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Hypolipidaemic Effects of *Ballota undulata* in Rabbits

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Abstract: The aim of to study the effect of *Ballota undulata* (70% EtOH) extract on lipid profile on Rabbits. The plant extract was orally administered to the atherogenic rabbits (atherogenic diet + cholesterol powder supplement 400 mg/kg/body weight/day dissolved in 5 mL coconut oil) at dose of 1.2 g kg^{-1} body weight/day. During the overall period of the experiment blood was collected and serum was analyzed for lipid profile. Animals were sacrificed; the heart and the liver were collected and kept at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) level were made for cholesterol, phospholipids and triglycerides. In addition blood serum was analyzed further for HDL-Cholesterol. All the results were statistically analyzed using students t-test. Hypolipidaemic nature of *Ballota undulata* (70% EtOH) extract was studied in hyperlipidaemic Rabbits. The increased cholesterol levels were brought to normal by administration of *Ballota undulata*. Serum cholesterol levels dropped from 940.7 to 230.41 (75.55%) and further to 119.2 (87.32%) by the end of the experiment. Similarly, phospholipids and triglycerides levels were observed to be also reduced. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. *Ballota undulata* possesses active hypolipidaemic constituents.

Key words: Hyperlipidaemic, atherosclerosis, HDL-cholesterol, *Ballota undulata*

INTRODUCTION

The vast majority of modern medications were derived originally from ancient herbal traditions (Yu *et al*., 2006; Lahans, 2007; Ngemenya *et al*., 2006). Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value (Ramoutsaki *et al*., 2002). There are numerous natural plant products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally (Lee *et al*., 2007). 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 (Shanker *et al*., 2007).

Ballota undulata (Lamiaceae) is a Mediterranean plant but has a more continuous distribution in a wider range of relatively moist microhabitats, up to 800 m above the sea level; it has less than 15 leaves, usually erects and undulates, with white to dark pink flowers (Citoglu *et al*., 2004). Its distribution is affected positively by elevation. *Ballota undulata* 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 (Citoglu *et al*., 2004; Zaghloul, 2003). The most important constituents of *Ballota undulata* are monoterpenes and sesquiterpenes (Bader *et al*., 2003).

This plant was suggested to exert anti-allergic, antispasmodic, antimicrobial and anti-inflammatory properties (Al-Bakri and Afifi, 2007). 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.

In this study, the aim is to determine the effect of oral administration of *Ballota undulata* plant extract on the cholesterol fed albino Rabbits.

MATERIALS AND METHODS

Animals: Adult healthy albino rabbits weighing 1.6-1.7 kg were housed individually in metallic cages in an air-conditioned room (26±2°C) were fed control diet (standard pellets). This diet was supplemented with green leafy vegetables and water *ad libitum*. The average consumption of diet was calculated 200 g day^{-1}.

Atherogenic diet was prepared by mixing wheat flour, milk powder, dried egg yolk. Hydrogenated fat, butter, salt jaggery and vitamins in the given proportion.

Component	Control (g %)	Atherogenic diet (g %)
Protein	20	15
Carbohydrate	65	60
Sucrose	3	3
Fat	5	15
Salts	4	4
Vitamin	1	1
Fiber	2	2

In addition to the atherogenic diet, the rabbits were fed with cholesterol 400 mg kg^{-1} body weight day^{-1} dissolved in 5 mL coconut oil.

Plant and treatment: Aerial parts of *Ballota undulata* plants were collected from Zoubia area (West-North of Jordan) during spring, 2006. The aerial parts were dried and grinded into powder in Philadelphia University. Each 500 g of dried and ground *Ballota undulata* was then refluxed in (2 L) 70% ethanol at 50°C for 36 h 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^{-1}. The concentrate was dissolved in a normal saline and used. The extract, 1.2 g kg^{-1}, was administered orally to rats using animal feeding intubation's needles (Popper and Sons, New York).

Determination of LD_{50} in mice: Determination of LD_{50} in mice was conducted to determine the dose to be given to rabbits. Graded doses of the aqueous extract of *Ballota undulata* 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 h (Hruskova *et al*., 1961; Litchfield and Wilcoxon, 1949).

Experimental design: Rabbits were divided in the following groups of six animals each:

Group A: Vehicle (5 mL normal saline) treated control (120 days).

Group B: Atherodiet + cholesterol feeding (120 days, 400 mg cholesterol/kg body weight/day in 5 mL coconut oil).

Group C: Atherodiet + cholesterol feeding (120 days, atherodiet withdrawn + *Ballota undulata* L. (70% EtOH) 1.2 g kg^{-1} body weight day^{-1} (120-150 days).

Group D: Atherodiet + cholesterol feeding (120 days, Atherodiet withdrawn + *Ballota undulata* L. (70% EtOH) extract 1.2 g kg^{-1} body weight day^{-1} (120-180 days).

All the animals were sacrificed and the heart, the aorta and the liver were removed, cleaned from the fat and adhering connective tissue and kept at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) were made for cholesterol (Zlatkis *et al*., 1953), Phospholipids (Zilversmit and Davis, 1950) and triglyceride (Gottfried and Rosenberg, 1973). In addition blood serum was analyzed further for HDL Cholesterol (Burnstein *et al*., 1970).

Statistical analysis: Data were expressed as mean±SD (statistical package for social sciences [SPSS, version 11.5]). Differences between control and *B. undulata* exposed groups were analyzed using either the Chi-square test, student t-test or nonparametric test, when applicable. A p-value of <0.05 was considered significant (Ipstein and Poly, 1970).

RESULTS AND DISCUSSION

A non-significant reduction in the body weights was noticed in rabbits fed with cholesterol diet and later treated with *Ballota undulata* L. extract (Groups C and D) in comparison with the initial body weights. A non-significant change in weight of heart was. Liver weight was significantly increased in cholesterol fed rabbits (Table 1).

Ballota undulata L. (70% EtOH) extract feeding (Groups C and D) resulted in a significant lowering of total cholesterol, triglycerides and phospholipids of liver and ventricular heart muscles in comparison with cholesterol fed rabbits. In group D the reduction was on higher side (Table 2).

An eight-fold increase was observed in serum cholesterol in treated rabbits fed with atherogenic diet ($p \leq 0.001$). In addition a significant reduction in the blood serum cholesterol was recorded in both *Ballota undulata* L. treatment group (Gr. C and D). Serum triglyceride increased significantly ($p \leq 0.001$) after cholesterol feeding but was subsequently reduced after *Ballota undulata* L. extract treatment. An increased in phospholipids and HDL cholesterol followed by cholesterol diet could be corrected by *Ballota undulata* L. extract feeding (Table 3).

The LD_{50} of the aqueous extract of *Ballota undulata* L. was 4.14 g kg^{-1} body weight (according to the Litchfield and Wilcoxon, 1949) method which represents 20.54 g of crude powdered plant material 1 kg body weight.

A positive correlation between cholesterol plasma concentration and the risk of coronary heart disease has been widely demonstrated by the lipid research Clinics Primary Prevention Trails (Choi *et al*., 1991). In order to find good means to decrease plasma cholesterol level with minimal toxicity.

The level of cholesterol in lipoprotein fractions has been shown to be a good indicator of atherosclerosis risk

Table 1: Change in body, liver and heart weight after cholesterol/*Ballota undulata* L. (70% EtOH) extract feeding in rabbits (8 animals per treatment)

	Body weight (kg)		Body weight (%)	
Treatments	Initial	Final	Liver	Heart
Group A	1.68±0.11	1.72±0.73	2.12±0.26	0.20±0.65
Group B	1.58±0.07	1.44±0.89	4.15±0.13	0.23±0.32
Group C	1.65±0.32	1.47±0.33	2.17±0.36	0.21±0.43
Group D	1.60±0.11	1.45±0.27	2.07±0.33	0.20±0.20

Table 2: Change in tissue lipids after cholesterol/*Ballota undulata* L. (70% EtOH) extract feeding in rabbits (8 animals per treatment)

	Cholesterol		Triglycerides (mg g^{-1})		Phospholipids (mg g^{-1})	
Treatments	Liver	Heart	Liver (g)	Heart (g)	Liver	Heart muscles
Group A	9.70±0.11	6.4±0.33	3.5±0.20	4.01±0.26	7.66±0.54	9.13±0.04
Group B	17.30±0.66[c]	8.1±0.70[c]	5.1±0.22[c]	12.40±0.34[c]	12.50±0.57[c]	9.92±0.02[c]
Group C	10.15±0.30[c]	10.1±0.10[c]	5.2±0.20[b]	4.28±0.81[d]	9.40±0.81[c]	8.38±0.06[c]
Group D	9.88±0.16[c]	7.9±0.70[c]	3.9±0.83[c]	3.92±0.44[c]	8.36±0.23[c]	7.99±0.07[c]

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p \leq 0.001$, [d]NS = Non-significant

Table 3: Change in serum analysis after cholesterol/*Ballota undulata* L. (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatments	Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Phospholipids (mg dL^{-1})	HDL cholesterol (mg dL^{-1})
Group A	97.8±4.00	63.70±2.88	168.00±7.50	24.80±2.80
Group B	807.4±7.40[c]	259.80±6.56[c]	252.32±8.57[c]	246.00±6.70[c]
Group C	203.8±8.66[b]	89.20±10.5[c]	173.76±7.33[b]	68.10±2.87[c]
Group D	104.7±9.33[c]	74.18±3.66[c]	112.60±8.91[c]	40.67±1.67[c]

[b]$p \leq 0.01$, [c]$p \leq 0.001$

in rabbits (Azzarito *et al*., 1996). Significant lowering of cholesterol after *Ballota undulata* L. feeding indicates a risk reduction action.

Plasma triglycerides and cholesterol carry the highest risk for ischemic heart disease (Carlson and Bottiger, 1985), HDL and LDL cholesterol are significant variables and indicator for coronary heart disease (Miller and Miller, 1975). It is reported that HDL is inversely related to total body cholesterol. Treatment with *Ballota undulata* L. extract reduces serum cholesterol and triglyceride by 7.8 and 3.5 times, respectively. HDL alters the balance of unesterified cholesterol between plasma and cell by increasing its utilization in the lecithin cholesterol acyl transferase (LCAT) system to form cholesterol ester which moves rapidly into the cells. Decreased total cholesterol and phospholipid after *Curcuma longa* extract feeding indicate the anti- atherogenic or hypolipidaemic nature of the plant product. Further reduction in total cholesterol, triglyceride and phospholipids of liver and ventricular heart muscle may be suggestive of a beneficial role of *Ballota undulata* L. in hyperlipidaemic subject.

The possible mechanism of lipid alteration might be cholestatic effect of *Ballota undulata* L. in liver enhanced removal or catabolism of lipoproteins (Brattsand, 1975) and/ or inhibition of lysosomal lipid hydrolytic enzymes secreted by the liver (Sherlock, 1998). In conclusion *Ballota undulata* L. possesses active hypolipidaemic constituents. Further chemical and pharmacological investigations are in progress.

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