda PON ve ARE enzim aktivitelerinde D grubuna kıyasla istatistiksel olarak anlamlı bir artış gözlendi, ancak K+QFE grubunda tam kan GSH-Px ve serum SOD düzeylerinde K grubuna kıyasla anlamlı bir artış bulundu. D+QIFE grubunda K grubuna kıyasla plazma, kalp, kas ve karaciğer doku MDA düzeylerinde istatistiksel olarak anlamlı bir azalma tespit edildi. Sonuç olarak, *Q. ithaburensis* meyve ekstraktının antihiperglisemik, antihiperlipidemik etkiye sahip olduğu, antioksidan sistemi güçlendirdiği, diyabete bağlı metabolik süreçleri ve buna bağlı komplikasyonları önleyen/iyileştiren iyi bir fitoterapötik ajan olduğu sonucuna varıldı.

Anahtar kelimeler: *Antioksidan, lipid peroksidasyonu, fitoterapötik ajan, oksidatif stres.*

1. Introduction

From ancient times to the present, it is known that herbal medicines or their extracts are used to protect or treat health. It is known that plants are widely preferred by the public because they are natural and present a reduced risk of side effects compared to synthetic drugs. What is considered natural among people is the polyphenols (such as flavonoids, lignans, stilbenes, and phenolic acids) found in the structure of plants and have strong antioxidant properties (Shweta et al., 2021; Mithun et al., 2022). Polyphenolic compounds have many effects, such as antioxidant, anticancer, antimicrobial (Ahmed et al., 2016), antithrombotic (Mirza et al., 2019), and antidiabetic effects (Ngan et al., 2020). Today, the antidiabetic effect of polyphenols is one of the most researched areas and the mechanisms of action of approximately five hundred plants have been studied and established (Ramesh et al., 2017; Lin et al., 2018; Tarique et al., 2020).

Corresponding authors: smeral@uludag.edu.tr

Type 2 diabetes (non-insulin-dependent diabetes) is one of the rapidly spreading chronic diseases in the world. Genetic and environmental factors are effective in the formation of this disease, and in both cases, heterogeneous insulin resistance and pancreatic beta-cell dysfunction are in question (Ralph et al., 2015). In addition, oxidative stress resulting from hyperglycemia, hyperlipidemia, obesity, and alterations in the antioxidant defense system contributes to the development of microvascular and macrovascular complications (Surapon, 2015; de Gaetano et al., 2018). Diabetes treatment is a life-long disease that needs to be followed closely. Therefore, it is necessary to consider that there are different strategies for the management of this disease and to evaluate it well. Among these, reducing and controlling postprandial hyperglycemia caused by carbohydrate intake is very common, especially in diabetes patients. For this reason, it is important to inhibit the catalytic activities of α-amylase and α-glucosidase which are effective in carbohydrate metabolism to control postprandial blood sugar (Bhandari et al., 2008; Jianwei et al., 2014). Another is preventing the formation of advanced glycation end products (AGEs) (Matsuda et al., 2003). Control of all of these is provided by synthetic inhibitory agents, but their side effects should not be ignored (Nissen et al., 2007; Neuman et al., 2012; Eugene et al., 2016). A further approach is to strengthen the antioxidant defense against oxidative stress in diabetes. In fact, our body creates the primary defense against oxidative stress through its antioxidant enzyme system, which includes enzymes like glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Pisoschi et al., 2015). Also, antioxidant vitamins (e.g., C, E, B1, and B6) and polyphenols, which are powerful antioxidants, play a crucial role in preventing and combating oxidative stress. Supplementing diabetic conditions with these vitamins and polyphenolscan help enhance our antioxidant defense (Sarandol et al., 2020; Tas et al., 2014; Tas et al., 2024; Zanza et al., 2019; Sen et al., 2010).

Quercus, one of the plants used in diabetic conditions, belongs to the Fagaceae family and is known as the oak tree. *Quercus* spp. consists of about 600 species worldwide, which produce some fruit commonly known as an acorn (Vinha et al., 2019). Acorn fruit is abundant in phenolic compounds (such as ellagic acid and gallic acid derivatives), flavonoids (quercetin, catechin, naringin), and tannins, as well as other unsaturated fatty acids such as linoleic, oleic, and palmitic acids (Rakic et al., 2006; Taib et al., 2020; Makhlouf et al., 2019, Ana et al., 2016). Additionally, acorns are rich in protein, fiber, and minerals and contain vitamins A and E (with tocopherols found in significant amounts) which support and strengthen their antioxidant structure (Özcan, 2007; Özcan, 2006; Özcan, 2005). *Quercus* species; bark, leaf, and fruit extracts have broad mechanisms of action such as antidiabetic (Yin et al., 2018) anti-inflammatory (Huang et al., 2016), anticancer (Zehra et al., 2019), neuroprotective (Gezici & Sekeroğlu, 2019), and antioxidant (Rakic et al., 2006).

There are many studies on the effects of *Quercus* species. However, no study has been found on the effects of *Q. ithaburensis* subsp. macrolepis (Kotschy) Hedge and Yalt fruit extracts on STZ-nicotinamide induced type 2 diabetic rats.

In this study, the effects of *Q. ithaburensis* subsp. macrolepis fruit extract on STZ-nicotinamide-induced type 2 diabetic rats were examined. To evaluate antioxidant mechanisms, serum PON and ARE activities, blood GSH-Px, and erythrocyte SOD levels were measured. Plasma and tissue MDA (heart, gastrocnemius muscle, liver, kidney) levels were measured to evaluate lipid peroxidation status. Also, blood glucose, serum insulin, and lipid levels were determined.

2. Material and Method

2.1. Experimental Design

Forty male Wistar albino rats, aged 3 months, were acquired from the Animal Center of Bursa Uludağ University (Ethic number 2018-04/11). The rats, grouped four per cage, were kept in an environment with a constant temperature of 25 ± 2°C and a 12-hour light/dark photoperiod throughout the experiment. During this period, the rats were given a standard pelleted diet and tap water.

The rats (n=10) were assigned to four distinct groups: Group 1: Normal control rats (C), Group 2: Control rats administered *Q. ithaburensis* fruit extract (C+QIFE), Group 3: STZ- Nicotinamide-induced diabetic rats (D), Group 4: STZ-nicotinamide-induced diabetic rats administered *Q. ithaburensis* fruit extract (D+QIFE).

2.2. Diabetes Induction

The STZ-nicotinamide diabetes model previously described by Masiello et al. (1998) was applied with a modified dosage. Rats were first injected with 45 mg/kg body weight of nicotinamide intraperitoneally (I.P.), and 15 minutes later, they were given a single intraperitoneal dose of 65 mg/kg body weight of STZ, prepared in citrate buffer (pH 4.5). Rats with blood glucose levels ≥200 mg/dL (measured with IME-DC blood glucose meters, Germany) 48 hours after injection were considered diabetic.

2.3. *Quercus ithaburensis* subsp. *macrolepis* Fruit Extract

Quercus ithaburensis subsp. macrolepis was collected from the skirts of Kaz Mountain, located to the north of Edremit Gulf, within the borders of Edremit District in Balıkesir Province, and *Q. ithaburensis* plant (above-ground part) extract was prepared by a commercial company (Kale Natural Bitkisel Ürünler, Edremit/Balıkesir). The QIFE extract came from the company in sterile dark glass bottles as 100mL/5g and we diluted it by pouring it into 400mL water. According to the water consumption per rat, 535mg/kg QIFE fruit extract was given to the drinking water of group II (C+QIFE) and group IV (D+QIFE) rats one week after STZ injection for 21 days. In our study, the *Q. ithaburensis* dose to be given to diabetic rats was evaluated as reference according to the administration doses of different Quercus species such as 25 mg/kg (Doğan et al., 2015), 200 and 800 mg/kg (Yin et al., 2018), 250 mg/kg, 500 mg/kg (Saini et al., 2012). The fluid and food intake of all groups of rats were measured daily, while body weight and blood sugar levels were measured weekly using an Abbott glucometer (USA).

2.4. Sample Preparation

Four weeks after the experimental procedure, blood

samples were taken from the rats by cardiac puncture under 3% isoflurane inhalation throughout the operation. Then, the heart, skeletal muscle (gastrocnemius muscle), liver, and kidney tissues were quickly removed, rinsed with a standard normal saline cold solution, and stored at -20°C. For plasma samples blood samples were collected into tubes containing EDTA and/or heparin. Plasma and serum samples were then centrifuged at 3000 rpm for 15 minutes and stored at -20°C until analyzed.

2.5. Determination of Biochemical Parameters

Serum lipid levels (TC, HDL-C, TG) were measured using an autoanalyzer (Abbott, Architect, USA). Serum insulin levels were determined using a Rat ELISA kit (Lab Science, E-EL-R2466, USA) and the results were expressed as ng/mL. In addition, serum SOD and blood GSH-Px levels were determined using a kit (YL Biotech, Shanghai) and the values were expressed as ng/mL. Serum PON1 and ARE enzyme activities were determined using a commercial kit and the units were expressed as U/L. (Rel Assay Diagnostics, Mega Tıp, Gaziantep, Türkiye). MDA levels of tissues (heart, skeletal muscle, liver, and kidney) were studied according to the method of Ohkawa et al. (1979) and the values were expressed as nmol MDA/mg tissue expressed. Also, according to the method of Young & Trimble (1991) plasma MDA concentration was determined. The results are expressed as nmol/mL.

2.6. Statistical Analysis

Statistical analyses were performed using SPSS 20.0 for Windows (SPSS, Chicago, IL), and the data are presented as the mean ± standard error of the mean (SEM). The Kruskal–the Wallis test followed by the Mann–Whitney U test was used. A level of $p<0.05$ was accepted as statistically significant.

3. Results

As presented in Table 1, food ($p<0.05$) and water intake ($p<0.01$), blood glucose ($p<0.01$), and total cholesterol (TC) levels ($p<0.05$) were significantly higher in group D rats compared to control rats, whereas a significant decrease in serum insulin levels was seen ($p<0.01$). D+QIFE group rats that were given QIFE fruit extract had a statistically significant decrease in food ($p<0.01$) and water intake ($p<0.05$), blood glucose ($p<0.01$), and TC levels ($p<0.05$) compared to group D rats, while a significant elevation in insulin levels was observed ($p<0.01$). Compared to group C, the increase in body weight in the C+QIFE group was not statistically significant. There was a significant increase in serum GSH-Px and SOD enzyme activities in the C+QIFE group compared to the C group ($p<0.01$ and $p<0.05$, respectively).

A significant reduction in blood glucose and TC levels ($p<0.05$) was detected in C+QIFE group rats compared to the control group rats. In the D group, SOD and blood GSH-Px levels were elevated ($p<0.05$) when compared to the C group rats but PON and ARE enzyme activities were found to be statistically lower ($p<0.01$). However, only PON and ARE enzyme activities were significantly higher in the D+QIFE group ($p<0.01$) compared to the D group. Additionally, in the D group rats, SOD and GSH-Px levels were significantly increased ($p<0.05$) compared to the C group rats (Table 2).

Table 1. Body weight, food and water consumption, serum glucose and insulin levels, and lipid profile of the study group

Group	C	C+QIFE	D	D+QIFE
Food intake (g/24s)	24.1 ± 1.3	22.8 ± 0.4	34.1 ± 0.5^{a*}	30.2± 0.46^{b**}
Water intake (mL/24s)	41 ± 1.3	45 ± 1.4	85 ± 1.3^{a**}	76 ± 2.3^{b*}
Body weight (g)	288 ± 7	300 ± 3.1	298 ± 4.5	289 ± 6
Glucose (mg/dL)	133.6± 3.2	118.5± 2.5^{a*}	294± 18^{a**}	278.4 ± 5.6^{b**}
Insulin (ng/mL)	1.7 ± 0.1	1.7 ± 0.2	0.6 ± 0.06^{a**}	1.02 ± 0.03^{b**}
TC (mg/dL)	60.2 ± 1.5	48 ± 1.7^{a*}	69.6± 1.8^{a*}	60± 2.6^{b*}
TG (mg/dL)	78.4 ± 2.2	73.5 ± 3.5	80.6 ± 3.6	79.5 ± 3.5
HDL-C (mg/dL)	50.8 ± 1.9	51.1± 1.3	54.5 ± 1.0	55.7 ± 1.6

TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, C: Normal control rats, C+QIFE: Control rats with orally administered *Q. ithaburensis* fruit extract, D: Streptozotocin-Nicotinamid induced diabetic rats, D+QIFE: Diabetic rats with orally administered *Q. ithaburensis* fruit extract. Values are presented as the mean±SEM (standard error of the mean) for ten rats in each group, Statistical comparison: ^{a} C vs C+QIFE, ^{b} C vs D, ^{c} D vs D+QIFE, Statistical significance, * $p < 0.05$, ** $p < 0.01$

Table 2. Serum paraoxonase and arylesterase activities, superoxide dismutase, and blood glutathione peroxidase levels in rats.

Group	C	C+QIFE	D	D+QIFE
Blood GPX (ng/mL)	8.3 ± 1.4	12.8 ± 0.53^{a**}	11.8 ± 0.85^{a*}	13.14 ± 0.66
Blood SOD (ng/mL)	0.94 ± 0.17	1.59 ± 0.14^{a*}	1.28 ± 0.03^{a*}	1.31 ± 0.32
PON (U/L)	136.5 ± 8.5	144.7 ± 9.8	52.3 ± 3.5^{a**}	160.1 ± 9.5^{b**}
ARE (U/L)	140.1 ± 2.4	143.1 ± 1.3	59.7 ± 2.4^{a**}	148.7 ± 7.4^{b**}

PON: Paraoxonase, ARE: Arylesterase, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, Values are expressed as mean ± SEM (standard error of the mean) for ten rats in each group, Statistical comparison: ^{a} C vs C+QIFE ^{b} C vs D, ^{c} D *vs* D+QIFE, Statistical significance, * $p < 0.05$, ** $p < 0.01$, for abbreviations of study groups, see Table 1.

According to C group rats, plasma (Figure 1) and tissue MDA heart, musle, liver, kidney (Figure 2) levels were significantly higher in the D group rats ($p<0.01$). Plasma, heart, muscle ($p<0.01$), and liver ($p<0.05$) tissue MDA levels were significantly diminished in the D+QIFE group compared with the D group. However, the reduction in

plasma and tissue MDA levels in the C+QFE group, compared to the C group rats, was not statistically significant.

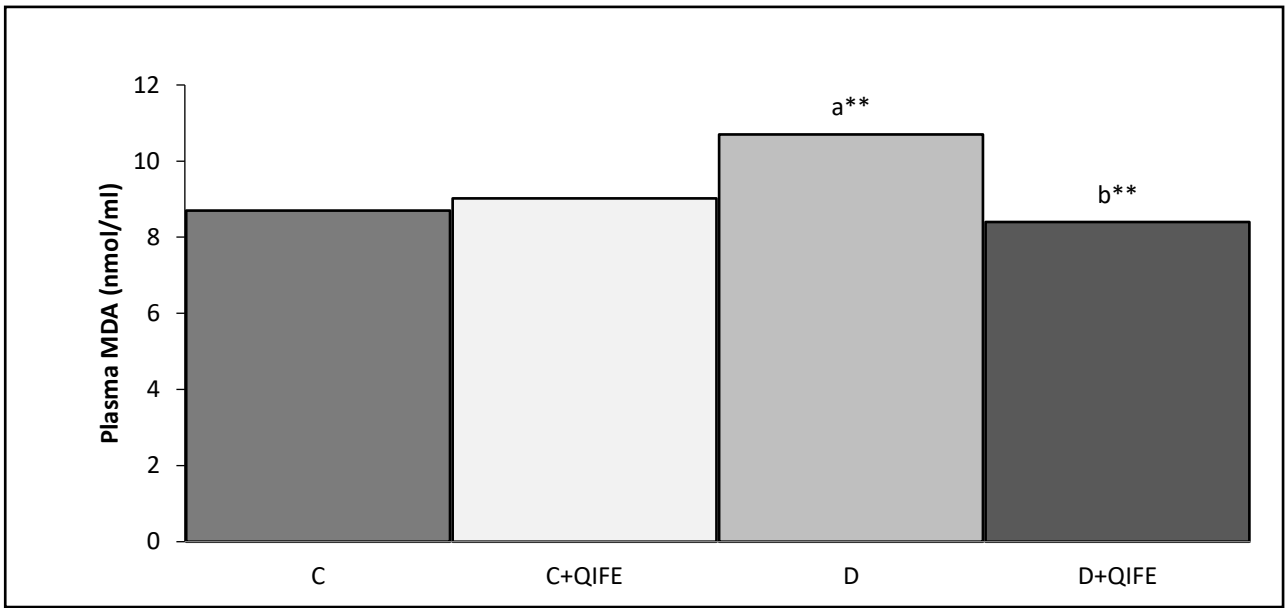

Figure 1. Plasma MDA (nmol/ml)

C: Normal control rats, C+QIFE: Control rats with orally administered *Q. ithaburensis* fruit extract, D: Streptozotocin-Nicotinamid induced diabetic rats, D+QIFE: Diabetic rats with orally administered *Q. ithaburensis* fruit extract. Values are expressed as mean ± SEM (standard error of the mean) for ten rats in each group, Statistical comparison: [a] C vs C+QIFE, [b] C vs D, [c] D vs D+QIFE, Statistical significance, ** $p < 0.01$

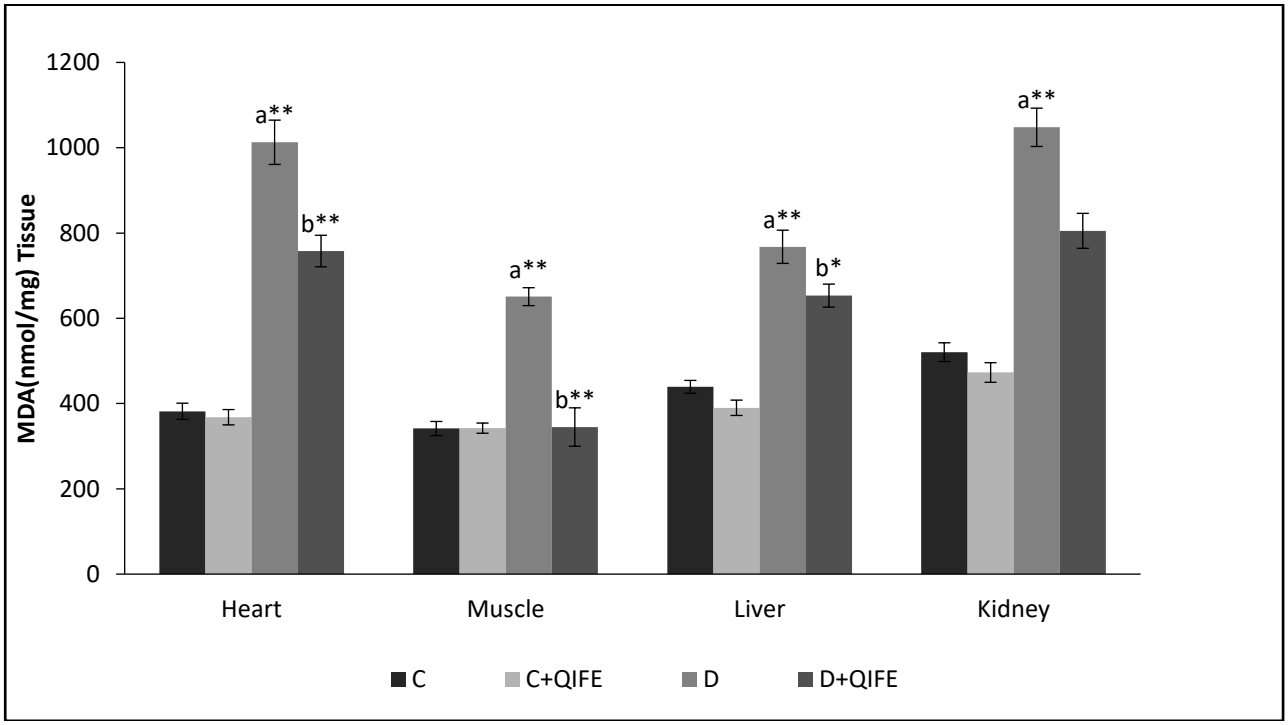

Figure 2. MDA(nmol/mg) Tissue

Values are expressed as mean ± SEM for ten rats in each group, Statistical comparison: [a] C vs C+QIFE [b] C vs D, [c] D vs D+QIFE, Statistical significance, * $p < 0.05$, ** $p < 0.01$, for abbreviations of study groups, see Figure 1.

4. Discussion and Conclusion

STZ-Nicotinamide administration is one of the commonly used methods for experimentally inducing type 2 diabetes in rats. While STZ selectively damages the insulin-secreting beta cells of the pancreas, nicotinamide (B3) partially protects the pancreatic β cells through nitric oxide-mediated mechanisms and causes moderate hyperglycemia in rats when administered together (Masiello et al.,1998; Szkudelski, 2012; Ghasemi et al., 2014). In this study, a moderate increase in fluid and food intake and a decrease in hyperglycemia, hyperlipidemia, and insulin levels in rats administered STZ-nicotinamide supported the findings of type 2 diabetes (Table 1). In addition, the increase in plasma and tissue MDA levels, SOD and GSH-Px antioxidant enzyme levels, and decrease

in PON and ARE enzyme levels in the diabetes group support research findings indicating elevated oxidative stress levels in diabetes (Sarandol et al., 2020; Bassalat et al., 2020; Tas et al., 2018; Tas et al 2024; de Gaetano et al., 2018).

Many plants are used for therapeutic purposes in diabetes and one of them is the *Quercus* species. It has been reported that phenolic acids (especially ellagic and gallic acids and their derivatives), flavonoids (especially flavan-3-ol), and tannins (Rakic et al., 2006; Taib et al., 2020; Makhlouf et al., 2019; Özcan, 2007) found in the structure of almost all *Quercus* species have therapeutic properties in diabetes (Doğan et al., 2015; Faten et al., 2022; Yin et al., 2018; Ema et al., 2020). In this study, we observed a significant decrease in serum glucose levels in the D+QIFE and C+QIFE groups, as well as a significant increase in insulin levels in the D+QIFE group. These effects may be attributed to the impact of flavonoids in *Q. ithaburensis* fruit extract on carbohydrate metabolism, when compared to the D and C groups, respectively. Because flavonoids have the ability to inhibit carbohydrate hydrolyzing enzymes such as α-amylase, α-glucosidase, and disaccharidase and/or affect insulin uptake by altering the GLUT4 mechanism (Bhandari et al., 2008; Jianwei et al., 2014; de Gaetano et al., 2018; Etxeberria et al., 2012; Anastasia et al., 2015). In addition, another reason for the decrease in blood glucose levels is the ability of flavonoids to regenerate the pancreas due to their strong antioxidant effects and this has been shown in many studies (Arora et al., 2021; Vinayagam & Xu, 2015; Matacchione et al., 2020; Attanayake et al., 2019). The increase in insulin levels in the D+QIFE group in this study confirms this. However, another important issue that we need to focus on in this study is that blood sugar levels were significantly lower in the C+QIFE group than in the control group. For this reason, QIFE extract may have a hypoglycemic effect on healthy individuals and its dose-related effects should be carefully evaluated and investigated. Also, we think that the decrease in TC levels in the C+QIFE and D+QIFE groups may be due to different action mechanisms of flavonoids in the QIFE extract such as reducing intestinal lipid absorption, bile acid chelation or inhibition of pancreatic lipase (Sun et al., 2020; Sugiyama et al., 2007; Gök et al., 2020). In addition, the hyperglycemia and hyperlipidemia we detected in the diabetes group in this study, and the changes in antioxidant enzyme levels may have contributed to the increase in plasma and tissue MDA levels in group D and these results are consistent with our previous studies (Taş et al., 2024, Taş et al., 2022; Taş et al., 2018; Bassalat et al., 2020). As it is known, one of the main targets of ROS is lipids and MDA is a product of lipid peroxidation and is one of the parameters widely used to indicate oxidative stress in the body (Tsikas, 2017; Wereski et al., 2022). Diabetes is a major risk factor for the development of atherosclerotic heart disease (Wereski et al., 2022; Katsiki, 2019; Shiyi et al.,2024). The decrease we observed in plasma and tissue MDA levels in the D+QIFE group in our study may be due to the direct antioxidant effect of the flavonoids found in *Q.ithaburensis* fruit extract as well as its antihyperglycemic and antihyperlipidemic effects as shown in this study. The importance of the antioxidant system in combating increased ROS in diabetes is very important, and antioxidant enzymes such as serum SOD and blood GSH-Px, CAT are very important in terms of their effects in preventing lipid peroxidation and the development of atherosclerosis (Chatuphonprasert et al., 2013; Sheweita et al., 2016). In this study, the increase in SOD and GSH-Px enzyme levels in the diabetic D group can be considered a response to increased oxidative stress. The increase in SOD and GSH-Px antioxidant enzyme levels in the C+QIFE group may result from the effect of *Q. ithaburensis* fruit extract on enzyme synthesis at the transcriptional level. The reason why there was no change in these enzyme levels in the D+QIFE group may have developed depending on the administration time or dose of this extract in the diabetic group.

Another antioxidant enzyme is PON 1; in our study, the decrease in PON and ARE levels in the diabetic group may be due to hyperglycemia, oxidative stress, and increased protein glycation in diabetes. It has been determined that paraoxonase binding to HDL is impaired due to changes in the regions where HDL binds to PON in diabetes (Abbott et al., 1995). When compared with this study, it is thought that the decrease in PON levels we found in our study may have developed due to this reason. PON1 is an HDL-dependent enzyme and exhibits atheroprotective properties by protecting both LDL and HDL from oxidation. In addition, ARE reflects the enzyme mass (Soran et al., 2015; Kotur-Stevuljević et al., 2020; Sun et al., 2017) and another factor that may contribute to decreased PON activity in diabetic conditions may be the decrease in ARE enzyme protein synthesis. The increase in both PON and ARE levels in the D+QIFE group that received *Q ithaburensis* fruit extract may be due to the antihyperglycemic and antihyperlipidemic properties of the QIFE extract as well as its ability to perform transcriptional enzyme synthesis as a powerful antioxidant.

Conclusion

We believe that *Quercus ithaburensis* subsp. *macrolepis* fruit extract exhibits antihyperglycemic, antihyperlipidemic, and hyperinsulinemic effects. Additionally, it significantly alleviates oxidative stress through its potent antioxidant activity as indicated by reduced levels of the lipid peroxide end product MDA (in plasma and tissues) and increased antioxidant enzyme activities. In addition, *Q. ithaburensis* fruit extract increased PON and ARE levels, which are very important in preventing the oxidation of lipoproteins, suggesting that it has a significant effect on the progression or prevention of micro-macrovascular complications in diabetes. However, another remarkable issue in this study is the significant decrease in blood sugar in the group receiving C+QIFE extract and the possibility that this may create the risk of hypoglycemia in healthy individuals. Therefore, when creating a treatment/support program, we recommend applying different dose and duration strategies well for healthy individuals and diabetic patients.

Thus, based on its strong antioxidant properties and ability to correct the impaired metabolism in diabetes, the fruit extract *Q. ithaburensis* can be considered a good phytotherapeutic agent to treat/support diabetes. However, further studies should be performed to fully understand its mechanisms of action.

Acknowledgements: This study covers the Master thesis of the first author (2018). (Bursa Uludag University Graduate School of Natural and Applied Science).

Ethics committee approval: All experimental procedures involving animal use adhered to approved ethical policies and procedures. (Bursa Uludag University, Ethics approval number: 2018-04/11).

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – S.T.; Design – S.T.; Supervision – S.T., B.Ö.; Fund – S.T., B.Ö.; Materials – S.T., B.Ö.; Data Collection and Processing – S.T., B.Ö.; Analysis Interpretation – S.T., B.Ö.; Literature Review S.T., B.Ö.; Writing – S.T., B.Ö.; Critical Review – S.T., B.Ö.

References

Abbott, C.A., Mackness, M.I., Kumar, S., Boulton, A. J., & Durrington, P.N. (1995). Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 15(11), 1812–1818. https://doi.org/10.1161/01.ATV.15.11.1812

Ahmed, S.I., Hayat, M.Q., Tahir, M., Mansoor, Q., Ismail, M., Keck, K., & Bates, R.B. (2016). Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia Angustifolia* Vahl. *BMC Complementary. Alternative Medicine*, 16, 460. https://doi.org/10.1186/s12906-016-1443-z

Ana, F.V., João, C.M.B., Anabela, S.G., Costa., & M Beatriz P.P. Oliveira (2016). A new age for *Quercus* spp. fruits: Review on nutritional and phytochemical composition and related biological activities of acorns. *Comprehensive Reviews in Food Science and Food Safety*, 15(6). https://doi.org/10.1111/1541-4337.12220

Anastasia, W.I., Sanro, T., Rizna, T.D., & Kazutaka, I. (2015). Antioxidant and α-glucosidase inhibitor activities of natural compounds isolated from *Quercus gilva Blume* leaves. *Asian Pacific Journal of Tropical Biomedicine,* 5(9), 748-755. https://doi.org/10.1016/j.apjtb.2015.07.004

Arora, S.K., Verma, P.R., Itankar, P. R., Prasad, S.K., & Nakhate, K.T. (2021). Evaluation of pancreatic regeneration activity of *Tephrosia purpurea* leaves in rats with streptozotocin-induced diabetes. *Journal of Traditional and Complementary Medicine,* 11, 435–445. https://doi.org/10.1016/j.jtcme.2020.05.008

Attanayake, A.P., Jayatilaka, K.A.P.W., & Pathirana, L.K.B. M. (2019). β-cell regenerative potential of selected herbal extracts in alloxan-induced diabetic rats. *Current Drug Discovery Technologies,* 16(3), 278–284. https://doi.org/10.2174/1570163815666180418153024

Bassalat, N., Taş, S., & Jaradat, N. (2020). *Teucrium leucocladum*: An effective tool for the treatment of hyperglycemia, hyperlipidemia, and oxidative stress in streptozotocin-induced diabetic rats. *Evidence-Based Complementary and Alternative Medicine*, 1–8. https://doi.org/10.1155/2020/1234567

Bhandari, M.R., Jong- Anurakkun N., Hong, G., & Kawabata, J. (2008). α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). *Food Chemistry*, 106(1), 247-252. https://doi.org/10.1016/j.foodchem.2007.05.077

Chatuphonprasert, W., Lao-Ong, T., & Jarukamjorn, K. (2013). Improvement of superoxide dismutase and catalase in streptozotocin-nicotinamide-induced type 2-diabetes in mice by berberine and glibenclamide. *Pharmacology and Biology,* 51(11), 1427–1433. https://doi.org/10.3109/13880209.2013.839714

de Gaetano, M., McEvoy, C., Andrews, D., Cacace, A., Hunter J., Brennan, E., & Godson, C. (2018). Specialized Pro-resolving Lipid Mediators: Modulation of Diabetes-Associated Cardio-,Reno-, and Retino-Vascular Complications. *Frontiers in pharmacology*, 9, 1488. https://doi.org/10.3389/fphar.2018.01488

Dogan, A., Celik, I., & Kaya, M.S. (2015). Antidiabetic properties of lyophilized extract of acorn (*Quercus brantii* Lindl.) on experimentally STZ-induced diabetic rats. *Journal of Ethnopharmacology,* 176, 243–251. https://doi.org/10.1016/j.jep.2015.01.031

Ema, B., Adrian, N., & Corneliu, T. (2020). A comprehensive review of phytochemistry and biological activities of *Quercus* species. Review. *Forests*, 11, 1-24. https://doi.org/10.3390/f11090904

Etxeberria, U., De La Garza, A.L., Campión, J., Martínez, J.A., & Milagro, F.I. (2012). Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. *Expert Opinion on Therapeutic Targets,* 16(3), 269–297. https://doi.org/10.1517/14728222.2012.669370

Eugene, H., Suk-Yong, J., Gyuri, K., Yong-Ho, L., Eun Yeong, C., Chung Mo, N., & Eun Seok, K. (2016). Rosiglitazone Use and the risk of bladder cancer in patients with type 2 diabetes. *Medicine (Baltimore)*, 95(6), e2786. https://doi.org/10.1097/MD.0000000000002786

Faten, M., Boutheina, S., Sondes, F., Faten, A., Lassaad Ben, S., Riadh, Ksouric., & Khaldi A. (2022). Phenolic profile and in vitro anti-diabetic activity of acorn from four African Quercus species (Q. suber, Q. canariensis, Q. coccifera and Q. ilex). *South African Journal of Botany*, 146, 771-775. https://doi.org/10.1016/j.sajb.2021.12.023

Gezici, S., & Sekeroglu, N. (2019). Neuroprotective potential and phytochemical composition of acorn fruits. *Industrial Crops and Products,* 128, 13–17. https://doi.org/10.1016/j.indcrop.2018.10.039

Ghasemi, A., Khalifi, S., & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (Review). *Acta Physiologica Hungarica,* 101(4), 408–420. https://doi.org/10.1556/APhysiol.101.2014.4.2

Gök, H.N., Deliorman Orhan, D., Gürbüz, İ., & Aslan, M. (2020). Activity-guided isolation of α-amylase, α-glucosidase, and pancreatic lipase inhibitory compounds from *Rhus coriaria* L. *Journal of Food Science,* 85(10), 3220–3228. https://doi.org/10.1111/1750-3841.15438

Huang, J., Wang, Y., Li, C., Wang, X., & He, X. (2016). Triterpenes isolated from acorns of *Quercus serrata* var. *Brevipetiolata* exert anti-inflammatory activity. *Industrial Crops and Products,* 91, 302–309. https://doi.org/10.1016/j.indcrop.2016.05.013

Hussain, S., Jan, F.G., Jan, G., Irfan, M., Musa, M., Rahman, S., …… & Ali, S. (2024). Evaluation of the Hypoglycemic and Hypolipidemic Potential of Extract Fraction of Quercus baloot Griff Seeds in Alloxan-induced Diabetic Mice *Current Pharmaceutical Design*, 30, 2978-2991. https://doi.org/10.2174/0113816128319184240827070016

Jeremiah, O.U., & Sogolo, L.L. (2020). Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: An updated review. *Oxidative Medicine and Cellular Longevity*, 3, 2020. https://doi.org/10.1155/2020/1356893

Jianwei, Z., Shan, Z., Peipei, Y., Linlin, Y., Jin, H., Lingling, S., Xiaojing, Z., Yujun, L., & Chao, M. (2014). α-Glucosidase inhibitory activity of polyphenols from the burs of *Castanea mollissima* Blume. *Molecules,* 19, 8373-8386. https://doi.org/10.3390/molecules19068373

Katsiki, N., Banach, M., & Mikhailidis, D.P. (2019). Is type 2 diabetes mellitus a coronary heart disease equivalent or not? Do not just enjoy the debate and forget the patient! *Archives of Medical Science,* 15(6), 1357–1364. https://doi.org/10.5114/aoms.2019.89618

Kotur-Stevuljević, J., Vekić, J., Stefanović, A., Zeljković, A., Ninić, A., Ivanišević, J., ... & Spasojević-Kalimanovska, V. (2020). Paraoxonase 1 and atherosclerosis-related diseases. *Biofactors,* 46(2), 193–205. https://doi.org/10.1002/biof.1549

Lin, Y., Lu, Y., Song, Z., & Huang, D. (2018). Characterizations of the endogenous starch hydrolase inhibitors in acorns of *Quercus fabri*. *Food Chemistry,* 258, 111–117. https://doi.org/10.1016/j.foodchem.2018.03.136

Makhlouf, F.Z., Squeo, G., Barkat, M., Pasqualone, A., & Caponio, F. (2019). Comparative study of total phenolic content and antioxidant properties of *Quercus* fruit: Flour and oil. *The North African Journal of Food and Nutrition Research,* 3(5), 148–155. https://doi.org/10.51745/najfnr.3.5.148-155

Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire, B.D., …. & Ribes, G. (1998). Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes,* 47(2), 224–229. https://doi.org/10.2337/diab.47.2.224

Matacchione, G., Gurău, F., Baldoni, S., Prattichizzo, F., Silvestrini, A., Giuliani, A., … & Sabbatinelli, J. (2020). Pleiotropic effects of polyphenols on glucose and lipid metabolism: Focus on clinical trials. *Ageing Research Reviews,* 61, 101074. https://doi.org/10.1016/j.arr.2020.101074

Matsuda, H., Wang, T., Managi, H., & Yoshikawa, M. (2003). Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorganic & Medicinal Chemistry, 11*, 5317–5323. http://doi.org/10.1016/j.bmc.2003.09.045

Mirza, B., Željan, M., Andrea, A., Ivana, B., & Maja, T. (2019). Antithrombotic activity of flavonoids and polyphenol-rich plant species. *Acta Pharmaceutica, 69*, 483-495. https://doi.org/10.2478/acph-2019-0050

Mithun, R.S.J., Khairnar, J.K., Abdulaziz, B.D., Mohammad, A.A, Mohammad, N.A., Fahad, M.A., … & Rajlakshmi, D. (2022). Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects. Antioxidant Potentials and

Mechanism(s) of Action. *Frontiers in Pharmacology*, 13, https://doi.org/10.3389/fphar.2022.806470

Neumann, A., Weill, A., Ricordeau, P., Fagot, J. P., All, F., & Allemand, H. (2012). Pioglitazone and risk of bladder cancer among diabetic patients in France: A population-based cohort study. *Diabetologia,* 55(7), 1953–1962. https://doi.org/10.1007/s00125-012-2538-9

Ngan, T., Bao, P., & Ly, L. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology,* 9(9), 252. https://doi.org/10.3390/biology9090252

Nissen, S. E., & Wolski, K. (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *The New England Journal of Medicine,* 356(24), 2457–2471. https://doi.org/10.1056/NEJMoa072761

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry,* 95(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Özcan, T. (2007). Characterization of Turkish *Quercus* L. taxa based on fatty acid compositions of the acorns. *Journal of the American Oil Chemists' Society,* 84(7), 653–662. https://doi.org/10.1007/s11746-007-1087-8

Özcan, T., & Baycu, G. (2005). Some elemental concentrations in the acorns of Turkish *Quercus* L. (Fagaceae) taxa. *Pakistan Journal of Botany*, 37(2), 361–371.

Özcan, T. (2006). Total protein and amino acid compositions in the acorns of Turkish *Quercus* L. taxa. *Genetic Resources and Crop Evolution*, 53(3), 419–429. https://doi.org/10.1007/s10722-004-1337-7

Saini, R., & PatilI, S.M. (2012). Anti-Diabetic Activity of Roots of Quercus Infectoria Olivier in Alloxan Induced Diabetic Rats. *International Journal Of Pharmaceutical Sciences And Research,* 3(4),1318-1321 ISSN: 0975-8232

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry,* 97, 55–74. https://doi.org/10.1016/j.ejmech.2015.04.040

Rakic, S., Povrenovic, D., Tešević, V., Simić, M., & Maletić, R. (2006). Oak acorn, polyphenols and antioxidant activity in functional food. *Journal of Food Engineering,* 74(3), 416–423. https://doi.org/10.1016/j.jfoodeng.2005.03.057

Ralph, A. D. F., Ele, F., Leif, G., Robert, R. H., William, H. H., Jens, J., … Ram, W. (2015). Type 2 diabetes mellitus. *Nature Reviews Disease Primers,* 1, 15019. https://doi.org/10.1038/nrdp.2015.19

Ramesh, C.G., Dennis, C., Srinivas, N., Alan, B., Kellie, B., & Basil, D.R. (2017). Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetology & Metabolic Syndrome,* 9(59). https://doi.org/10.1186/s13098-017-0254-9

Saini, R., & PatilI, S.M. (2012). Anti-Diabetic Activity of Roots of Quercus Infectoria Olivier in Alloxan Induced Diabetic Rats. *International Journal Of Pharmaceutical Sciences And Research,* 3(4),1318-1321. ISSN: 0975-8232.

Sarandol, E., Taş, S., Serdar, Z., & Dirican, M. (2020). Effects of thiamine treatment on oxidative stress in experimental diabetes. *Bratislava Medical Journal,* 121(3), 235–241. https://doi.org/10.4149/BLL_2020_036

Sen, S., Chakraborty R., Sridhar C., Reddy Y.S.R., & De B. (2010). Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research,* 3(1), 91-100. ISSN 0976 – 044X

Sheweita, S.A., Mashaly, S., Newairy, A.A., Abdou, H.M., & Eweda, S.M. (2016). Changes in oxidative stress and antioxidant enzyme activities in streptozotocin-induced diabetes mellitus in rats: Role of *Alhagi maurorum* extracts. *Oxidative Medicine and Cellular Longevity,* 2016, 5264064. https://doi.org/10.1155/2016/5264064

Shiyi, T., Lintong, Yu., Jun, L., Li, H., Tiantian, X., Deshuang, Y.,…… & Chao M. (2024). Multiple triglyceride-derived metabolic indices and incident cardiovascular outcomes in patients with type 2 diabetes and coronary heart disease. *Cardiovascular Diabetology*, 23, 359. https://doi.org/10.1186/s12933-024-02446-1

Shweta, P., Aleena M., Hinal, C., Shiv, K., & Rahul, M. (2021). Classifications of polyphenols and their potential application in human health and diseases. *International Journal of Physiology, Nutrition and Physical Education*, 6(1), 293-301. https://doi.org/10.22271/journalofsport.2021.v6.i1e.2236

Soran, H., Schofield, J.D., & Durrington, P.N. (2015). Antioxidant properties of HDL. *Frontiers in Pharmacology,* 6, 222. https://doi.org/10.3389/fphar.2015.00222

Sugiyama, H., Akazome, Y., Shoji, T., Yamaguchi, A., Yasue, M., Kanda, T., & Ohtake, Y. (2007). Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *Journal of Agricultural and Food Chemistry,* 55(11), 4604–4609. https://doi.org/10.1021/jf070569k

Sun, C., Wang, L., Sun, J., Wang, Z., & Tang, Z. (2020). Hypoglycemic and hypolipidemic effects of rutin on hyperglycemic rats. *Journal of Traditional Chinese Medicine,* 40(4), 640–645. https://doi.org/10.19852/j.cnki.jtcm.2020.04.012

Sun, T., Hu, J., Yin, Z., Xu, Z., Zhang, L., Fa, L., & Li, Y. (2017). Low serum paraoxonase1 activity levels predict coronary artery disease severity. *Oncotarget,* 8(12), 19443–19456. https://doi.org/10.18632/oncotarget.15525

Surapon, T. (2015). Oxidative stress, insulin resistance, dyslipidemia, and type 2 diabetes mellitus. *World Journal of Diabetes*, 6(3), 456–480. https://doi.org/10.4239/wjd.v6.i3.456

Szkudelski, T. (2012). Streptozotocin–nicotinamide-induced diabetes in the rat: Characteristics of the experimental model. *Experimental Biology and Medicine,* 237(5), 481–490. https://doi.org/10.1258/ebm.2012.011372

Taib, M., Rezzak, Y., Bouyazza, L., & Lyoussi, B. (2020). Medicinal uses, phytochemistry, and pharmacological activities of *Quercus* species. *Evidence-Based Complementary and Alternative Medicine*, https://doi.org/10.1155/2020/1920683

Tarique, H., Bie, T., Ghulam, M., Gang, L., Najma, R., Muhammad, S.K., ….& Yulong, Y. (2020). Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. *Pharmacological Research*, 152, 104629. https://doi.org/10.1016/j.phrs.2020.104629

Taş, S., Sarandöl, E., & Dirican, M. (2014). Vitamin B6 supplementation improves oxidative stress and enhances serum paraoxonase/arylesterase activities in streptozotocin-induced diabetic rats. *Scientific World Journal,* 2014, 351598. http://doi.org/10.1155/2014/351598

Taş, S., Taş, B., Bassalat, N., & Jaradat, N. (2018). In-vivo, hypoglycemic, hypolipidemic and oxidative stress inhibitory activities of *Myrtus communis* L. fruits hydroalcoholic extract in normoglycemic and streptozotocin-induced diabetic rats. *Biomedical Research,* 29, 2727–2734. http://doi.org/10.4066/biomedicalresearch.29-18-708

Taş, S., Sarandöl, E., Tekin, C.N., Tosunoğlu, A., Vatan, Ö., Hürriyet, H., … & Dirican, M. (2022). Monofloral *Brassica nigra* pollen improves oxidative stress and metabolic parameters in streptozotocin-induced diabetic rats. *Journal of Applied Biological Sciences,* 16(3), 563–574. https://doi.org/10.5281/zenodo.7114334

Taş, S; Tosunoğlu, A; Dirican, m., Tekin, C. N., & Sarandöl, E. (2024). Ameliorative effects of monofloral *Cistus creticus* bee pollen on the oxidant-antioxidant systems in streptozotocin-induced diabetic rats. *Palestinian Medical and Pharmaceutical Journal*, 9(3), 337-346. https://doi.org/10.59049/2790-0231.1215

Tsikas, D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical Biochemistry,* 524, 13–30. https://doi.org/10.1016/j.ab.2016.10.021

Vinayagam, R., & Xu, B. (2015). Antidiabetic properties of dietary flavonoids: A cellular mechanism. *Nutrition & Metabolism,* 12, 60. https://doi.org/10.1186/s12986-015-0042-5

Vinha, A., Barreira, J., Ferreira, I., & Oliveira, M. (2019). Therapeutic, phytochemistry, and pharmacology of acorns (Quercus nuts): A review. *In Bioactive Compounds in Underutilized Fruits and Nuts*, pp. 1–15.

Wereski, R., Kimenai, D.M., Bularga, A., Taggart, C., Lowe, D.J., Mills, N.L., & Chapman, A. R. (2022). Risk factors for type 1 and type 2 myocardial infarction. *European Heart Journal,* 43(2), 127–135. https://doi.org/10.1093/eurheartj/ehab581

Yin, P., Wang, Y., Yang, L., Sui, J., & Liu, Y. (2018). Hypoglycemic effects in alloxan-induced diabetic rats of the phenolic extract from Mongolian oak cups enriched in ellagic acid, kaempferol and their derivatives. *Molecules,* 23(5), 1046. https://doi.org/10.3390/molecules23051046

Young, I., & Trimble, E. (1991). Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorimetric detection. *Annals of Clinical Biochemistry,* 57(3), 318–325. https://doi.org/10.1177/0004563220911639

Zanza, C., Thangathurai J., Audo, A., Muir, H.A., Candelli, M., Pignataro, G., Thangathurai D., Cicchinelli, S., & Franceschi, F. (2019). Oxidative stress in critical care and vitamins supplement therapy: "a beneficial care enhancing" *European Review for Medical and Pharmacological Sciences*, 23(17), 7703-7712. https://doi.org/10.26355/eurrev_201909_18894

Zehra, B., Ahmed, A., Sarwar, R., Khan, A., Farooq, U., Ali, S.A., & Al-Harrasi, A. (2019). Apoptotic and antimetastatic activities of betulin isolated from *Quercus incana* against non-small cell lung cancer cells. *Cancer Management and Research,* 11, 1667–1683. https://doi.org/10.2147/CMAR.S186956