

Effects of *Ballota nigra* on glucose and insulin in alloxan-diabetic albino rats

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Submitted: April 16, 2007 Accepted: April 28, 2007

Key words: ***Ballota nigra*; insulin; glucose; alloxan; diabetes mellitus; albino rats**

Neuroendocrinol Lett 2007; 28(4):470–472 PMID: 17627273 NEL280407A01 © 2007 Neuroendocrinology Letters • www.nel.edu

Abstract The hypoglycemic effect of *Ballota nigra* extract on Albino rats was investigated. Alloxan-induced diabetes mellitus was accompanied by several fold increases in plasma glucose. Administration of aqueous extract of *B. nigra* extract significantly reduced glucose in both healthy and diabetic rats. These results suggest that *B. nigra* possess hypoglycemic effects in rats and therefore, can be useful for the treatment of diabetes mellitus.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value [1]. 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 phyto-medicines [2].

The use of medicinal plants for the treatment of diabetes mellitus dates back to the Ebers papyrus, 1550 BC [3]. 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 [3,4].

Through the ages, physicians have attempted the treatment of diabetes mellitus with indigenous plant [5]. Many herbs and plants that exhibit hypoglycemic activity when taken orally have been described [6]. Some of these plants have also been pharmacologically proven to have a value in the treatment of diabetes mellitus [7].

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 [8,9]. The most important constituents of *Ballota nigra* are monoterpenes and sesquiterpenes [10].

This plant was suggested to exert anti-allergic, antispasmodic, antimicrobial and anti-inflammatory properties [11]. Herein, the effect of oral administration of this plant extract on blood glucose and insulin levels in diabetic albino rats was investigated.

MATERIALS AND METHODS

Forty 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.

Determination of LD₅₀ 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 intraperitoneally 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 [12,13].

Rats were divided into four groups of ten rats each. These groups were treated as follows: Group one (normoglycemic): was given 2 ml of distilled water. Group two (normoglycemic + *B. nigra*): was given the crude extract of *Ballota nigra*, in a concentration of 400 mg/kg

body weight (1 ml volume) as single dose. Group three (hyperglycemic): was injected alloxan monohydrate (150 mg/kg body weight [Sigma, St. Louis, MO, USA]) intraperitoneally. Group four (hyperglycemic + *B. nigra*): was injected alloxan monohydrate (150 mg/kg body weight, (Sigma, St. Louis, MO, USA)) intraperitoneally and rats were fed 400 mg/kg body weight of the crude extract of *Ballota nigra*. Distilled water or *B. nigra* extract were given to all rats orally using special animal feeding intubation needles (Popper and Sons, New York).

Diabetes was induced in both groups 3 and 4 by the single intraperitoneal injection of alloxan monohydrate [14,15]. Animals were then fed 50% glucose at (5 ml/animal/5 hour interval for 24 hours). After 72 hours of alloxan injection, *Ballota nigra* extract was then orally introduced as described above. Blood samples were collected from the tail vein of rats at 0, 1, 3 and 6 hour intervals and blood glucose was determined using commercial kits (Promega Inc., Madison, WI, USA).

Data were expressed as mean \pm 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 [16].

RESULTS

The aqueous extract of *Ballota nigra*, showed a significant decrease in plasma glucose levels in both normoglycemic and alloxanized rats (Table 1). The decrease in glucose level was time-dependent and the most was observed 6 hours after administration of the *B. nigra* extract (32% and 22.3% in normoglycemic and alloxanized rats, respectively). Distilled water did not affect plasma glucose level in the control rats and those levels were statistically equivalent at various time intervals. On the contrary, blood glucose levels of untreated diabetic rats remained high throughout the experimental period.

Ballota nigra aqueous extract produced no significant change in spontaneous motor activity and rectal temperature and did not produce any noticeable changes in behavior, food, water intake and the morphology of the viscera.

Table 1. Effect of *Ballota nigra* aqueous extract on plasma glucose (Mg\dl) in normal and alloxan-diabetic rats.

Treatment	0 hour	+ 1 hours	+ 3 hours	+ 6 hours	Change after 6 hours (%)
Normoglycemic + normal saline	94.3 \pm 1.1	97.18 \pm 1.2	98.03 \pm 1.5	96.43 \pm 1.5	
Normoglycemic + <i>B. nigra</i>	90.4 \pm 1.18	88.6 \pm 1.13	77.3 \pm 1.7*	61.7 \pm 2.01**	-32
Hyperglycemic + normal saline	328.2 \pm 3.44	331.17 \pm 3.23	336.17 \pm 3.17	323.6 \pm 3.53	
Sever hyperglycemic + <i>B. nigra</i>	349.3 \pm 3.66	331.4 \pm 3.87	303.19 \pm 4.1**	271.56 \pm 3.78**	-22.3

*p<0.01, **p<0.001

DISCUSSION

Beta cells of the islets of Langerhans, which produce insulin, are destroyed in alloxan-induced diabetes [17]. Hyperglycaemia occurs because the insulin dependent tissues are unable to utilize plasma glucose and also because of the enhanced hepatic gluconeogenesis from amino acids derived from muscle proteins [18,19].

In the present investigation we have shown that *Ballota nigra* aqueous extract exhibits significant hypoglycemic activity in both healthy and alloxan-diabetic rats.

Up to date, the hypoglycemic effect of this plant has not been reported. Interestingly, the decrease in the level of blood glucose was consistent and time-dependent. However, the mechanism for lowering plasma glucose in both normoglycemic and alloxanized animals is not yet clear and additional investigation are needed. Further studies are in progress to isolate the active components and to determine their mechanisms of action.

These data clearly indicate that *Ballota nigra* extract, probably without metabolic transformation, is capable of reducing high blood glucose mainly through enhancing insulin secretion. The insulinotropic agent of *Ballota nigra* has been purified to homogeneity in our lab and further work is in progress to elucidate its chemical structure.

ACKNOWLEDGMENT

This research project was supported by the Deanship of research, Jordan University of Science and Technology, School of Medicine.

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