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Origanum majorana L. extract effects on kidney tissue in STZ-induced DM rats

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ABSTRACT: Diabetes mellitus is a chronic disease characterized by decreased insulin synthesis or increased blood glucose due to insulin resistance. In this study, we aimed to investigate the protective role of *Origanum majorana* L. on the biochemical parameters of rats induced by streptozotocin (STZ). Male Sprague-Dawley (SD) rats weighing 300-400 g were used. Rats were divided into 3 groups control, diabetes mellitus, and diabetes mellitus + *Origanum majorana* L. Citrate buffer was used i.p. in the control group. To induce experimental diabetes in rats, streptozotocin (60 mg/kg) was injected intraperitoneally (i.p.). After 48 hours, rats with blood glucose values higher than 200 mg/dL were considered diabetic. Diabetic rats were divided into two groups. While the first group was not treated, the second group received 200 mg/kg of *Origanum majorana* L. extract for 6 weeks. In this study, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and creatinine values were measured in serum. Malondialdehyde (MDA) and glutathione (GSH) levels were measured in kidney and liver tissues. It was found that AST, ALT, and creatinine values increased in diabetes and decreased significantly in the treatment group. MDA levels were increased and GSH values were decreased in diabetes. *Origanum majorana* L. treatment decreased lipid peroxidation and resulted in a significant increase in GSH levels. In conclusion, our findings show that *Origanum majorana* L. treatment has a protective effect against nephropathy in diabetes by preventing oxidative damage.

KEYWORDS: Diabetes mellitus; liver; kidney; *Origanum majorana* L.; oxidative damage.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most severe metabolic disorders with chronic hyperglycemia, insulin resistance, and defects in carbohydrates, lipids, and protein metabolism [1]. DM affects more than 2 million patients around the world [2]. Coronary heart disease, stroke, and peripheral arterial disease are among the macrovascular complications associated with DM. Also, diabetic kidney disease, retinopathy, and peripheral neuropathy are among the microvascular complications associated with DM. As individuals with DM live longer, they become susceptible to several other complications, and the risk of developing the forenamed complications also increases [3]. According to the International Diabetes Federation (IDF), the prevalence of DM (diagnosed or undiagnosed) has been estimated that 536.6 million people in 2021, and this number is forecasted to increase by 46%, which means approximately 783.2 million people in 2045 [4]. Hyperglycemia causes repeated acute changes in intracellular metabolism (activation of the polyol pathway, activation of diacylglycerol-protein kinase C, increased oxidative stress), and cumulative long-term changes in the structure and function of macromolecules, through the formation of advanced glycation end-products [5].

Oxidative stress plays a key role in developing different metabolic diseases such as DM, Alzheimer's Disease, cancer, and renal diseases. Hyperglycemia induces the overproduction of various reactive oxygen species (ROS) leading to tissue damage through different signaling pathways. ROS can lead to oxidative damage to macromolecules such as lipids, proteins, and DNA [6]. In this context, the increase in the formation

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of free oxygen radicals triggers oxidant damage and, is responsible for the development of many diabetic complications including cardiomyopathy, retinopathy, nephropathy, and erectile dysfunction [7].

Over the last few years, researchers have focused on investigating the pharmacological analysis of Turkish traditional medicinal plants, especially anti-diabetic plants. *Origanum majorana* L. (OM) belongs to the Lamiaceae family and is a self-supporting growth habit medical plant. In traditional medicine, OM was used for anti-cooling, against allergies, fever, flu, hypertension, antidiabetic, and headache. OM is rich in phytochemicals such as carvacrol, arbutin, vitexin, sitosterol, limonene, and apigenin [8]. The OM shows its antidiabetic activity by increasing plasma insulin levels, stimulating hepatic glycogen synthesis and glucokinase activity. In addition, OM methanolic extract is safe in toxicological studies and hepatoprotective, antimutagenic, and gastrointestinal effects have been reported [8]. The literature contains studies on many Origanum species. But experimental DM studies on OM are limited. Few studies were found on the protective effects of OM in a different model of DM. However, there are no studies on the impact of OM in rat kidneys with DM [9]. This study revealed the effect of OM on nephropathy, one of the complications of DM, for the first time in the literature.

This study aimed to investigate the pharmacological effects of OM on blood glucose levels in both normal and STZ-induced diabetic rats. Also for the first time, the effect of OM on diabetic complications such as diabetic nephropathy was evaluated by serum creatinine and kidney MDA/GSH levels. Also, ALT, AST, and creatinine levels of serum were measured using the ELISA method.

2. RESULTS

2.1. *Origanum majorana* L. maintained body weight and normoglycemia

Figure 1 is shown the effects of OM on body weight and blood glucose level. Although animals of similar body weights were used for the experiment STZ induced DM was associated with significant weight loss and a rise in blood glucose level (Fig. 1). At the end of the study the body weight of the OM group significantly increased compared to DM group (Fig. 1a; ****p<0.0001). Moreover, OM restored the body weights of rats to values comparable to those of the C group (Fig. 1a). As compared to the C group, the blood glucose level was markedly elevated in DM rats (Fig. 1b). Although blood glucose levels did not decrease to group C levels in rats given OM, they decreased significantly compared to the DM group (Fig. 1b; *p<0.05). Consequently, therapy with OM maintained body weight and led to the maintenance of blood glucose levels in the DM.

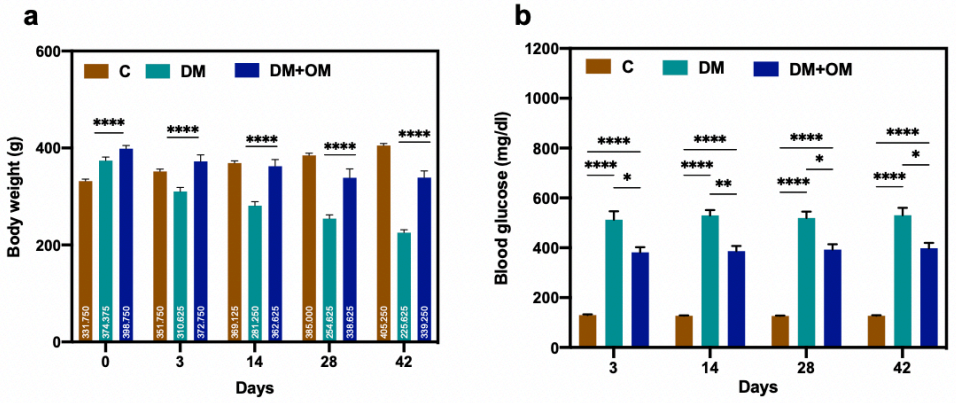

Figure 1. Effect of *Origanum majorana* L. on (a) body weight and (b) blood glucose levels of diabetic animals. The value of each group (n=6) was given as mean ± standard error. Data analysis was performed using two-way ANOVA and Bonferroni test. C=Healthy control; DM= Diabetes mellitus; DM+OM = Diabetic rats treated with *Origanum majorana* L. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to every other group.

2.2. *Origanum majorana* L. improves ALT, AST, and creatinine serum profile

Figure 2 is shown the effects of OM on plasma levels of ALT, AST, and creatinine. As compared to the C group, there was a significant increase in ALT and AST in animals in that DM group (Fig. 2a and 2b). In the OM-treated group compared to the DM group ALT and AST levels were significantly decreased (Fig. 2a and 2b; ***p<0.001). In order to test the renal function we measured plasma levels of creatinine. As compared to the C group, there was a significant increase in creatinine in animals in that DM group (Fig. 2c). However OM group compared to the DM group creatinine levels were significantly decreased (Fig. 2c; ***p<0.001).

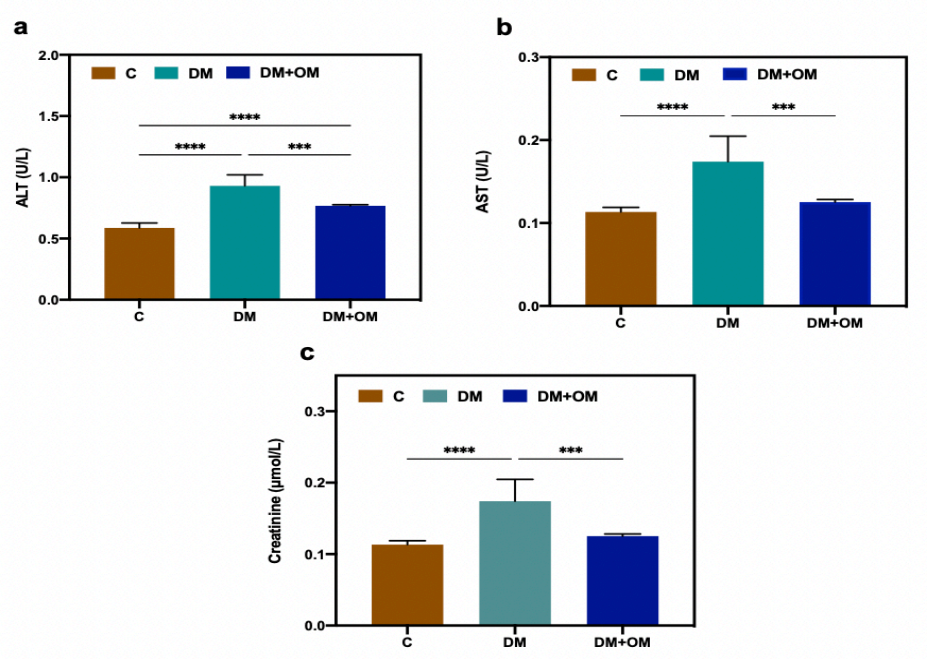

Figure 2. Serum biochemical parameters. Levels of (a) ALT, (b) AST, and (c) Creatinine. The value of each group (n=6) was given as mean ± standard error. Data analysis was performed using one-way ANOVA and Tukey's test. C=Healthy control; DM= Diabetes mellitus; DM+OM = Diabetic rats treated with *Origanum majorana* L. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to every other group.

2.3. *Origanum majorana* L. effects on MDA and GSH

Figure 3 is shown the effects of OM on kidney levels of MDA and GSH. MDA levels, which is a marker of oxidative damage in the DM group, were significantly increased in kidney tissue compared to group C (Fig. 3a; ****p<0.0001). In contrast, MDA levels were significantly reduced in the OM-treated group (Fig. 3a; ***p<0.001). While GSH levels decreased in the DM group compared to group C, they increased significantly in the OM group compared to DM (Fig. 3b; ***p<0.001). These results suggest that OM is protective against oxidative damage in DM.

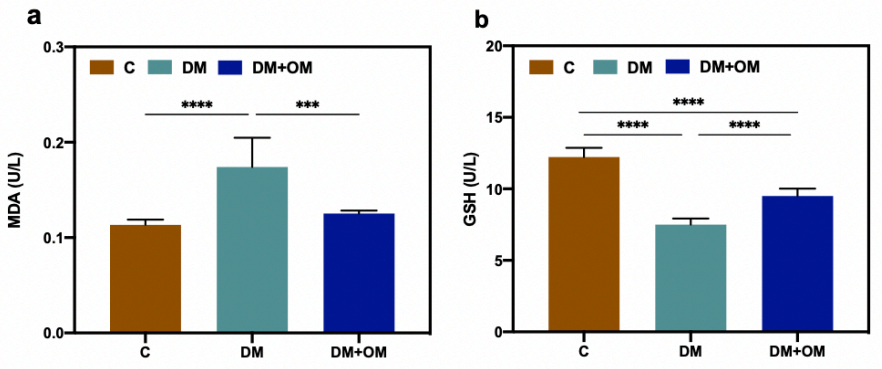

Figure 3. Effect of *Origanum majorana* L. on (a) MDA and (b) GSH in kidney levels. The value of each group (n=6) was given as mean ± standard error. Data analysis was performed using one-way ANOVA and Tukey's test. C=Healthy control; DM= Diabetic control; DM+OM = Diabetic rats treated with *Origanum majorana* L. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to every other group.

3. DISCUSSION

DM is a metabolic disorder characterized by lipoprotein abnormalities, hyperglycemia, disruption of reactive oxygen species-scavenging enzymes, and oxidative stress [10]. Uncontrolled DM in time causes microvascular and macrovascular complications (i.e. cardiovascular disease, nephropathy, neuropathy, and

retinopathy) and end-organ damage such as wound healing disorder [10, 11]. Hyperglycemia-characterized DM can cause reactive oxygen species (ROS) so DM complications occur by oxidative stress [11].

Numerous natural products are researched as preventive and therapeutic against DM complications [11]. Previous studies are shown that OM exists in different pharmacological activities. OM oils and extracts have anti-inflammatory, hepatoprotective, antioxidant, antidiabetic, and nephroprotective. For all these reasons it was used in traditional medicine. In addition, OM contains active bioactive compounds such as flavonoids, terpenes, and phenolic acid. When evaluated in terms of toxicology, it was found to be harmless and safe for medicinal use [8]. It is known that OM has therapeutic efficacy in DM and its complications, but there are unclear regarding its effect on DM [10].

The findings of this study are consistent with the effects in the literature that OM lowers MDA levels and increases GSH levels in the kidney tissue, lowers blood glucose, and decreases serum ALT, AST, and Creatinine levels. As has been well described, OM possesses a wide variety of pharmacological activities. Furthermore, it has been shown to exert protective effects against the advancement of DM by ameliorating a variety of pathogenic alterations.

In the our study, assessed the effects of OM in rats with STZ-induced DM. Our findings showed that OM significantly decreased blood glucose levels and increased body weight. Consistent with previous studies, in our study, blood glucose in rats treated with OM showed a significant decrease compared to the DM group [9]. One of the roles of OM in the treatment of DM is to lower high blood glucose levels.

As an indicator of hepatic function, serum ALT and AST enzyme levels are measured and used to assess the extent of liver damage. In DM, ALT and AST levels increase and leak from the liver cytosol into the bloodstream and become a marker of damage [13, 14]. In this study, serum ALT and AST levels were elevated in STZ-induced DM rats but significantly decreased in OM-treated rats. Previous studies have also shown that OM reduces ALT and AST levels [15].

In the present study, MDA and GSH in the kidney tissue and creatinine levels in the serum were examined as markers of renal function. Endogenous antioxidants such as catalase (CAT), superoxide dismutase (SOD), and GSH scavenge free radicals and thus convert them into harmless products [16]. The antioxidant defense system, which consists of GSH, CAT, and SOD, represents the severity of oxidative stress generated by STZ-induced hyperglycemia [17]. OM treatment significantly raised the renal antioxidant molecule GSH compared to the DM group. The present finding is consistent with the antioxidant effect of OM in the literature [16]. Also, hyperglycemia following STZ administration led to the production of ROS in the DM group, there was an increased level of MDA. In previous studies, it has been proven that OM has a lowering effect on MDA levels in kidney tissue in STZ-induced rats [18].

Urea, uric acid, blood urea nitrogen (BUN), and creatinine are serum kidney biomarkers used in the evaluation of kidney damage. In the present study, serum creatinine levels are assessed. Other investigations show decreased serum creatinine levels with treatment OM. In the present study, there was a significant increase in the serum creatinine of DM group rats compared to group C. Serum creatinine levels were significantly decreased in the group treated with OM compared to the DM group. In previous studies, the effect of OM on serum creatinine levels is consistent with our study. All these results suggest that OM is a protective molecule against kidney damage [19, 20].

Together with these findings, our study suggests that OM is a significant product in the DM with antioxidant, hepatoprotective, and nephroprotective effects. More studies are needed to understand the pathways related to the nephroprotective and hepatoprotective activity of OM in DM.

4. CONCLUSION

When we evaluate these results, we concluded that OM can be added to the treatment of diabetic nephropathy, which is one of the important complications of DM, by scavenging free radicals due to its antioxidant effect. In addition, we believe that OM can be used as an alternative to many synthetic drugs with side effects for protection against oxidative damage, instead of food supplements and traditional drugs, and it may be beneficial to add it to the treatment.

5. MATERIALS AND METHODS

5.1. Preparation of herbal formulation

The air-dried aerial parts of the endemic *Origanum majorana* L. (OM), whose protective effects we investigated in DM, were left to maceration with MeOH (methyl alcohol) at room temperature. The solvent

was evaporated in a rotary evaporator at a temperature not exceeding 50°C, and the remaining non-volatile solvent was dried on the hot plate. The OM extract was prepared and standardized by Turgut Taskın from the Marmara University Faculty of Pharmacy, Pharmacognosy Department. The extracts were stored at +4°C to prevent deterioration.

5.2. Animals and experimental outline

All animals were maintained at 50% humidity, a constant temperature (25±2°C), and were subjected to a 12 h light/dark light cycle with fed ad libitum with standard rat chow and tap water. The study was approved by Marmara University Animal Care and Use Committee and animals (021.2016.mar) were obtained from Marmara University Experimental Animal Center as male (300-400 g).

The animals fasted the day before DM induction. Streptozotocin (STZ) injected via i.p. at a dose of 60 mg/kg (dissolved in 0.1 M citrate buffer, pH adjusted to 4.5) was administered to rats to induce diabetes. STZ was dissolved in 0.1 M fresh citrate buffer, pH 4.5. Blood samples were taken by the tail vein after 48 hours of administrating STZ injection and blood glucose levels were measured with a glucometer. Fasting blood glucose levels greater than 200 mg/dL were considered diabetic.

Rats (n=18) were equally divided into three groups: diabetic (DM), control (C), and *Origanum majorana* L.-treated diabetic rats (OM). The control group (n=6) received 0.1 M citrate buffer intraperitoneally (i.p.) for six weeks. In the DM group, rats whose blood glucose level was above 200 mg/dl after 48 hours of STZ administration (i.p.), were given 10 ml/kg saline per-orally (p.o.) daily for six weeks. In the OM group (n=6), rats whose blood glucose level was above 200 mg/dl 48 hours after STZ administration, were given 200 mg/kg Origanum extract dissolved in saline p.o. daily for 6 weeks. Hyperglycemia was confirmed by measuring blood glucose levels using a glucometer 48 h after STZ administration. Rats with blood glucose levels of 200 mg/dl were considered diabetic. After six weeks, blood samples were collected into a non-heparinized tube and were centrifuged at 3000 rpm for 5 min. Serum was collected and stored frozen until needed for biochemical assay, also kidney and liver tissues were removed. Part of the tissues was stored at -80°C for the measurement of glutathione activity and lipid peroxidation (malondialdehyde levels). Malondialdehyde (MDA) and glutathione (GSH) measurements were made in kidney tissue samples to examine the oxidant damage. In serum samples, creatinine was measured to examine kidney functions, while ALT and AST were measured to test liver functions.

5.3. Biochemical assays

5.3.1. Malondialdehyde and glutathione analysis

MDA and glutathione GSH determinations were made to examine oxidant damage in liver and kidney tissues. MDA was determined by the Buege method. GSH determination was made by the Ellman method. A fasting blood glucose value higher than 200 mg/dL (11.1 mmol/L) were considered as diabetic.

5.3.2. ELISA analysis

Biochemical analyzes were performed from blood samples taken from the heart under light ether anesthesia and from kidney and liver tissues after decapitation. Serum levels of creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using ELISA (Enzyme Linked Immunoobbent Assay) kits to examine liver function and to idintify liver tissue damage.

5.4. Statistical analysis

All data of this study are expressed as mean ± S.E.M. P-value <0.05 is considered statistically significant. Values and calculations were performed using the computer statistical program Graphpad prism 9.0 (GraphPad Software, San Diego; CA; USA). Statistical analysis was performed using one-way or two-way analysis of variance (ANOVA) followed by Tukey and Bonferroni tests.

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Conflict of interest statement: The authors declared no conflict of interest.

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