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Effects of the Hydro-Ethanol Extract of *Myrtus communis* L. On Blood Glucose Level and Histopathological Changes in Alloxan-Induced Diabetic Rats

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Abstract: Diabetes mellitus is a growing health concern worldwide. Traditional plants are widely used in the Middle East in order to treat the diabetes mellitus. Over all Plant drugs are frequently considered to be less toxic and free from side effects compared to the synthetic ones. *Myrtus communis* L. (Myrtaceae) leaves were traditionally used as antidiabetic medications in Mediterranean countries. The present study aims to evaluate the various effects of the ethanol extraction of *Myrtus communis* L. on the serum concentration of glucose and the pancreatic β-cell mass in Alloxan induced diabetes in rats. Materials and Methods: Thirty adult female Albino rats (200±20 g) were used in this experiment. Diabetes was induced in 20 rats by simple intraperitoneal injection of 120 mg/kg 10% alloxan tetrahydrate. The rats were randomly assigned into two diabetic groups (each group containing ten rats) and one non-diabetic control group (n=10). On the 7th day, one of the diabetics groups was treated with the leaves extract of *Myrtus communis* L. (300 mg/kg), while the second diabetics group remained untreated. After the intraperitoneal administration of alloxan, the serum glucose of the diabetic rats increased significantly, in comparison to the normal rats ($P<0.05$). Administration of *Myrtus Communis* L. extract at a dose of 300 mg/kg body wt. tended to significantly bring the serum glucose toward the normal value, while the serum glucose of the normal rats remained significantly high. Histopathologically, alloxan resulted in severe necrotic changes in the pancreatic islets, especially in the central area of the islets. Tissue sections of the pancreas in the treated animals demonstrated hyperchromic nuclei, enhanced regeneration of B cells and increased size of the pancreatic islets. The present study indicated a significant anti-hyperglycemic effect of *Myrtus communis* L. leaves and supported its traditional usage in the treatment of diabetes mellitus.

Key words: Diabetes Mellitus · *Myrtus communis* L. · Histopatological Change · Pancreatic Islets

INTRODUCTION

Diabetes mellitus is a common endocrine disease which is defined as a group of metabolic diseases characterized by chronic hyperglycemia, which results from defects in insulin secretion, insulin action or both and leads to impaired carbohydrate, lipid and protein metabolism and increased risk of cardiovascular diseases [1]. It is a growing health concern worldwide; and the number of the diabetics is increasing due to the increase in sedentary lifestyle, consumption of energy-rich diets and obesity [2]. Moreover, it is recognized as one of the leading causes of morbidity and mortality in the world. While about 2.5 to 7 percent of the world's population have been diagnosed with diabetes mellitus, it is still expected to increase in future [2]. Despite significant effect of the anti-hyperglycemic drugs and insulin sensitizers, side effects, such as hypoglycemia at higher dose administration [3], low oral bioavailability due to the degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity and poor permeability across the intestinal epithelium [4], make it necessary to find other alternatives. This leads to an increasing demand for natural products with the anti-diabetic activity and fewer side effects.

Herbal therapy has been used for patients with insulin-dependent and non-insulin-dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy and other consequences of this metabolic disease [3]. In fact Herbal drugs are widely prescribed because of their

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effectiveness, fewer side effects and relatively low cost [5].In addition traditional plant medicines are used throughout the world for a range of diabetic presentations. The plants may effect on the blood glucose through different mechanisms some of which may influence the insulin's activity. *Myrtus communis* L. *(*Myrtaceae) leaves and the volatile oil obtained from the leaves are used to lower the blood glucose level in type-2 diabetic patients in Turkish traditional medicine [6]. On the other hand oxidative stress plays an important role in the chronic complications of diabetes mellitus. Hyperglycemia is involved in the generation of oxygen-free radicals [7]. Therefore, the present study aims to examine the influence of oral administration of *Myrtus Communis L.* on the serum concentration of glucose and pathological changes in tissues of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Animals: 40 Adult female Albino rats (200±20 g) were obtained from the university animal house. The animals were housed in cages under the standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/dark cycle) and were maintained with free access to water and *ad libitum* standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids). The rats were randomly assigned into two diabetic groups as well as one control group (n=10).

Preparation of Alloxan-Induced Diabetic Rats: Alloxan tetrahydrate (Sigma, St. Luis, MO, USA) was dissolved in sterile distilled water. Diabetes was induced in 20 rats by intraperitoneal injection of 120 mg/kg of alloxan tetrahydrate (10%). The rats were fasted 12h before and after the injection of alloxan. The range of the diabetogenic dose of alloxan is quite narrow; even light overdosing may be toxic and result in the loss of many animals [8]. This dose was chosen according to the studies conducted by Mansour *et al*.[9] and Sheweita *et al*. [10]. Each animal of the control group was injected with the same amount of normal saline. The amount of blood glucose was assayed in both the control and the study groups after making sure that diabetes mellitus is induced in study group.

Preparation of the *Myrtus Communis* L. leaves Extract: One kilogram of fresh *Myrtus Communis* L. leaves was obtained from the local market. The dried leaves of *Myrtus Communis* L. were grinded into powder. The powder was then extracted by 1 L of hydro-ethanol mixture (80/20, v/v) for 8 hours. This step was repeated for four times. Afterwards, the filtrate was pooled and concentrated under the vacuum at a temperature not exceeding 60°C [11]. The obtained *Myrtus Communis* L. alcoholic extract was stored at -20°C before being used.

Experimental Design: The 30 rats of the present experiment were assigned into the following three groups.

Group 1: Normal control group (n=10). The rats of this group were administrated with 1ml distilled water.

Group 2: Untreated diabetic group (n=10). The rats of this group were intraperitoneally injected with 120 mg/kg, 10% alloxan tetrahydrate and were left untreated.

Group 3: Treated diabetic group (n=10). The rats of this group were intraperitoneally injected with 120 mg/kg, 10% alloxan tetrahydrate and were then daily administered, through the oral route, with *Myrtus Communis* L. leaves extract (1ml/kg body weight, equivalent to 300 mg/kg), using an intragastric tube, for 15 days.

Blood Glucose Measurement: Blood glucose was measured every day until the end of the experiment. Blood was collected from the tail of animals after 12 h fasting with a glucometer Easy Gluco (Ames, Korea). The accuracy of the glucometer was checked by the Orthotolidin method.

Histopathological Examination: Twenty two days after the diabetes induction and at the end of the fifteen days treatment, the animals of all the groups were euthanized by Na-thiopental (50 mg/Kg), Xylazin (20 mg/Kg) and Ketamin HCl (300 mg/Kg). The study was approved by the local ethics committee of our faculty, in accordance with the ethics standards of "Principles of Laboratory Animal Care".

Appropriate tissue samples were obtained from pancreas and liver, were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 ìm thickness and stained with hematoxylin-eosin and Gomeri aldehyde-fuchsin (GAF)- a beta cell specific staining for light microscopic examination. The sections were quantitatively (morphmetric) and qualitatively (morphological) evaluated. For quantitative analysis the following factors were considered:

- The volume density of the islets in 200 microscopic fields was determined in order to evaluate the proportion of the islets tissue to total tissue sections.
- The volume density of the B cells in the islets tissue was obtained by counting approximately 500 parts of islet and determining the proportion B cells to the total islet cells.
- The percent of the B cells of 4 islets of each tissue section from an animal and totally 40 islets of each group were counted.
- The number of the islets per square millimeter was calculated.
- The average area of the islets was determined by measuring the diameter of 4 islets in each section and a total of 40 islets in each group.

Statistical Analysis: Descriptive statistics including the mean, standard error, median, minimum and maximum were calculated for all variables. Different parameters were compared through the one-way ANOVA followed by the Tukey post hoc test. The data were analyzed through the SPSS statistical software (version 16) and of $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Effect of *Myrtus Communis* L. on Serum Glucose: The effect of the *Myrtus Communis* L. leaves extract on the serum glucose in the diabetic rats has been shown in Fig. 1.

In comparison to those of the normal rats, the serum glucose of the diabetic rats of Groups 2 and 3 was significantly higher at all intervals after intraperitoneal administration of alloxan ($p<0.05$). While the glucose concentration of the untreated rats remained high at all intervals, administration of *Myrtus Communis* L. leaves extract at doses of 300 mg/kg body wt tended to bring the serum glucose significantly toward the normal values from third day onwards. Nevertheless, the normal rats did not exhibit any significant alterations in their serum glucose concentration during the study intervals.

Histopathological Findings: The histopathologic sections of the pancreas of the untreated diabetic rats showed that alloxan resulted in severe necrotic changes of the pancreatic islets, particularly the cells in the center of the islets. Nuclear changes such as pyknosis, karorhexis, karyolysis and disappearance of nucleus and, in some places, the residues of the destroyed cells were visible.

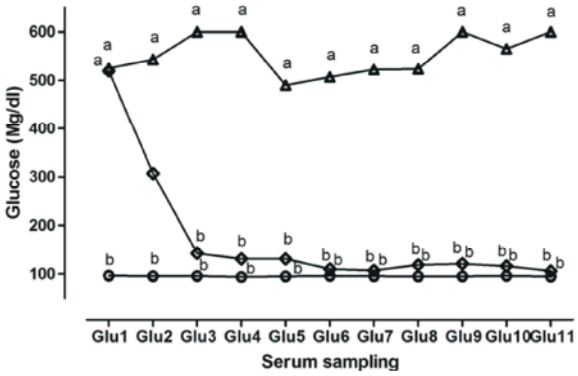

Fig. 1: Comparative effects of *Myrtus Communis* L. (Δ; n=10 on serum the glucose of alloxan induced diabetic rats with nondiabetic (O; n=10) and untreated diabetics (♦; n=10) rats. Different letters in each serum sampling period showed significant differences between the groups ($P<0.05$). Values are presented as mean.

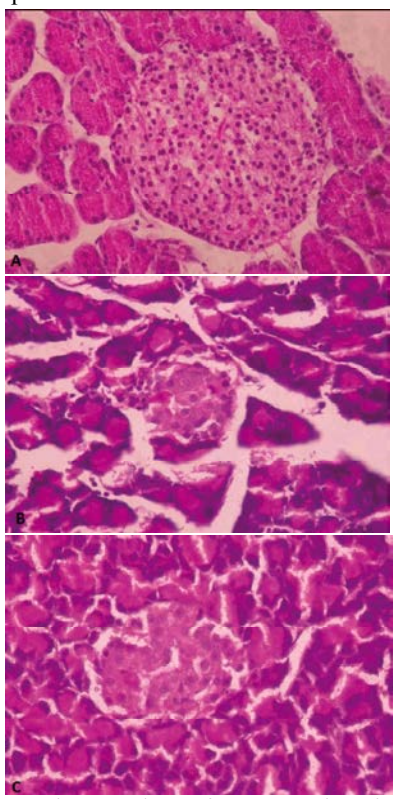

Fig. 2: Photomicrographs of pancreatic tissues (A) normal rat pancreatic tissue (B) diabetic un-treated control rat (C) diabetic rat treated with *Myrtus Communis* L. 300 mg/kg showing restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia

Table 1: Effect of *MyrtusCommunis* L. extract on the histomprphometric parameters in pancreas

Group	Volume density of B cells in islets	Volume density of islets	Percent of B cells	Volume density of B cells in pancreas	Number of islets (per cm^2)
Control positive	8.2±2.240	0.95±0.04	3.85±1.50	0.09±0.004	42.6±4.45
Control negative	58.72±6.80	1.9±0.22	32.55±2.95	1.25±0.340	80.0±6.72
Myrtus Communis L.	15.25±3.65	1.2±0.24	10.50±1.45	0.30±0.020	52.0±4.41

Moreover, a relative reduction in the size and the number of the islets, especially those around the central vessel, together with a severe reduction in beta cells was demonstrated in these animals- the pancreas of the treated diabetic animals showed increased size of pancreatic islets. Moreover, it revealed to have some cells with hyperchromic nucleus and regeneration of B cells in the sections which were stained with haematoxylin and eosin.

The results of the histomorphometric study are summarized in Table 1. The number of the pancreatic islets (per cm^2), volume density of islets, volume density of B cells in pancreas, volume density of B cells in pancreas and volume density of B cells in the pancreatic islets in the diabetic groups were all significantly lower than the normal group. However, the histologic sections of pancreas of the treated animals showed signs of regeneration so that there was no significant difference between the histomorphometry of the treated and the untreated diabetic rats at this stage.

The histopathologic sections of the second group showed degenerative changes represented by the disorganization of the hepatic cords, congestion of the central veins as well as sinusoids and mild nonspecific inflammation with hepatocellular necrosis. Also the hepatocytes showed morphological changes such as pyknosis, karyorrehexis, chromatolysis and cytoplasmic vacuolization. However, the liver of the treated diabetic animals revealed a significant improvement of the hepatic tissue compared to those of the untreated diabetic ones; therefore, except for the presence of a few mildly degenerated hepatocytes around the central vein of the treated rats which still had some cytoplasmic vacuoles, other hepatocytes and portal as well as sinusoid areas were almost normal. In addition, there was no evidence of hemorrhages or inflammatory cells infiltration in the livers of the treated diabetic rats.

The histopathologic sections of kidneys of the non-diabetic control animals were normal and the proximal and the distal convoluted tubules, renal corpuscles, glomerulus and glomerular capsule had normal structure. The microscopic examination of the kidney of the treated and the untreated diabetic rats showed moderate, degenerative and necrotic changes in the glomerular epithelium with diffused interstitial and glomerular hemorrhage and a mild tubular necrosis.

No lesions was not found in the heart, lungs, stomach, large as well as small intestine and spleen of either normal controls, treated or untreated diabetic animals.

Diabetes is possibly the world's fastest growing metabolic disease. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs. While the knowledge of the heterogeneity of this disorder increases, the need for more appropriate therapies increases, as well [12, 13]. Traditional medicinal plants are used throughout the world for a wide range of diabetic complications. Therefore, studying such medicines might help the researchers a great deal in finding new anti-diabetic drugs [14]. Many herbs and herbal products are believed to possess a hypoglycemic effect. Plant drugs are frequently considered to be less toxic and free from side effects than the synthetic ones. *Myrtus Communis* L. is traditionally used as a medicinal plant in tropical and subtropical countries and, according to a review of the literature it is believed to have hypoglycemic properties as well as anti- diabetic activities [15-20].

The chemical composition of *Myrtus Communis* L. was reported as follows [21]; 1,8-cineol (18.2%), linalool (16.3%), myrtenyl acetate (14.5%), linalyl acetate (6.7%), α-terpineol (6.5%), α-pinene (6.4%), geranyl acetate (5.5%), limonene (3.4%), geraniol, neryl acetate and methyl eugenol (between 1 and 2%).

The results of the present study showed that the oral administration of 300 mg/kg ethanol extract of *Myrtus Communis* L. for 15 days in the alloxan-induced diabetic rats reduced the elevated serum glucose concentrations to the normal range. The antidiabetic effect of *Myrtus Communis* L. extract could be linked to more than one mechanism such as the reversible inhibition of alpha-glucosidases present in the brush-border of the small intestinal mucosa, higher rate of glycolysis as envisaged by the higher activity of glucokinase, as one of the key enzymes of glycolysis- and an enhanced rate of glycogenesis- as evidenced by the higher amount of liver glycogen was observed after the extract administration [17].

Consistent with the findings of the present study, the hypoglycemic effect of *Myrtus Communis* L. on rabbits was shown in another study [17]. Besides Önal *et al.* [22]

reported the *Myrtus Communis* L. leaves to possess a potential α-glucosidase inhibitory effect; and it is suggested that plasma insulin levels are not affected by α-glucosidase inhibitors [23]. In addition, myrtle has been reported to possess hypoglycaemic, antimicrobic and, antihemorrhagic proprieties [17, 21]. *Myrtus Communis* L. may produce its hypoglycemic effect mainly by influencing the mechanism of intestinal transport of carbohydrates. In particular, *Myrtus Communis* L. might inhibit α-glucosidases of the small intestine, thus reducing the intestinal absorption of glucose and delaying the glucose release from the complex carbohydrates. Also, the higher rate of glycolysis as envisaged by the higher activity of glucokinase and the enhanced rate of glycogenesis- as evidenced by the higher amount of liver glycogen- was observed after the *Myrtus Communis* L. administration [17].

The histopathological findings of the pancreas of the treated animals of the present experiment, showed the increased size of the islets, hyperchromic nucleus and regeneration of the B cells. These findings reveal that the anti hypoglycemic effect of *Myrtus Communis* L. is due to its through the insulin-like substances, such as saponin, or the activation of the insulin receptors. In addition also this plant is able to increase the number of the B cells in the islets of Langerhans.

Liver as an insulin-dependent organ has a major role in glucose and lipid homeostasis. Several mechanisms are implicated in the pathogenesis of the functional and morphological alterations of the liver of the diabetic patients [24]. In this experiment, the liver of the untreated diabetic rats showed disorganization of the hepatic cords with vacuolization and pyknotic changes in the nuclei of the hepatocytes. Following the *Myrtus Communis* L. intake, the livers of the animals of the present study showed improvement in their histological structure with the persistence of the cytoplasmic vacuoles in some of the hepatocytes close to the central vein which could be attributed to the residual adverse effect of the diabetic affliction. Herbal hepatotoxicity has been recognized for many years, but new agents are constantly being identified [25]. Histopathological evaluation of heart, lungs, stomach, large as well as small intestines and spleen was carried out in this experiment, but the obtained results were not in line with those of the studies mentioned above.

CONCLUSION

Myrtus Communis L. (Myrtaceae) leaves were traditionally used as antidiabetic medications in Mediterranean countries. The present study indicated a significant antihyperglycaemic effect of *Myrtus Communis* L. leaves. The pancreases and livers of the treated rats showed an improvement in the histological structure. The obtained results support the traditional usage of *Myrtus Communis L.* in the treatment of diabetes mellitus and the decrease in its complications.

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