Effects of *Ballota nigra* on blood biochemical parameters and insulin in albino rats

Mohamad K. Nusier [1], Hameed N. Bataineh [2], Ziad M. Bataineh [3] & Haytham M. Daradka [4]

1. Department of Biochemistry and Molecular Biology, Jordan University of Science and Technology, School of Medicine, Irbid, Jordan
2. Department of Physiology, Jordan University of Science and Technology, School of Medicine, Irbid, Jordan
3. Department of Anatomy, Jordan University of Science and Technology, School of Medicine, Irbid, Jordan
4. Department of Biology, Faculty of Science, Jarash National University, Jarash, Jordan

Correspondence to: Mohamad Nusier, MD., PhD.
Department of Biochemistry and Molecular Biology,
Jordan University of Science and Technology, School of Medicine,
Irbid 22110, Jordan
FAX: +962 2 7095010
EMAIL: mick@just.edu.jo

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Abstract

Ingestion of aqueous 70% ethanol extract of *Ballota nigra* (400 mg/kg body weight for 7 days) by Albino rats (n=10) was investigated to study its effects on glucose, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), troponin I (TnI), serum creatine kinase (CK), total protein, total bilirubin and blood urea. *Ballota nigra* extract caused a significant decrease in blood glucose, total serum cholesterol and CK levels. Blood levels of TnI, AST, ALT, triglycerides, total bilirubin, total protein and blood urea were unchanged.

The hypoglycemic effect of *Ballota nigra* extract on Albino rats was further investigated by conducting a glucose tolerance test intraperitoneally (IPGTT). Healthy rats that were fasting for 18 hours followed by administration of a dose of 400 mg/kg body weight of the crude extract of *Ballota nigra*, orally. A significant decrease in blood glucose levels (after 15, 30, and 45 minutes) with a significant increase in serum insulin level (after 15 and 30 minute) was noted. These results suggest that, the crude extract of *Ballota nigra* have hypoglycemic, insulin-releasing and cholesterol lowering effects in rats.

ORIGINAL ARTICLE

INTRODUCTION

The vast majority of modern medications were derived originally from ancient herbal traditions [1–3]. Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value [4]. There are numerous natural plant products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally [5]. Several plants containing volatile oils, polyphenols and alkaloids as active constituents are utilized as popular folk medicines, while others gained popularity in the form of finished products collectively named phytomedicines [6].

The use of medicinal plants for the treatment of diabetes mellitus dates back to the Ebers papyrus, 1550 BC [7]. Even after the discovery and use of insulin and other modern oral hypoglycaemic agents, the search for safer and more effective drugs of plant origin for the treatment of diabetes has continued [7,8].

Through the ages, physicians have attempted the treatment of diabetes mellitus with indigenous plant [9]. Many herbs and plants that exhibit hypoglycemic activity when taken orally have been described [10]. Some of these plants have also been pharmacologically proven to have a value in the treatment of diabetes mellitus [11].

Ballota nigra (Lamiaceae) is a Mediterranean plant but has a more continuous distribution in a wider range of relatively moist microhabitats, up to 800 meters above the sea level; it has less than 15 leaves, usually erects and undulates, with white to dark pink flowers [12]. Its distribution is affected positively by elevation. *Ballota nigra* prefers low-pH soils, share soil microhabitats with high clay and silt and organic matter. These habitats have low sand content, low pH, and relatively high soil moisture [12,13]. The most important constituents of *Ballota nigra* are monoterpenes and sesquiterpenes [14].

This plant was suggested to exert anti-allergic, antispasmodic, antimicrobial and anti-inflammatory properties [15]. Herein, the effect of oral administration of this plant extract on blood glucose, lipid profile and other biochemical parameters in albino rats was investigated in albino rats.

MATERIALS AND METHODS

Twenty adult male and female Albino rats weighing approximately 300 g were raised in the Animal House Unit at Jordan University of Science and Technology, School of Medicine (JUST), between April and September 2006. Rats were maintained at a controlled temperature of 21 ± 1 °C and under a 12-hr-light: 12-hr-dark schedule. Food and water were supplied *ad libitum*.

Aerial parts of *Ballota nigra* plants were collected from Zoubia area (west-north of Jordan) during spring, 2006. The aerial parts were dried and grinded into powder. Each 500 g of dried and ground *Ballota nigra* was then refluxed in (2 L) 70% ethanol at 50 °C for 36 hours in continuous extraction (soxhlet) apparatus. Ethanol extract was filtered and concentrated under reduce pressure at 50 °C using a rotary evaporator. The net yield was 30 g/kg. This material was then dissolved in distilled water and administered orally to 10 rats using animal feeding intubation needles (Popper and Sons, New York) in a concentration of 400 mg/kg body weight (1 ml volume) as single daily dose for 7 days. Determination of LD_{50} in mice was conducted to determine the dose to be given to rats. Graded doses of the aqueous extract of *Ballota nigra* in 0.2 distilled water was administered intraperitonealy to six groups of six non fasted male albino mice (25–30 g each). They were housed in transparent plastic cages at 24 °C. Mortality was noted after 1 hour [16,17]. Ten control rats received gastric infusions of 1 ml distilled water, the same way the experimental rats did. All rats were healthy and continued to receive their respective drinking water and food throughout the experimental period. On day 8, twenty four hours after the last dose, animals were weighed and autopsied under light ether anesthesia. Blood was collected by heart puncture using a sterile syringe and serum was separated for biochemical analysis. Glucose, total cholesterol, triglycerides, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum creatine kinase (CK), total protein, total bilirubin and blood urea were measured using commercial kits from Cis BIO International) Gif sur Yvette, France), serum troponin I (cTnI) (Beckman Coulterkit, Galway, Ireland), Concentrations were determined using UV/Visible spectrophotometer.

To test the hypoglycemic effect of crude extract of *Ballota nigra*, twenty more Albino rats were randomly assigned to either control or experimental groups. The control group received 2 ml of distilled water orally and the experimental groups were fed 400 mg/kg body weight crude extract of *Ballota nigra* orally. A specific stainless feeding needle was used for administration of either distilled water or the crude extract. Five minute after administration of either distilled water or extract, glucose tolerance test (IPGTT) was conducted on all rats by intraperitoneal administration of glucose (1.5 g/kg body weight). Blood glucose and insulin levels were then measured using commercial kits from Cis BIO International (Gif sur Yvette, France) after 0, 15, 30, 45 and 60 minutes.

Data were expressed as mean ± SD (statistical package for social sciences (SPSS, version 11.5)). Differences between control and *B. nigra* exposed groups were analyzed using either the Chi-square test, student t-test or nonparametric (Kruskal-Wallis) test, when applicable. A p-value of <0.05 was considered significant [18].

RESULTS

The crude extract of *Ballota nigra* caused a significant decrease blood glucose and in total serum cholesterol level ($p<0.01$). On the other hand, serum triglycerides levels were not affected. Also, the hepatic enzymes, AST

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and ALT concentrations were not significantly changed. The cardiac enzymes, CK was significantly decreased ($p<0.05$) in the treatment group in comparison with the controls, while TnI was unchanged. The concentrations of serum total protein, total bilirubin and urea were not altered (Table 1).

Data from the IPGTT experiment (Table 2) shows that administration of the crude extract of *Ballota nigra* had caused a significant decrease in blood glucose level at 15, 30, and 45 minutes. A significant increase in insulin level was observed after 15 and 30 minutes of the administration of the crude extract in the treated animals in comparison with the controls.

DISCUSSION

Diabetes mellitus is possibly the word fastest growing metabolic disorder and as knowledge of heterogeneity of this disease increases, so does for more appropriate therapies [19,20]. Oral administration of crude extract of *Ballota nigra* showed a significant reduction in blood glucose and an elevation of insulin concentration after different time-intervals in the treated rats when compared to the controls. These data suggest a significant role of *Ballota nigra* in the stimulation of β cells to secrete more insulin. Even though the mechanism is not yet clear, but may be due to the biological effect of this plant to increase secretion of glucagon-like peptide 1, which acts on β cells of the pancreas to stimulate insulin secretion as well as increase the insulin gene transcription [21,22]. Therefore, further fractionation techniques are needed to explain these results.

Traditional medicinal plants are widely used in folk's medicine to treat diabetes mellitus and the study of such plants might offer a natural key to unlock a dialectologist's pharmacy for the future [20].

The hypoglycemic effect of *Ballota nigra* in rats is the first to be reported in the literature. It is clear from the present study that *Ballota nigra* aqueous extract exhibits a hypoglycemic activity when given orally. The decrease in blood glucose level was also consistent and time-dependent. The plant extract, probably without metabolic transformation, is capable of reducing high blood glucose mainly through enhancing insulin secretion. An insulinotropic agent *of Ballota nigra* has been purified to homogeneity in our laboratory and further work is in progress to elucidate its chemical structure and properties.

Positive correlation between cholesterol plasma concentration and the risk of coronary heart disease has been widely demonstrated [23]. The search for new cholesterol lowering agents with minimal or no toxicity never stops. Therefore, the effect of *Ballota nigra* on the lipid profile was tested in albino rats of Sprague Dawley strain. Toxicity of *Ballota nigra* was investigated by testing for blood biochemical parameters such as; total bilirubin, total protein, blood urea, CK, TnI, AST and ALT. Our results demonstrated that oral administration of this extract significantly decreased the total serum cholesterol with no evident changes in triglycerides levels. To explain the cholesterol lowering effect that usually obtained using various drugs, different mechanisms were suggested including; inhibition of hydroxymethylglutaryl Coenzyme A (HMG CoA) reductase, the key regulatory enzyme in cholesterol biosynthesis [23], decrease in cholesterol absorption by the intestinal wall [24] and/or induction of LDL-receptors within the peripheral tissue [25–27]. The mechanism by which *Ballota nigra* extract decreased the

Table 1. Serum biochemistry of *Ballota nigra* fed albino rats (Mean ± S.E.)

Treatment Group	Glucose Mg\dl	Cholesterol Mg\dl	Triglyceride Mg\dl	AST IU\L	ALT Mg\dl	CK IU\L	TnI IU\L	Total Protein Mg\dl	Total Bilirubin Mg\dl	Urea Mg\dl
Control group (n=10)	92.44±2.62	193.1±5.67	97.5±5.62	123.38±7.66	48.6±2.72	431±3.51	12.5±3	7.4±1.31	0.37±0.21	57±3.11
Treated group (n=10)	77.37±3.11**	144±7.21**	83.0±4.83	121.78±4.33	46.4±1.96	348.1±9.6*	15±2	6.2±2.1	0.41±0.18	60±3.45

When Treatment Group was compared to controls: *p<0.05, **p<0.01

Table 2. The effect of *Ballota nigra* on IPGTT (1.5 g glucose/kg body weight) in 18 hour fasting normal rats. The extract was given at –5 minutes. Glucose and insulin were tested at 0.0, 5, 15, 30, 45, 60 minute intervals (Mean of ± S.E.)

	0.0 minute		5th minute		15th minute		30th minute		45th minute		60th minute	
	Glucose Mg/dl	Insulin µU	Glucose Mg/dl	Insulin µU	Glucose Mg/dl	Insulin µU	Glucose Mg/dl	Insulin µU	Glucose Mg/dl	Insulin µU	Glucose Mg/dl	Insulin µU
Control group (n=10)	113±5.67	18±2.85	116±4.44	19±2.43	230± 4.66	67±3.01	190±3.83	59.55±3.56	185±3.54	38.7±2.45	150±5.12	23±2.69
Treated group (n=10)	114±5.33	17±2.77	115±4.17	19±2.58	140±4.27***	83±2.87**	133±3.19**	71.12±3.11**	128±3.17**	36.4±2.18	120±5.32	20±2.34

When Treatment Group was compared to controls: **p<0.01, ***p<0.001

blood cholesterol level will be tested in the future work. In contrary to total cholesterol, AST, ALT levels as well as total bilirubin, total protein and blood urea were not changed. On the other hand, a significant reduction in the serum level of CK was observed. It seems likely that the administered dose of *Ballota nigra* extract was not toxic. The decrease in serum CK is unclear. However, this could be a possible effect of the plant having an inhibitory effect on the expression of CK. Several studies have reported such effect of other compounds on different enzymes [28–31].

Ballota nigra extract seems to reduce serum blood glucose, cholesterol levels with no evident toxicity. Therefore, this plant might have an important application as a hypoglycemic as well as a cholesterol lowering agent and thus might decreases the risk for coronary artery disease.

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