

The Effect of *Myrtus communis* Extract on Liver Enzymes and Blood Biochemical Factors in Diabetic Adult Male Rats

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Article information	Abstract
<p>Article history: Received: 28 Aug 2013 Accepted: 18 Sep 2013 Available online: 5 Feb 2014 ZJRMS 2014 Oct; 16(10): 12-17</p> <p>Keywords: <i>Myrtus caumunis</i> Liver Blood sugar Rats</p> <p>*Corresponding author at: Department of Physiology, Darab Branch, Islamic Azad University, Darab, Iran. E-mail: hjowhary@yahoo.co.uk</p>	<p>Background: The aim of this study was the effect of <i>Myrtus communis</i> extract on liver enzymes and blood biochemical factors in diabetic adult male rats.</p> <p>Materials and Methods: This study has been carried out experimentally and completely random. Seventy adult male Wistar rats were divided in 7 groups including: control which received no treatment, sham who received 2 mL of distilled water, the 1st, 2nd and 3rd experimental groups which received 0.75, 1.5 and 3 mg/kg <i>Myrtus communis</i> leaf extract respectively, the 4th experimental group as the diabetic control group who received streptozotocin (60 mg/kg) and the 5th experimental group as the diabetic treatment group who received 3 mg/kg of extract. This experiment lasted 14 days with prescript orally. After this period, all the rats, were weighted, anesthetized and blood samples were taken from the heart centrifuged and sera were evaluated for the concentration of various factors. In addition liver were removed and sliced.</p> <p>Results: According to the obtained results, the plasma concentration of liver enzyme (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase), cholesterol and glucose presented a significant decrease at ($p \leq 0.05$). Whereas no significant change were seen in body weight, triglyceride, urea, albumin and total protein. Histological studies of the liver tissue showed no significant difference among various groups.</p> <p>Conclusion: <i>Myrtus communis</i> is comprise of collections of flavonoids and other various components with antioxidant and anti inflammatory properties. Thence it can effective in treatment of liver diseases and decrease of blood sugar and cholesterol in diabetes mellitus patients.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Myrtle has been known as a medicinal herb by Iranians for a long time and many medicinal properties are listed for this plant [1]. It contains various chemical compounds such as tannins, flavonoids, saponins and vitamin C and also no alkaloids and glycosides. Myrtle leaves contain terpinolene, cineole, terpineol and linalyl acetate in addition to the previously mentioned compounds [2, 3]. Myrtle leaf extract can cause hypoglycemia [4]. Moreover, investigations have revealed its antioxidative effects [5-7]. Phenolic compounds such as carvacrol, rosmarinic acid and thymol that are present in the leaves of myrtle cause a threefold increase in MGMT (O-methylguanine-DNA methyltransferase).

MGMT is the first defense mechanism against DNA damage and physically it deals with cell proliferation and nucleus antigen and nucleus polymerase during the DNA replication. Also, a slight increase of MGMT can be sufficient for protection against damage and mutation. Thus, phenolic compounds can be used as dietary supplements for MGMT irregularities in human tissues and reduce cancer risk. It should be noted that the highest level of MGMT is in the liver and the lungs, and the lowest level is in the brain. Hepatic level of MGMT is 20

times more than the brain or bone marrow [8, 9]. Dosage forms of myrtle are topical ointment (Myrtoplex) which mostly contains tannins, polyphenols, myrtol, syncol and flavonoids, and topical solution (Myrtex) which contains cineole, myrtle oil, citronellol, menthol and flavonoid quercetin [2, 3]. Lobule is the functional unit of the liver which is created by connection of the networks of hepatocyte plates; and the most important liver enzymes are ALT (alanine aminotransferase), AST (aspartate aminotransferase) and ALP (alkaline phosphatase) which increase along with liver diseases [10]. Since urea is produced in the liver, the amount of the blood urea reduces with the liver damages; also the level of urea may decrease in the second or third trimester of pregnancy [11]. The aim of this study was the effect of *Myrtus communis* extract on liver enzymes and blood biochemical factors in diabetic adult male rats.

Materials and Methods

This study has been carried out experimentally and completely random. *Myrtus communis* samples were obtained from the Agricultural and Natural Research Center of Fars province. Identification of genus and species samples was done by plant taxonomy experts

from the College of Science/Shiraz University. Hydro alcoholic extracts of *Myrtus communis* were obtained using the method of Erdemoglu et al. [12]. This research was conducted as a laboratory work in which the code of professional ethics regarding laboratory animals was entirely complied with. Animals used in this study were 70 adult male Wistar rats, weighing approximately 210-230 g and aging approximately 2.5-3 months which were taken from Animal Breeding Center of Shiraz University and kept at the same location. Environment temperature was $23\pm 2^{\circ}\text{C}$ and constant throughout the day, and the light-dark cycles were 12 h of light and 12 h of darkness. Pellet and water were provided for the rats in the entire period of research and without restrictions. Rats were kept in polycarbonate cages with mesh roofs of stainless steel, so that 10 rats were kept in each cage. Some sawdust was shed in the cage floors and was replaced every three days. The lethal dose was determined. Doses were determined based on LD50 (Lethal Dose fifty) method. Seventy rats were divided into 7 groups of 10 animals each. These groups are as follows:

1. The control group who did not receive any substance or drug during the testing.
2. The sham group who received 2 mL of distilled water orally each day.
3. The first experimental group as the minimum treatment group who orally received 0.75 mg/kg/day of extract of Myrtle.
4. The second experimental group as the average treatment group who orally received 1.5 mg/kg/day of extract of Myrtle.
5. The third experimental group as the maximum treatment group who orally received 3 mg/kg/day of extract of Myrtle.
6. The fourth experimental group as the diabetic control group who received an intraperitoneal (i.p.) injection of 60 mg/kg of streptozotocin.
7. The fifth experimental group as the diabetic treatment group who orally received 3 mg/kg/day of extract of Myrtle in addition to an i.p. injection of streptozotocin.

After 14 days, rats were weighed and blood samples were obtained by sampling directly from the right ventricle of the heart after applying ether for anesthesia. The samples were incubated for 15 minutes at 37°C and then centrifuged at 3000 rpm for 15 min, after blood coagulation. Finally, serum was separated from each sample by using a Pasteur pipette and poured into another tube, and then the tube was sealed by Para film. These tubes were stored at -20°C until assessment of the desired factors. Liver resection was performed for each animal and after washing the liver with distilled water to remove excess tissue and blood, it was placed in a container with lid, containing formalin fixator; stained microscope slides were prepared in later stages. ALP, AST and ALT enzymes and other factors such as total protein, albumin, bilirubin, urea and glucose of serum were measured using the spectrophotometry method. Having gone through the stage of tissue processing, each sample was stained by Hematoxylin and Eosin method. Finally, the isolated slides were examined through an optical microscope

(Nikon 8ii, made in Japan) in terms of Histological modifications. Obtained results were analyzed by SPSS-16 software (SPSS Inc. Chicago, IL). Average body weight and other assessed factors in various groups were compared with the control group using the *t*-test and ANOVA methods and $p\leq 0.05$ was regarded as a significant difference.

Rates of urea, cholesterol, triglyceride, albumin, total protein, glucose, ALP, AST, ALT in serum were measured by related kits Iranian Pishtaz Teb Kit (REF: A21854), (REF: IM1188) with auto analyzer (Technicon, model RA-1000) by spectrophotometry method.

Results

The mean body weight in the extract receiving groups does not show a significant change at $p\leq 0.05$ level compared to the mean body weight in the corresponding control groups. Average concentration of alanine aminotransferase and alkaline phosphatase enzymes in the extract receiving groups of average and maximum doses in non-diabetic and diabetic groups are significantly lower than the corresponding control groups while in case of the aspartate aminotransferase enzyme, only the extract receiving group of maximum dose in non-diabetic and diabetic groups show a significant decrease compared to the corresponding control group.

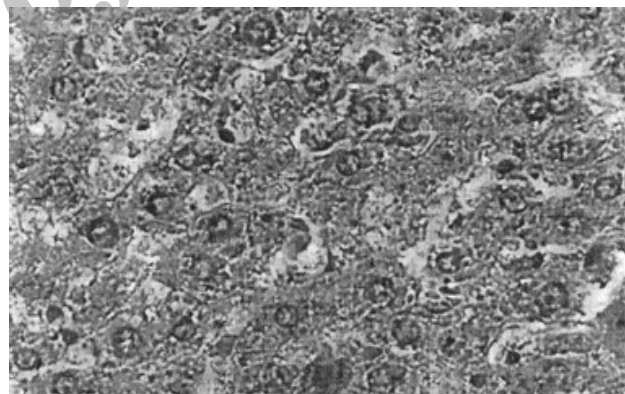


Figure 1. Liver tissue in the control group (x400)

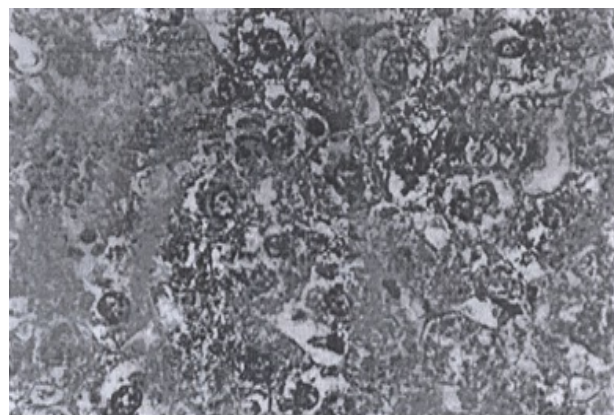


Figure 2. Liver tissue in the first experimental group that received extract of *Myrtus* at amount of 0.75 g/kg (x400)

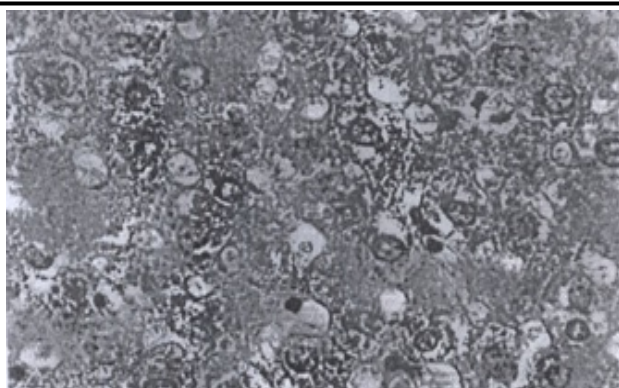


Figure 3. Liver tissue in the second experimental group that received extract of Myrtus at amount of 1.5 g/kg (x400)

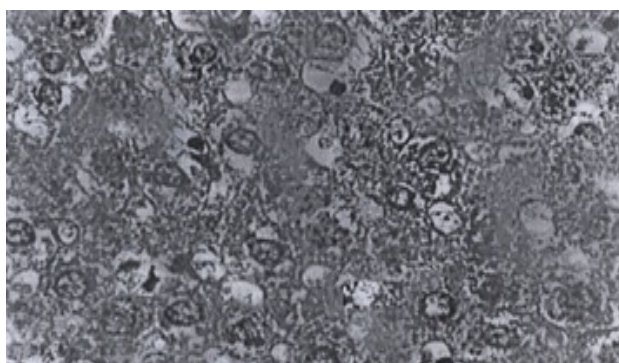


Figure 4. Liver tissue in the third experimental group that received extract of Myrtus at amount of 3 g/kg (x400)

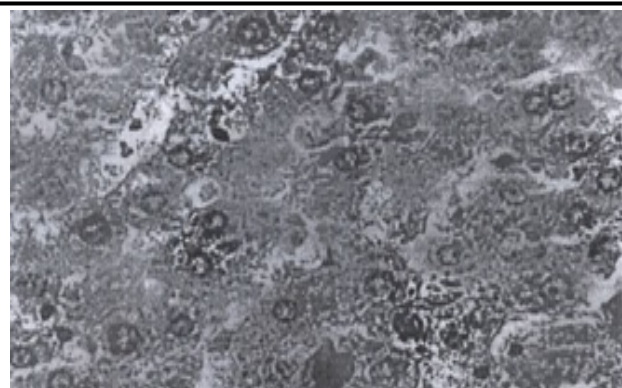


Figure 5. Liver tissue in the fourth experimental group as the diabetic control group that received streptozotocin at amount of 60 mg/kg (x400)

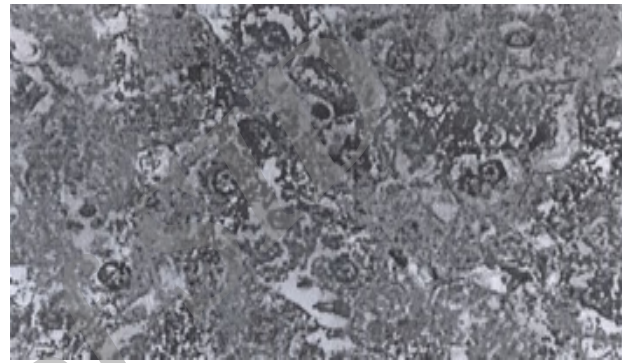


Figure 6. Liver tissue in the fifth experimental group as the diabetic treatment group that received extract of Myrtus at amount of 3 g/kg (x400)

Table 1. Comparison of control group, sham group and five experimental groups in terms of body weight, ALT, AST, ALP, Urea, cholesterol, triglyceride, albumin, total protein and glucose

Groups	Control (Mean±SD)	Sham (Mean±SD)	(Mean±SD) I	(Mean±SD) II	(Mean±SD) III	(Mean±SD) IV	(Mean±SD) V
Body weight (g)	222.49±8.73	225.45±12.46	223.37±9.34	221.23±11.26	224.98±7.94	216.46±9.38	219.61±8.49
ALT (U/I)	59.10±6.65	5.400±7.80	52.00±7.45	*44.25±5.25	*32.12±5.50	*63.45±6.7	*36.00±7.20
AST (U/I)	245.46	246.34±15.43	243.22±12.54	240.67±16.23	190.00±11.45	255.32±14.21	205.55±13.12
ALP (U/I)	504.43±19.33	500.76±28.34	502.64±33.42	430.85±24.52	401.96±32.63	517.67±34.78	398.23±29.43
Urea (mg)	29.45±3.08	28.98±2.49	28.33±2.77	29.46±2.68	27.86±3.05	35.89±2.58	34.33±2.85
Cholesterol (mg/dL)	265.05±28.89	246.76±31.59	263.10±29.53	263±25.92	231.74±29.86	409.68±40.64	205.97±21.12
Triglyceride (mg/dL)	79.34±17.43	78.65±9.59	78.10±8.63	79.74±11.79	75.69±8.94	69.78±6.35	75.86±7.48
Albumin (mg/dL)	0.03±0.01	0.04±0.01	0.03±0.02	0.04±0.01	0.04±0.01	0.03±0.02	0.03±0.01
Total protein (mg/dL)	0.092±0.04	0.091±0.03	0.087±0.04	0.087±0.05	0.081±0.04	0.086±0.03	0.083±0.03
Glucose (mg/dL)	130±14.68	129±15.55	130±12.45	131±12.88	122±9.56	565±33.48	310±21.34

* Indicates a significant difference between the control and experimental groups ($p \leq 0.05$). ALT (alanine aminotransferase), ALP (alkaline phosphatase), AST (aspartate aminotransferase)

In addition, the concentrations of urea, triglycerides, albumin and total protein in various extract receiving groups do not show significant changes compared to their averages in the corresponding control groups. On the other hand, in the case of cholesterol and glucose, only the diabetic group treated with the extract show a significant change compared to the diabetic control group.

Histological studies of the liver did not show particular changes in different groups, but cellular order and preservation of the radial mode and naturalness of the liver cells (single nucleus or double nucleus, abundant euchromatin and distinct nucleolus) are obvious in the extract receiving groups.

Discussion

The results of body weight changes indicate that the Myrtle leaf extract receiving groups do not show

significant changes compared to the corresponding control groups. Flavonoid compounds found in the Myrtle leaves such as rosmarinic acid, caffeic acid, thymol and carvacrol are free radical absorbents. Free radicals cause lipid peroxidation, and these compounds are effective against lipid peroxidation. Also NADPH is required for fatty acid synthesis which is provided in three ways:

1. Through malic acid.
2. Through isocitrate dehydrogenase.
3. Through NADPH who is produced in pentose phosphate or phosphogluconate pathways, which is also considered the main way for providing NADPH [2, 3].

Vanz et al. proved that carvacrol, which is one of the major constituents of the myrtle leaf, increases the regulation enzyme of pentose phosphate pathway, and with the increase of this enzyme and increased activity of this pathway, the required energy for lipogenesis will be

provided [13]. On the other hand, Ariza et al. caused an increase in the weight and growth of the pigs that their growth had been delayed, by feeding them oregano leaf extract which has compounds similar to Myrtle [14]. Therefore, we expected that oral administration of Myrtle leaf extract will cause weight gain but given the short duration of extract feeding in this study, in order to observe significant changes, longer period of time is required.

In case of ALP and ALT enzymes, significant decrease was observed in the extract receiving groups of average and maximum doses compared to the corresponding control groups and in case of AST enzyme the extract receiving group of maximum dose has a significant decrease compared to the corresponding control group. Alanine aminotransferase enzyme is in the cytoplasm and aspartate aminotransferase enzyme is in the cytoplasm and mitochondria of hepatocytes; when cells become necrotic or their membrane permeability changes due to damages such as viral hepatitis, decreased oxygen supply to the liver cells or hepatocyte damages under the influence of drugs or poisons, these enzymes are released into the blood in large quantities. On the other hand, natural alkaline phosphatase of serum is composed of several different isoenzymes which can be found in the liver, bone and placenta and less commonly in the small intestine. The level of this enzyme increases more than 4 times in bile duct obstructions and visceral liver diseases such as cancer and bone diseases where this increase is specific, however, the increase of this enzyme can be seen in other diseases of liver fewer than three times of the natural level, which is not specific [15, 16].

Catalase and glutathione peroxidase are in the category of hypo peroxidase enzymes and have antioxidant properties and cause neutralization of hydrogen peroxide; and glutathione peroxidase neutralizes free radicals in the presence of vitamin A and C.

According to Yaam et al. and Janbaz et al. researchs, it is proven that caffeic acid prevents increase of serum enzymes against liver injury induced by methane tetrachloride [17, 18]. According to EL-Ashmawi et al. study, with the increase of the antioxidant property of liver, ALT and AST enzymes are reduced [19]. Matsuura et al. showed that flavonoids present in Myrtle leaf such as rosmarinic acid are able to neutralize DPPH free radical and to prevent its damaging effects due to their antioxidant property [20]. Stimulation of DNA polymerase by the flavonoid compounds increases rRNA synthesis and thereby reconstruction of liver cells [2, 3]. Timolol and carvacrol are two important compounds of Myrtle leaf and increase glucose-6-phosphate dehydrogenase enzyme which is the main regulatory enzyme of pentose phosphate or phosphogluconate pathway. This pathway provides NADPH necessary for the decomposition of hydrogen peroxide. Hydrogen peroxide is removed by glutathione peroxidase which acquires its required hydrogen by oxidizing glutathione and the reduction of glutathione is performed by glutathione reductase which receives the required energy from phosphogluconate pathway. With increased activity

of the pentose phosphate pathway, elimination of hydrogen peroxide increases where this response is accompanied by the increase of superoxide dismutase, catalase and glutathione peroxidase [21].

According to the studies of Prins et al. and Jaeschke et al., lithospermic acid B, jasmonic acid, ursolic acid and other phenolic compounds can reduce liver inflammation by inhibiting the lipoxygenase cycle and inhibiting the production of leukotrienes and free radicals in liver Kupffer cells [22, 23]. With the increase in antioxidant defense system in the liver cells, the decrease of ALT, AST and ALP enzymes can be justified. Diabetes leads to increased concentrations of glucose in the extracellular fluid, which causes disposal of water from the cells and dehydration. In addition, the excretion of glucose in urine causes diuresis because glucose prevents reabsorption of liquids in the tubules due to creating high osmotic pressure in them, which intensifies cell dehydration [24]. Urine increased in treated diabetic rats compared with non-diabetic and diabetic control groups, which indicates that myrtle leaf extract has diuretic effects. Probably the increase in urine excretion leads to increased urea excretion and thereby reduced plasma concentrations of urea.

Albumin is a major plasma protein that is exclusively synthesized by hepatocytes. This protein has a long life of 15-20 days and almost 4% of it is demolished daily. Since the production and degradation rates of albumin are low, its serum level is not a good indicator of moderate or severe liver dysfunction. In acute liver diseases such as viral hepatitis, drug-induced hepatotoxicity and obstructive jaundice the serum level of albumin changes only slightly and usually severe liver damage reflects albumin production. Albumin and serum total protein are indicators of biosynthesis function of the liver. Mantoro and others proved that carvacrol has increased regeneration rate of the liver tissue. Myrtle extract having antioxidant properties, increases liver protective enzymes and improves liver function [5-7]. But given that albumin production and degradation rate is low the ineffectiveness of the extract on the levels of albumin and total protein in a short time can be justified.

Average cholesterol concentrations in diabetic control group significantly increased compared to non-diabetic control group. In fact, diabetes causes changes in plasma lipoproteins and increase of their oxidation and changes in fatty acid composition and changes in density of the plasma lipoproteins. Diabetes also reduces a protein called apoprotein B-100. Low-density protein receptors which are present on cell membranes throughout the body recognize apoprotein B-100 and force them to adhere to these cells. Adhesion is necessary for lipoproteins to be able to deliver their cholesterol load. In case of return of most of the cholesterol to the liver cells, the internal feedback mechanism of liver cells produces cholesterol along with the formation of further LDL [25, 26].

The results of this study showed that the cholesterol level was significantly reduced in the treated diabetic group compared with the diabetic control group, which may be due to the presence of compounds such as linoleic

acid, oleic acid and stearic acid. Apparently, saturated fatty acids suppress the clearance of LDL mediator-receptor and even inhibit expression of LDL receptors and thereby increase the total cholesterol. Therefore, it is possible that oleic acid returns the activity of LDL receptors to normal mode, and also reduces cholesterol reabsorption. On the other hand, it was shown that stearic acid not only does not increase LDL, but also reduces it, although it is a saturated fatty acid and is expected to increase LDL, because it quickly becomes saturated and converts to oleic acid [27, 28]. With regard to the abundance of linoleic acid and oleic acid in the myrtle extract, its reduction effect toward cholesterol can be attributed to these compounds.

Biologically active compounds such as flavonoids, terpenoids and alkaloids which have anti-diabetic effects can be seen in the leaves of myrtle. These compounds can exert their effects by several mechanisms such as inhibition of glycogenesis, stimulation of glycolysis, stimulation of insulin release and inhibition of glucose absorption from the intestinal tract. Also another possibility is that these compounds cause obstruction of ATP-dependent potassium channels in pancreatic beta-cells and increases intracellular calcium by reducing the voltage of the cell membrane. Increase in intracellular calcium in turn triggers insulin release, therefore decreases concentrations of glucose. Therefore a significant decrease can be seen in the glucose concentration in diabetic treatment group. Study of microscopic slides of the liver tissue showed that there are

no significant changes in any of the experimental and control groups, and in myrtle leaf extract receiving group cellular order and preservation of the radial mode and naturalness of the liver cells (single nucleus or double nucleus, abundant euchromatin and distinct nucleolus) are obvious. Thence, the concentrations of urea, triglycerides, albumin and total protein in various extract receiving groups do not show significant changes compared to their averages in the corresponding control groups. *Myrtus communis* can effective in treatment of liver diseases and decrease of blood sugar and cholesterol in diabetes mellitus patients.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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