Physiology and Pharmacology

Physiol Pharmacol 20 (2016) 24-30 **www.phypha.ir/ppj**

Original Article

Antihyperglycemic and antihyperlipidemic effects of hydroalcoholic extract of *Melissa officinalis* (Lemon Balm) in alloxan-induced diabetic rats

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Abstract

Introduction: Diabetes mellitus is a metabolic disorder of the endocrine system leading to increased blood glucose concentration in the patients. As a basic treatment for managing the blood glucose level, insulin or hypoglycemic medications are used but herbal medicines are more favored. The design of this research project was to study the therapeutic effect of hydroalcoholic extract of *Melissa officinalis* (HEMO) in diabetic rats.

Methods: Twenty-five Wistar male rats weighing 220±25 grams were distributed semi-randomly into five groups of five each. Group 1 and 2 was respectively the control and diabetic animals. Group 3, 4 and 5 were the diabetic animals treated with HEMO either at 20, 100 or 500 mg/Kg of body weight. To induce diabetic rat models, each animals received a single intraperitoneal injection of alloxan at the dose of 120 mg/Kg. All treatments with HEMO performed daily via gavage for a period of 4 weeks. Then, blood samples were collected from all animals to measure the blood glucose level, cholesterol, triglycerides, LDL and HDL.

Results: The results of this study indicated significant ($P<0.05$) decreases in blood sugar level, cholesterol, triglycerides and LDL in diabetic rats treated with HEMO. In addition, significant ($P<0.05$) increase in HDL level was observed in HEMO treated diabetic rats compared with the non-treated ones.

Conclusion: HEMO has significant effects on attenuating the blood sugar level, serum lipids and lipoproteins levels, whereas it improves the HDL level. These effect might be attributed to the antioxidant benefits of flavonoids which are present in HEMO.

Keywords:
Diabetes;
Hydroalcoholic Extract;
Melissa Officinalis;
Alloxan;
Rat

Received:
6 Oct 2015
Accepted:
26 Apr 2016

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Introduction

Diabetes mellitus is a metabolic disorder resulting from defects in insulin secretion, insulin action, or both. Insulin deficiency leads to increased blood glucose levels (hyperglycemia) associated with impaired metabolism of carbohydrate, fat and protein (Fröde and Medeiros, 2008; Lachin and Reza, 2012;

American Diabetes Association, 2008; Nesteruk et al., 2007). By the progress of diabetes, damages to blood vessels lead to severe complications such as retinal damage (retinopathy), peripheral nerve damage (neuropathy), kidney and cardiovascular complications and wound healing inhibition (Lachin and Reza, 2012; American Diabetes Association, 2008). Despite the well-known anti-diabetic drugs in the pharmaceutical industry, diabetes and its associated complications, remain a major problem in medicine in both developing and developed societies (Zimmet et al., 2001). It has recently been reported that some herbs are experimentally used as anti-diabetic treatment around the word. Anti-diabetic effects of these plants are caused by their ability to regenerate pancreas tissue that leads to increase in insulin secretion, inhibition of intestinal absorption of glucose and facilitation of insulin metabolism (Kim et al., 2006). High level of reactive oxygen species (ROS) is in association with pathogenesis of diabetes (Lachin and Reza, 2012; Demin et al., 2012). Since, oxidative stress is the main cause of necrosis of pancreatic beta cells, therapeutic administration of antioxidants and clearance of ROS can harness the progression of diabetes and its complications (Lukivskaya et al., 2004; Asgary et al., 2012).

Melissa officinalis (MO) is a species from Lamiaceaes family that also called Lemon balm because of its lemon like scent. Scientific research haa revealed that Melissa officinalis possesses many beneficial effects such as anxiolytic (Kennedy et al., 2004), anti-inflammatory (Bounihi et al., 2013), neuroprotective (Soulimani et al., 1991) and antioxidant (Ribeiro et al., 2001). Recently MO has been used to treat Alzheimer's disease (Akhondzadeh et al., 2003). Among more than one hundred chemicals which have been identified in this plant (Duke, 2006), its main ingredients include citral, citronellal, geraniol, linalool and β-caryophyllene-oxide. The odor of the plant is mainly because of citral and citronellal. There are also plenty of flavonoids in lemon balm exerting antioxidant effects (Ribeiro et al., 2001; Adzet et al., 1992; Shabby et al., 1995). Because of its applications in pharmaceutics and food and health industry, MO has been considered as one of the commercially significant plants in the past few decades (Aharizad et al., 2012). In this study we evaluated antihyperglycemic and antihyperlipidemic effects of different doses (20, 100, 500 mg/kg) of hydro-alcoholic extract of lemon balm in the diabetic rats.

Materials and methods

Material

Lemon balm leaves were obtained from Irani-Nosh company (Isfahan, Iran), genus and species authenticated by Department of Plant Biology, University of Isfahan. Dried leaves were ground by means of electrical mill before extraction.

Preparation of lemon balm extracts

150g of shattered leaves was extracted by stirring with 600 ml of EtOH 96% at lab temperature (25°c) for 24 hours (Yessoufou et al., 2013). After filtrating through a filter paper, residuum was added EtOH 70% for 24 hours. First and second filtrate mixed together and then concentrated to one- third by means of a rotary evaporator (Laborata 4000, Heidolph Germany) at 50°c and 70 rpm (El-Demerdash et al., 2005). Eventually the extract was dried with an oven at 45°c. Before experiments extract powder was dissolved in normal saline and was given to animals by gavage procedure.

Animals

Male Wistar rats (weight 200-250g) were obtained from Isfahan University of Medical Science (Isfahan, Iran). The animals were housed in plastic cages at a constant temperature of 23°c ± 1 and 12 hours light/dark cycle with free access to water and food (Pars company, Tehran, Iran). Animals were acclimatized for one week prior to experimental beginning. The experimental protocol was approved by the Animal Care and Use Committee of the University of Isfahan.

Alloxan induced diabetic rats

For inducing diabetes, a single intraperitoneal (i.p) injection of 120 mg/kg of Alloxan monohydrate (Sigma), dissolved in normal saline, was performed in overnight fasting animals (El-Demerdash et al., 2005; Aragão et al., 2010; Asgary et al., 2008). Three to five days after injection, diabetic symptoms such as polydipsia, polyuria and weight loss were emerged. Serum glucose level was monitored by a glucometer

Table 1: Effect of HEMO on serum glucose level.

	Control	Diabetic	Diabetic + 20 mg/kg	Diabetic + 100 mg/kg	Diabetic + 500 mg/kg
Before experiment	2.3±91.8	1.41±95	2.38±91.2	1.58±89	1.92±90.2
After Alloxan injection	1.8±90.8	*3.03±423.8	*2.58±420.4	*3.11±421.2	*2.3±424.6
At the end of experiment	6.3±90.4	*2.85±551.2	#244.4±5.31	#188.2±5.11	#222±3.74

Results are presented as mean ± SEM; n=6 in each group. MANOVA followed by tukey test, significance level considered as P<0.05. *: Significant difference compared to control group, #: Significant difference compared to diabetic group.

(Easy Gluco, Germany) and animals with serum glucose levels over than 250 mg/dl were considered as diabetic animal.

Treatment groups

Along with one control group of non-diabetic rats (n=6), alloxan-induced diabetic rats were randomly divided into 4 groups (n=6). Group 1 or control group received only normal saline via gavage. Group 2 or diabetic group received only one i.p injection of alloxan monohydrate. Group 3, 4 and 5, first received i.p injection of alloxan monohydrate and then were daily treated orally by gavage with 20, 100 and 500 mg/kg of extract (respectively) for 4 weeks.

Blood samples

Fasting (16 hours) blood samples were taken 3 times: time 0 was before experiment, time 1 was one week after alloxan injection and time 2 was at the end of experiment (4 week after alloxan injection). Blood glucose level was measured by means of glucometer (Easy Gluco, Germany). At the end of experiment, overnight fasted animals sacrificed by inhalation of chloroform and blood samples were collected directly from heart. After 40 minutes resting at lab temperature, blood samples were centrifuged at 3000 rpm for 15 minutes. Serum levels of cholesterol, triglyceride, HDL and LDL were determined by taking advantage of commercially available kits (Pars-Azma kit, Iran).

Statistical analysis

Data were analyzed by SPSS (version 20) software and differences between groups were tested by multivariate analysis of variance (MANOVA) and tukey posthoc. Results are presented as mean±SEM, significance level considered as P<0.05.

Results

Effect of HEMO on alloxan-induced hyperglycemia

As shown in Table 1, alloxan injection significantly (P<0.05) increased blood glucose level. Treatment with HEMO (20, 100, 500 mg/kg) significantly (P<0.05) attenuated alloxan-induced hyperglycemia and reduced blood glucose level compared to untreated group but this effect was not dose dependent. Maximum effect was observed in the group with 100 mg/ Kg of HEMO (table 1).

Effect of HEMO on alloxan-induced hyperlipidemia

Alternation of serum level of lipids is one of the diabetes symptoms. There was a significant (P<0.05) increase of cholesterol (Fig.1), triglyceride (Fig.2), LDL (Fig.3) and decrease of HDL (fig.4) in alloxan-induced diabetic rats compared to control group. Four weeks treatment with HEMO could moderate alloxaninduced hyperlipidemia and significantly (P<0.05) decreased cholesterol, triglyceride and LDL compared to untreated group, Furthermore HEMO treatment significantly (P<0.05) increased serum level of HDL compared to untreated group.

Discussion

Results illustrate that single i.p injection of Alloxan monohydrate, 120 mg/kg, leads to diabetes in rats hence serum glucose level was increased form 90.4 ±6.30 to 551.2±2.58 mg/dl. Alloxan also increases

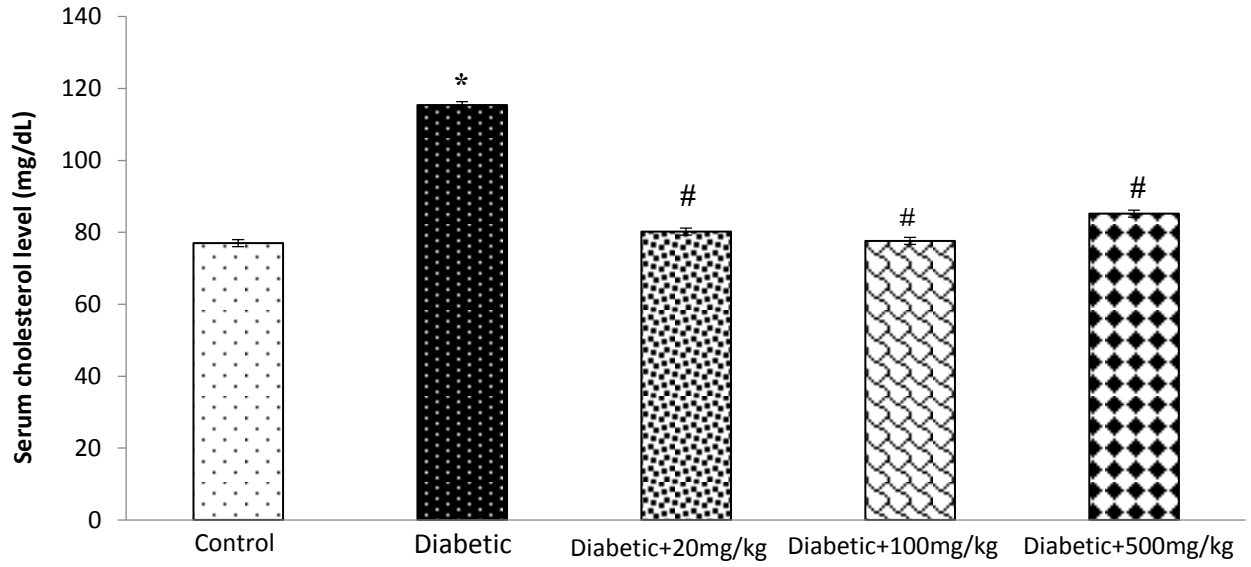

Fig.1. Effect of HEMO on serum cholesterol level. Results are presented as mean ± SEM, n=6 in each group. MANOVA followed by tukey test, significance level considered as P<0.05. *: Significant difference compared to control group, #: Significant difference compared to diabetic group. Effect of HEMO on serum cholesterol level. Results are presented as mean ± SEM, n=6 in each group. MANOVA followed by tukey test, significance level considered as P<0.05. *: Significant difference compared to control group, #: Significant difference compared to diabetic group.

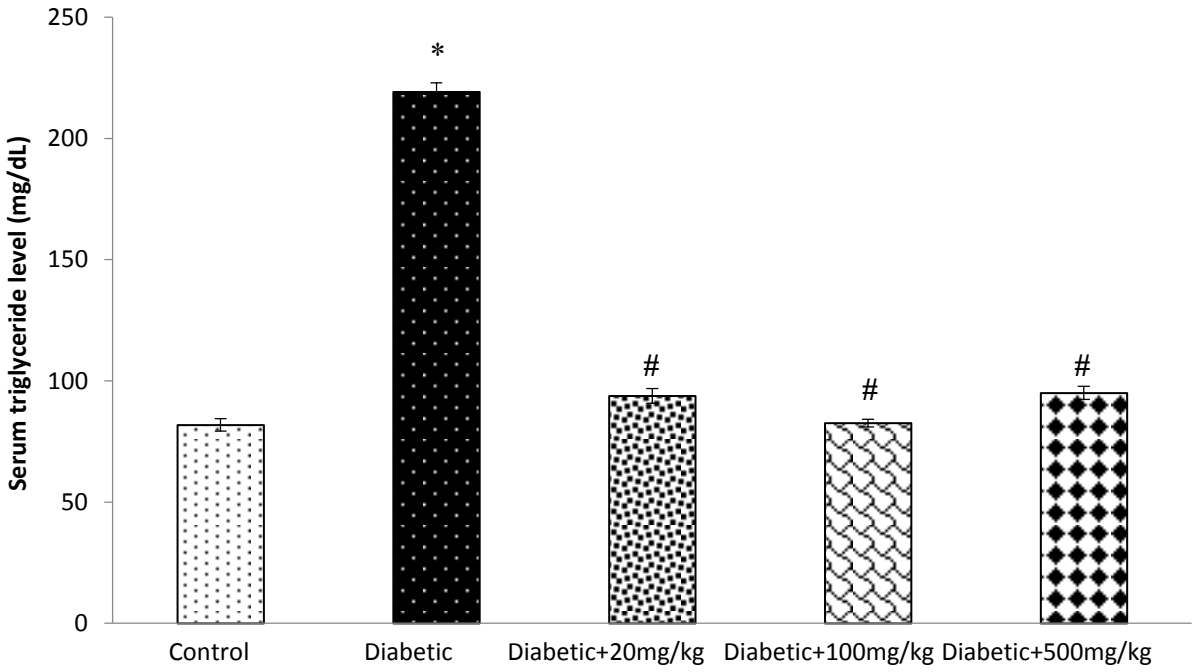

Fig.2. Effect of HEMO on serum triglyceride level. Results are presented as mean±SEM, n=6 in each group. MANOVA followed by tukey test, significance level considered as P<0.05. *: Significant difference compared to control group, #: Significant difference compared to diabetic group.

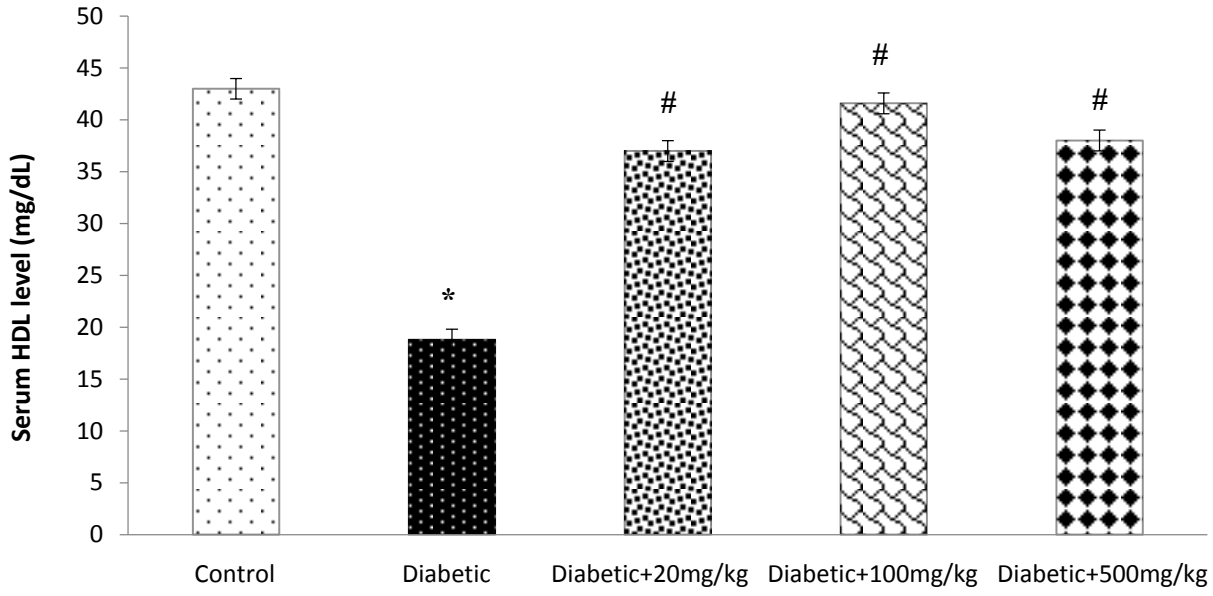

Fig.3. Effect of HEMO on serum HDL level. Results are presented as mean ± SEM, n=6 in each group. MANOVA followed by tukey test, significance level considered as P<0.05. *: Significant difference compared to control group, #: Significant difference compared to diabetic group.

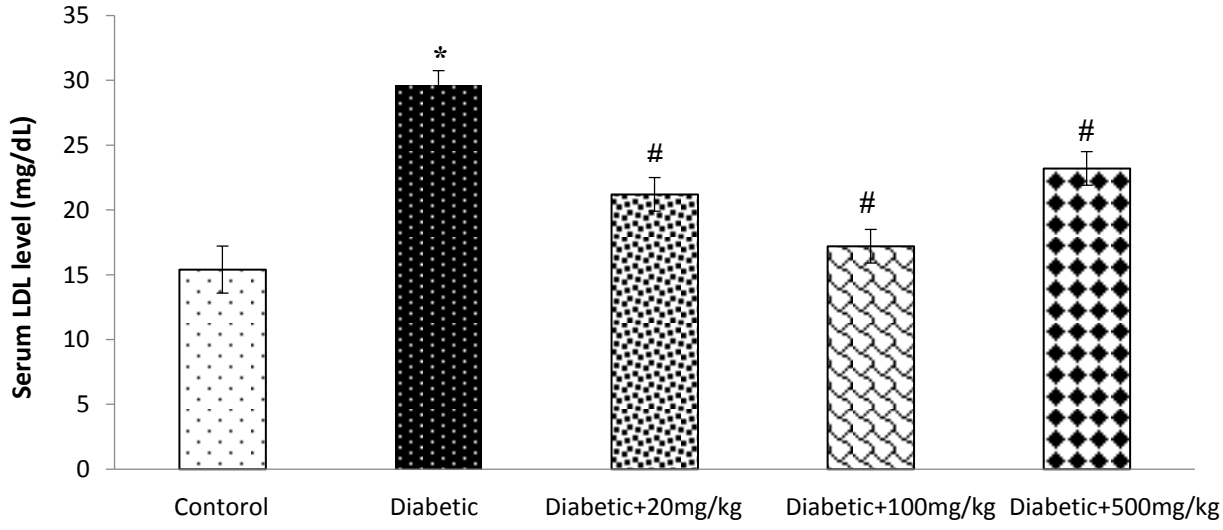

Fig.4. Effect of HEMO on serum LDL level. Results are presented as mean ± SEM, n=6 in each group. MANOVA followed by tukey test, significance level considered as P<0.05. *: Significant difference compared to control group, #: Significant difference compared to diabetic group.

serum levels of cholesterol, triglyceride and LDL and decreases serum HDL level significantly, in accordance with previous studies (Anthony and Adebimpe, 2009; Sreelatha and Inbavalli, 2012). Alloxan through destroying beta cells in the islets of Langerhans causes a massive reduction in insulin secretion. The absolute lack of insulin in animals results in metabolic changes including increased blood sugar and increased blood cholesterol (Sreelatha and Inbavalli, 2012; Rathnakar et al., 2011). For centuries, herbs are used for diabetes treatment. Previous studies have shown that several classes of chemical compounds found in plants are potentially effective in the treatment of diabetes (Kim et al., 2006; Rathnakar et al., 2011). In traditional medicine, many herbs are prescribed for diabetes

treatment (Yeh et al., 2003; Shukia et al., 2000) however the effectiveness of only some of them has been proven scientifically. This is while, herbal medicines have fewer potential side effects than chemical drugs (Rathnakar et al., 2011). Chemical analysis of the lemon balm has demonstrated that its leaves are rich in polyphenolic compounds such as rosmaric acid, trimeric compounds and some of flavonoids. These compounds can remove free radicals and have antioxidant properties which can inhibit oxidative stress induced cell death (Bayat et al., 2012; Lin et al., 2012). Main oils derived from lemon balm have high amount of tocopherols and phenolic compounds (Carnat et al., 1998) and therefore have robust antioxidant activity (Lin et al., 2012). Administration of lemon balm extract increases GSH level in the liver and blood of rats with hyperlipidemia (Bolkent et al., 2005) and may prevent damages caused by oxygen free radicals. Lemon balm contains high level of flavonoids (Bayat et al., 2012) with a variety of biochemical and pharmacological activities such as lipid lowering effects, antioxidant and cardioprotective properties (Lin et al., 2012; Wattanapitayakul and Bauer, 2001; Duh et al., 2004). Oxidative stress is a reflection of the concentration of intercellular oxidants such as H_2O_2 and O_2^-, and glutathione (GSH) serves as an endogenous antioxidant molecule. Low level of GSH is known to be associated with a number of diseases in which reactive oxygen species are produced in large quantities, including arthrosclerosis, heart failure, diabetes and neurological disorders (Wattanapitayakul and Bauer, 2001; Duh et al., 2004). Lipid lowering effect of lemon balm extract is related to its flavonoids content (Chung et al., 2010).

Conclusion

The results of this study indicated that three different doses of lemon balm extract is effective on lowering blood sugar in diabetic rats. Anti-diabetic properties of this plant may be attributed to its flavonoids and terpen contents. The results suggest that the extract at the middle dose tested (100 mg/kg) was more effective than doses of 20 and 500 mg/kg. The reason why dose of 100 mg/kg was more effective than two other doses should be examined in terms of cellular mechanisms.

Acknowledgment

The results presented in this article were extracted from the thesis of Sedigheh Khodsooz (MSc. graduate of University of Isfahan).

Conflict of Interest

The authors declare that they have no conflicts of interest.

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