Original Article

Effects of Aqueous Extract of*Myrtus Communis* L. Leaves on Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Background: Leaves, oil and fruit of *Myrtus communis* L. (MC) have therapeutic effects on diabetes mellitus (DM). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are liver enzymes associated with hepatic injury caused by DM.

Aims: In this study, we aimed to investigate the antidiabetic and antioxidant effects of the aqueous extract of MC leaves on normal and diabetic rats induced with streptozotocin (STZ).

Material &Methods: A total of thirty rats divided into six groups as each composed of five rats were used. DM was induced by a single 40 mg/kg dose of STZ in diabetic control group (Group II) and DM groups (Group IV,V and VI). Three different doses (150, 300 and 600 mg/kg) of aqueous extract of MC leaves were administered to the DM groups for 14 days. Serum samples and liver homogenates were obtainedin order to determine serum glucose levels and superoxide dismutase (SOD) activity, glutathione (GSH) and malondialdehyde levels (MDA), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) levels. Serum glucose levels were determined by a commercial glucose monitor with disposable dry reagent strips. SerumSOD activity, GSH and MDA levels were measured by commercial ELISA kits. Serum ALT, ALP, and AST levels were measured by biochemical and immune-enzyme analyzers with respective standard kits.

Results: Serum glucose, AST, ALT and ALP levels were reduced by MC administration in all diabetic groups. MC administration provided significance increment in SOD activity and GSH level, and significant reduction in MDA levels compared to controls ($p<0.05$) in all diabetic groups for all parameters, being highest at the dose of 600 mg/kg ($p<0.001$).

Conclusion: Aqueous extracts of MC leaves at the doses of 150, 300 and 600 mg/kg decreased blood glucose, serum ALT, AST and ALP levels. Besides, all extracts have antioxidant effects being highest at 600 mg/kg dose.

Key Words: *Myrtus communis* L., leaves, diabetes mellitus, antioxidant

INTRODUCTION

There is growing interest on herbal medicines in order to prevent disease symptoms and provide benefits additionally to conventional therapy [1-3].

All leaves, fruits, brunches, flowersand volatile oil of *Myrtus communis* L. (MC) have been traditionally widely used for the treatment of various diseases in Mediterranean regionand Turkey [4].In-vivo and in-vitro studies have demonstrated anti-inflammatory, anti-diabetic, anti-mutagenic, pro-apoptotic, cardiovascular, anti-atherogenicity, against hepatic ischemia, anti-ulcer, insecticidal, molluscicidal, protoicidal and some other pharmacological effects of MC [5].

The present study aims to investigate the antidiabetic and antioxidant effects of the aqueous extract of MC leaves on the diabetic rats induced with streptozotocin (STZ).

METHODS

Animals

A total of thirty 6-week-old male Albino Wistar rats, obtained from the Medical Experimental Research Centre, Ataturk University, Erzurum, Turkey

weighing between 180 and 200 g, were used for this study. Animals were fed under normal conditions (22^{o}C) in separate groups. All experiments were performed in the same laboratory under the same standard conditions. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the Ethics Committee of the Experimental Animal Teaching and Researcher Center of Ataturk University, Erzurum, Turkey.

Plant material

The aerial parts ofMC were collected from Antalya, a city located in the Mediterranean region of Turkey, in August 2012.The powdered leaves of herb was percolated in pure water. The solvent was filtered and evaporated under vacuum using a rotary evaporator. The obtained water extract was stored after lyophilization.

Experimental Design

Thirty Albino Wistar male rats were randomly divided into six groups of five animals each: Group I = control (healthy control); group II = DM control (diabetic control); group III = 600 mg/kg MC (600 mg/kg MC administrated healthy rats); group IV =DM+150 mg/kg MC (diabetic rats which were administrated 150 mg/kg MC); group V = DM+300 mg/kg MC; (diabetic rats which were administrated 300 mg/kg MC); group VI = DM+600 mg/kg MC (diabetic rats which were administrated 600 mg/kg MC).Type II Diabetes mellitus was induced by a single 40 mg/kg dose of STZwith intraperitoneal injection.Aqueous extract of MC leaves was administered orally to the groups 150, 300 and 600 mg/kg (I, II and III doses, respectively) for 14 days, starting the third day of STZ administration. Animals were sacrificed and the livers were rapidly removed to determine antioxidant levels in tissue homogenate.

Rat livers were kept in -80 °C for 3 days to determine tissue superoxide dismutase (SOD) enzyme activity and total glutathione (GSH) and malondialdehyde (MDA) levels. To prepare the tissue homogenates, the liver tissues were ground with liquid nitrogen in a mortar; 0.1 g was weighed and then treated with 4.5 ml of an appropriate buffer. This mixture was homogenized on ice using an Ultra-Turrax homogenizer for 5 min. Homogenates were filtered and centrifuged by using a refrigerator centrifuge at 4 ° C. These supernatants were then used to determine SOD and MDA levels with highly sensitive ELISA kits (Cayman Chemical, Cell Biolabs OxiSelect™ TBARS Assay STA-330 Kit, respectively). Kits were specifically designed for rat cytokines, and all measurements were performed according to the manufacturers' instructions. Cytokine assays for each animal and its correlated control were run in the same lot. All assays were carried out at room temperature in dublicate.

The GSH levels in the kidney tissues were measured with the method created by Sedlak and Lindsay [6]. For this assay, the liver tissue homogenized in 2 mL of 50mM Tris-HCl buffer containing 20mM EDTA and 0.2M sucrose, pH 7.5. The homogenate was centrifuged at 4200 rpm for 40 min at 4 °C, and then the supernatant was used to determine GSH using5,5-dithiobis(2-nitrobenzoic acid). Absorbance was measured by spectrophotometric method at 412 nm.

The activity of SOD and the levels of GSH and MDA in the tissues were expressed as mmol/min/mg tissue, nmol/mg tissue and nmol /g tissue, respectively.

Biochemical Investigation of Serum

Blood glucose levels were measured by Accu-Chek Active blood glucose monitor with disposable dry reagent strips which is suitable for glucose oxidase technique. Serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) levels were measured by biochemical and immune-enzyme analyzer GBG ChemWell 2990 (USA) with respective standard kits to determine possible hepatic injury.

Chemicals

Streptozotocin ($C_8H_{15}N_3O_7$) (STZ) and all other chemicals were purchased from Sigma Chemical Co. (Germany).

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of five animals per group. Differences among the groups were determined by One-Way Anova test followed by Fisher's least significant difference (LSD) Post-hoc test and *p* values less than 0.05 was considered significant, using SPSS 20.0 software.

RESULTS

The aqueous extract of MC at the dose of 600 mg/kg provided significant decrease in blood glucose levels ($p<0.001$) (Table 1).

MC administration reduced serum AST, ALT and ALP levels in all diabetic groups (Table 2).

SOD activity and GSH level were found to be increased and MDA level were found to be

significantly decreased in MC administration groups compared to control group ($p<0.05$). This antioxidant activity was dose dependent and found to be highest at the 600 mg/kgdose of MC aqueous extract. MDA and GSH levels and SOD activity measured by administration of MC at the dose of 600 mg/kg were similar to the levels in the normal control group (Table 3).

Table 1: Effects of MC administration on blood glucose levels in diabetic rats

Groups	Blood glucose level before MC administration (mg/dL)	Blood glucose level after MC administration (mg/dL)
Control (n=5)	80,20±4,43	81,00±4,79†
Diabetic control (n=5)	262,60±10,35*	459,40±17,81
MC 600 mg/kg (n=5)	89,20±9,28	88,40±8,29†
DM+ MC 150 mg/kg (n=5)	250,00±8,86*	232,80±9,33†
DM+ MC 300 mg/kg (n=5)	260,40±9,81*	217,40±8,87†
DM+ MC 600 mg/kg (n=5)	264,2±11,34*	161,00±8,91†

Results are means±SD. MC: Myrtus communis L aqueous extract, DM: Rats with diabetes mellitus (Diabetes is induced only in diabetic control and DM groups), comparisons: *control *vs* other groups *: $p<0.001$, †diabetic control *vs* other groups †: $p<0.001$.

Table 2: Effects of *Myrtus communis* L. administration on serum ALT, AST and ALP levels in diabetic rats

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control (n=5)	28,43±1,55†	69,86±5,85†	93,73±6,55†
Diabetic control (n=5)	44,75±0,48	98,85±8,31	151,31±10.10
MC 600 mg/kg (n=5)	25,74±0,92†	45,03±6,43†	80,10±4,44†
DM+ MC 150 mg/kg (n=5)	42,18±1,90*	88,16±0,91*	141,53±5,99*
DM+ MC 300 mg/kg (n=5)	36,41±2,14†	82,84±4,59*	132,38±7,43†
DM+ MC 600 mg/kg (n=5)	29,21±1,57†	72,42±15,10†	106,23±5,45†

Results are means±SD. MC: Myrtus communis L aqueous extract, DM: Rats with diabetes mellitus, (Diabetes is induced only in diabetic control and DM groups), comparisons: diabetic control group *vs* other groups, *:$p<0.05$, †: $p<0.001$.

Table 3: Effects of *Myrtus communis* L. administration on liver tissue SOD activity and GSH, MDA levels in diabetic rats

Groups	SOD (U/mg tissue)	GSH (µM/mg tissue)	MDA (µM/mg tissue)
Control (n=5)	0,248±0,015 †	115,303±7,501 †	6,115±1,168 †
Diabetic control (n=5)	0,132±0,005	84,964±6,832	11,119±1,133
MC 600 mg/kg (n=5)	0,250±0,016 †	116,782±5,439 †	7,123±1,525 †
DM+ MC 150 mg/kg (n=5)	0,121±0,012	94,440±8,756*	9,579±0,737*
DM+ MC 300 mg/kg (n=5)	0,176±0.009 †	120,166±5,519 †	9,149±0,685*
DM+ MC 600 mg/kg (n=5)	0,204±0,012 †	126,613±7,175 †	8,294±1,267 †

Results are means±SD, MC: *Myrtus communis* L aqueous extract, DM: Rats with diabetes mellitus (Diabetes is induced only in diabetic control and DM groups), comparisons: diabetic control group *vs* other groups, *:$p<0.05$, †: $p<0.001$. Diabetes is induced only in diabetic control and DM groups.

DISCUSSION

DM related hyperglycemia and hyperlipidemia may cause many metabolic syndromes and disorders in different body organs including the liver. It is well known that there is a strong relationship between DM and liver disorders. It has been reported that not only DM patients represent a high prevalence of liver disease, but also patients with liver disease represent a high prevalence of DM [7].

Evaluating liver enzyme levels is a useful approach for determining possible liver injury. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most significant enzymes taking place in the group of aminotransferases or transaminases which mediate the transformation of amino groups between amino acids and oxoacids. Elevated serum ALT and AST activity is a sign of hepatocyte membrane permeability defect which may further lead to cell death. Alkaline phosphatase (ALP) is a hydrolase type enzyme which catalyses removing of phosphate group from in dephosphorylation reactions. The elevated serum AST, ALT and ALP levels are considered as indicators of liver damage [8].Based on the finding that liver was necrotized in STZ-induced diabetic rats, the elevated AST, ALT and ALP activities in the serum of diabetic rats are

considered to be arise from the outflow from hepatocyte cytosol into the blood stream as a result of hepatocyte membrane permeabilityinjury [9].

In our study the elevated AST, ALT and ALP activities induced by STZ, were diminished with MC administration. Our results were in accordance with results of different studies [2,10]. Probably, MC extract might have provided reducement in the leakage of these enzymes from hepatic cells to bloodstream. Furthermore, researchers have suggested that elevated serum AST and ALT activities could be explained with excessive accumulation of glutamate and alanine caused by a mobilization of amino acids from protein stores [11]. In a study conducted by Elfellah et al., administration of MC ethanol-water extract had significantly reduced blood glucose in STZ-induced diabetic mice, whereas the extract had no hypoglycemic effect on normal mice [12]. Our study revealed this finding with the result that MC extract showed considerable hypoglycemic effect in STZ-induced diabetic rats, being highest at the dose of 600 mg/kg. In a study with similar approach to our study, researchers have determined the most effective anti-diabetic dose of MC as 800 mg/kg rather than the dose of 400 mg/kg on STZ induced diabetic rats [13]. The forthcoming studies will be performed between different from 600 mg/ kg to 800 mg/kg could be beneficial to determine the accurate anti-diabetic dose of MC.

In diabetic conditions, auto-oxidation of excessive glucose leads to formation of reactive oxygen species (ROS). Overproduction of reactive oxygen species (ROS) is implicated to be related with the complications associated with DM. This knowledge has been supported with studies reporting improvement in diabetic complications through antioxidant treatment including antioxidant molecules [14] and plant extracts [15]. Indeed, the underlying DM inducement mechanism of STZ is based on formation of superoxide radicals in the pancreatic beta cells in consequence of several reactions [16].

GSH is considered as a non-enzymatic antioxidant and SOD is considered as an enzymatic antioxidant which hasfree radical scanvenging potential in the cellular defense system [17]. MDA is an end product of lipid peroxidation and is a marker of oxidative stress related membrane damage [18].

The activities of enzymatic antioxidants are reported to be diminished in diabetic rats [19]. In our study, elevated MDA levels and decreased GSH and SOD activities in diabetic rats proved the key role of oxidative stress in the pathophysiology of liver damage in DM. This mechanism is mentioned to be related with the development of several complications of DM in-vitro [20] and in-vivo [21].

MC extracts are constituted of polyphenolic compounds which are known to have antioxidant properties. One of these compounds, quercetin, is a flavonoid and is capable to inhibit glucose absorption in the intestines [22]. Thus, the hepatoprotective effect of MC extract can be attributed to its both hypoglycemic and antioxidant effects.

The increment in SOD activity and GSH levels and the reducement in MDA levels provided by MC administration in our study suggest that, MC reduces the oxidative stress in liver.

CONCLUSION

Our study demonstrated that treatment with MC extract have proved a substantial hypoglycemic, antioxidant and hepatoprotective effect on STZ-induced diabetic rats.These effects may be a result of synergistic effects of many active phytochemicals of MC extract.

Dietary consumption of MC, noting the possible long-term toxic effects, may be useful in avoiding or lessening the complications of oxidative stress related hepatic injury of DM.Further studies are needed to determine accurate effective dose of MC extract or its active components as antidiabetic agents or adjuvant agents to conventional antidiabetic treatment.

Previous presentation

This study was presented as poster in ISOPS 11th International Symposium on Pharmaceutical Sciences, June 9-12, 2015, Ankara, Turkey.

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