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Hypotensive Activity of *Melilotus Officinalis* (L.) Pallas

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Abstract

Melilotus officinalis (L.) Pallas., is used traditionally to treat hypertension and chronic venous insufficiency. The high prevalence of hypertension and the side effects of antihypertensive drugs potentiate us to evaluate *Melilotus officinalis*. The hypotensive effect of *Melilotus officinalis* butanolic fraction (MOBF) was investigated in anaesthetized normotensive rats. The doses of 60, 80 and 100 mg/kg, i.v., produced a significant ($P < 0.05$) decrease in blood pressure. The statistically non significant reduction in blood pressure was showed by 20 and 40 mg/kg, i.v. These results suggest that *Melilotus officinalis* exhibited hypotensive effect in normotensive rats. However further evaluation of compound related activity is required.

Keywords: *Melilotus officinalis* (L.) Pallas, mean arterial pressure, hypotension.

Introduction

Melilotus officinalis (L.) Pallas, (Fabaceae) is an annual to biennial erect or decumbent plant, 30-250 cm tall. The plant is distributed in Pakistan, Kashmir, India, Tibet, Russia, China, Turkey,

Middle and Southern Europe [1]. The herb has aromatic, emollient and carminative properties [2]. Traditionally it is used to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances and for the symptomatic treatment of bruises, sprains and insect bites[3], in liver diseases and as a diuretic[4]. The aerial parts of plant are traditionally reported to use in hypertension and chronic venous insufficiency in Montenegro[5]. The dried leaves and flowers have been used in Europe as a treatment of arthritis, bronchitis, hemorrhoids and rheumatism[6]. The anticoagulant properties of this plant is due to the presence of sweet smelling coumarins[7]. *Melilotus officinalis* (L.) Pallas, possesses melilotin and other coumarin glycosides and flavonoid pigments [8]. It contains fatty acids, triterpenes, glycosides [9], flavones, volatile oils, resins and tannins[4]. The chemical structure of reported coumarin, flavonoids and glycosides are mentioned in Figure.1. The presented study is an attempt to evaluate the hypotensive effect of *Melilotus officinalis* (L.) Pallas., in anaesthetized normotensive rats.

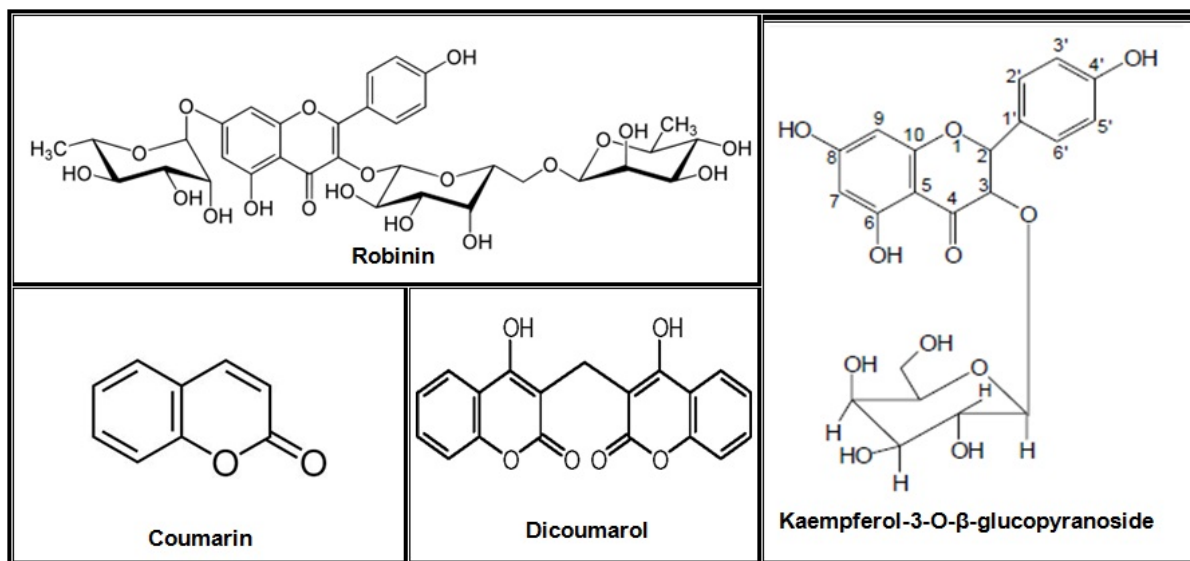


Figure 1. Chemical structure of constituents isolated from *Melilotus officinalis*

Material and Methods

Plant material

Plant material was collected in September 2012 from University of Karachi. The sample specimen was kept in department of Pharmacognosy for further reference

Extraction

The plant material was dried in shade and soaked in ethanol for a week at room temperature. The filtered ethanolic extract was partitioned with *n*-butanol.

Animals

Albino Sprague-Dawley rats, of either sex weighing 220-250g, were used. They were housed in plastic cages with saw dust as beddings at room temperature and given food and water *ad libitum*. The animals were treated complying with the international standards for dealing the experimental animals duly approved by the legal bodies of the University of Karachi. Animals were randomly divided into control and treated groups of five animals each. The groups of animals were transferred in different cages and marked with their identification.

Hypotensive activity

Normotensive rats were anaesthetized with sodium pentothol. Spontaneous respiration was maintained by inserting tracheal canula (polyethylene tube of 92.75mm diameter), after tracheotomy. The left jugular vein was cannulated with polyethylene tube (1 mm diameter) for intravenous administration of drug and flushing of 0.9% NaCl [10]. For arterial blood pressure measurements, a polyethylene canula (polyethylene tube of 1mm diameter) filled with heparin was placed into right carotid artery. The exposed surface with the cannula was covered with cotton moist in warm saline. The cannulation of the carotid artery was performed in the same manner as the cannulation of jugular vein and the polyethylene tube (1 mm diameter) filled with heparin

sodium in saline solution was used [11]. The arterial blood pressure was measured from the left carotid arterial cannula connected to a Research grade blood pressure transducer (Harvard, 60-3003) with Oscillograph (Harvard) for recording[12]. The animal was allowed to equilibrate for at least 30 minutes before commencing the experiment. Blood pressure was monitored until steady baseline levels were obtained. Crude butanolic extract of *Melilotus officinalis* (20,40, 60, 80 and 100 mg/kg, i.v.) were then administered and flushed in with 0.2 ml saline[13].

The oscillograph was adjusted by keeping its channel amplifier's knob on DC, and +ve polarity. The pen position on chart paper was adjusted using zero knobs slightly above the lowest pen position. The pressure transducer was calibrated by using "Sphygmomanometer" to standardize 1 mm pen deflection on chart paper for its equivalency with pressure (mmHg) [14]. The mean arterial Blood pressure (MABP) was calculated using the following formula [15]:

$$\text{MABP} = \text{DP} + 1/3 (\text{SP}-\text{DP})$$

Where, DP= diastolic pressure; SP= systolic pressure

$$\% \text{ fall in MABP} = \text{MABP} [\text{Control} - \text{Treated} / \text{Control} \times 100]$$

The response of mean arterial blood pressure (MABP) was expressed as percent change from the control measurement taken immediately before injection[16].The methodology is mentioned in Figure-2.

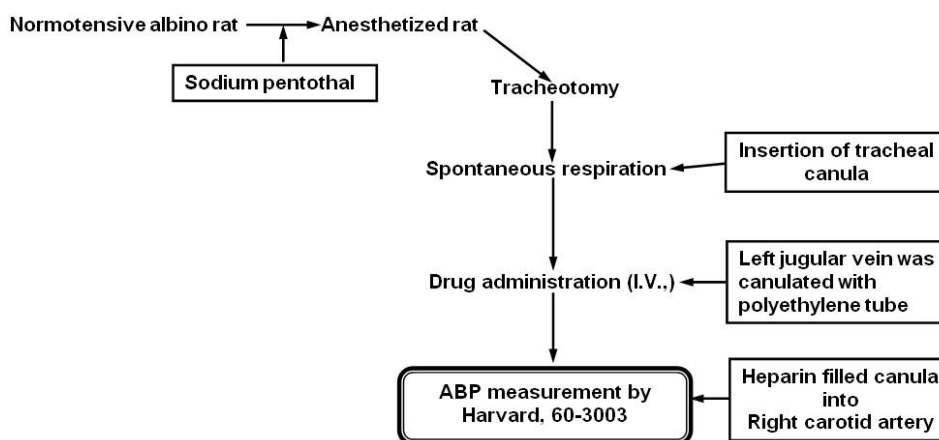


Figure 2. Methodology of Hypotensive activity

Statistical Analysis

The values were expressed as mean \pm standard error of mean. The statistical significance of the difference was determined by an unpaired Student's *t*-test. The *p*-values less than 0.05 ($P < 0.05$) vs. control are considered statistically significant.

Results

The effect of MOBE in a dose of 20,40, 60, 80 and 100 mg/kg, i.v. on MABP of normotensive rats is shown in figure 3 & 4. The 60, 80 and 100 mg/kg i.v., produced a significant ($P < 0.05$) decrease in blood pressure. The doses of 20 and 40 mg/kg, i.v., showed statistically non significant reduction in blood pressure. The dose of 80 mg/kg i.v., showed maximum (57.14) % fall in MABP whereas 40 mg/kg i.v., showed minimum % fall in MABP (15.9).

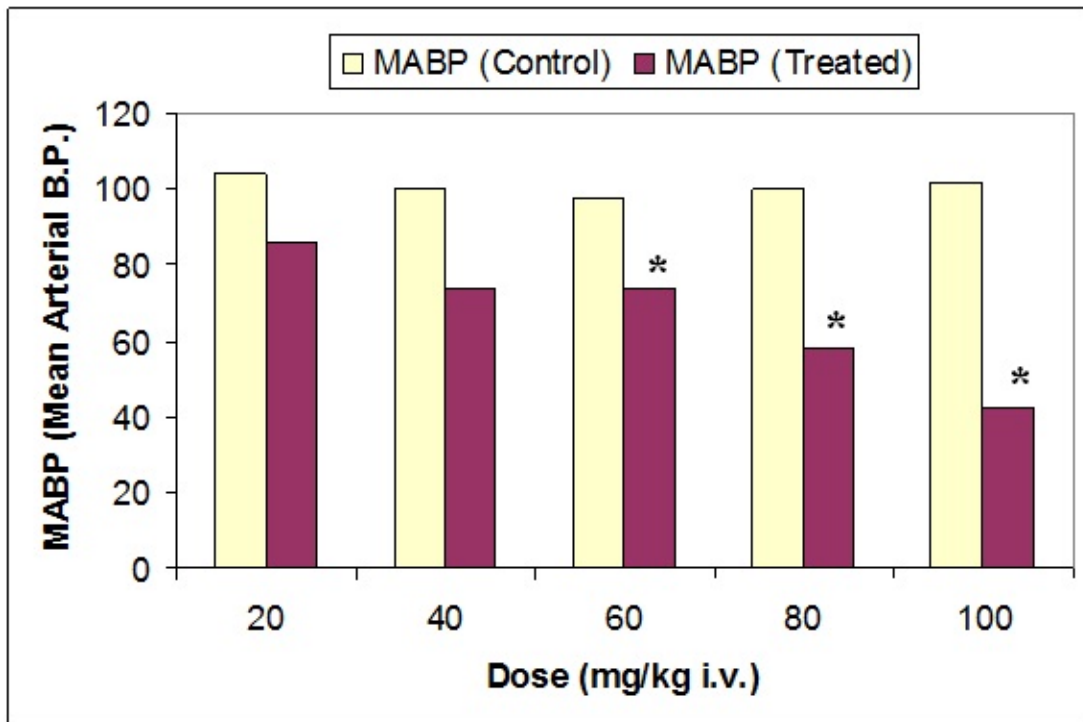


Figure.3. Effects of *Melilotus officinalis* on MABP of anaesthetized rats in different doses. n= 5, values= mean± S.E.M., * = P<0.05

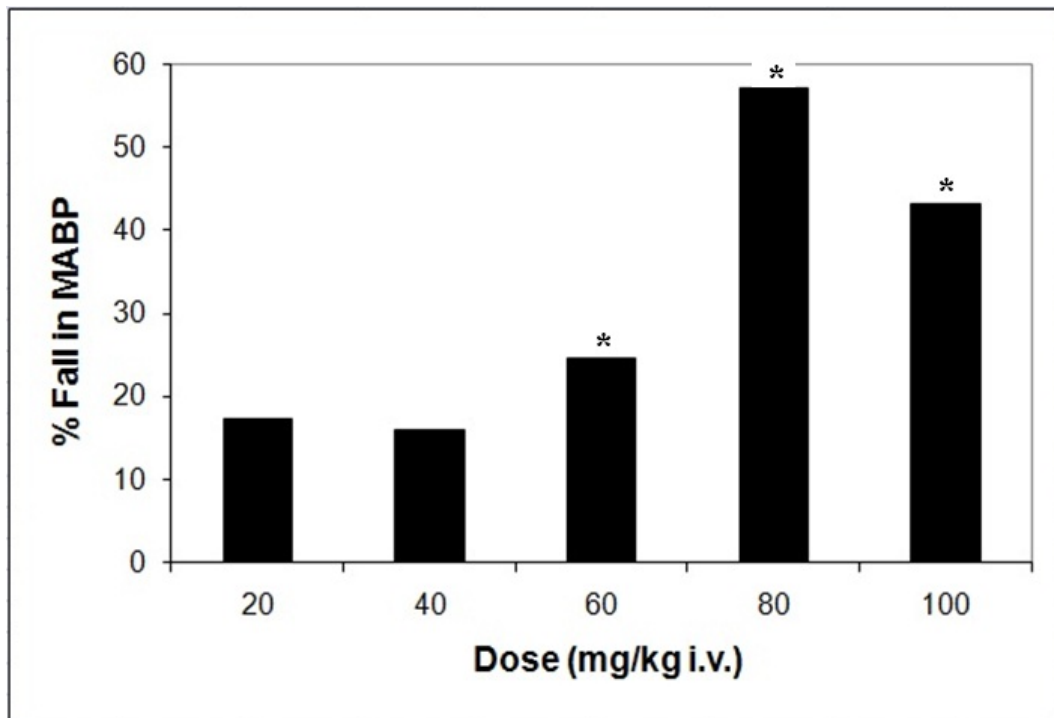


Figure.4. Effects of *Melilotus officinalis* on % fall in MABP of anaesthetized rats in different doses. n= 5, values= mean± S.E.M., * = P<0.05

The presented study scientifically proves the traditional use of *Melilotus officinalis* (L.) Pallas in hypertension [5]. The coumarin glycosides [17] and flavonoids [18, 19] possess antihypertensive activity. Chemically *Melilotus officinalis* contains coumarin glycosides [7] and flavonoids [4]. So, it may be possible that hypotensive effect of MOB is due to the presence of coumarin glycosides and / or flavonoids figure.5. However, the present study is on preliminary level and further

investigation is required to confirm the hypotensive effect of *Melilotus officinalis* and also determine the compounds responsible for this activity with their possible mode of action.

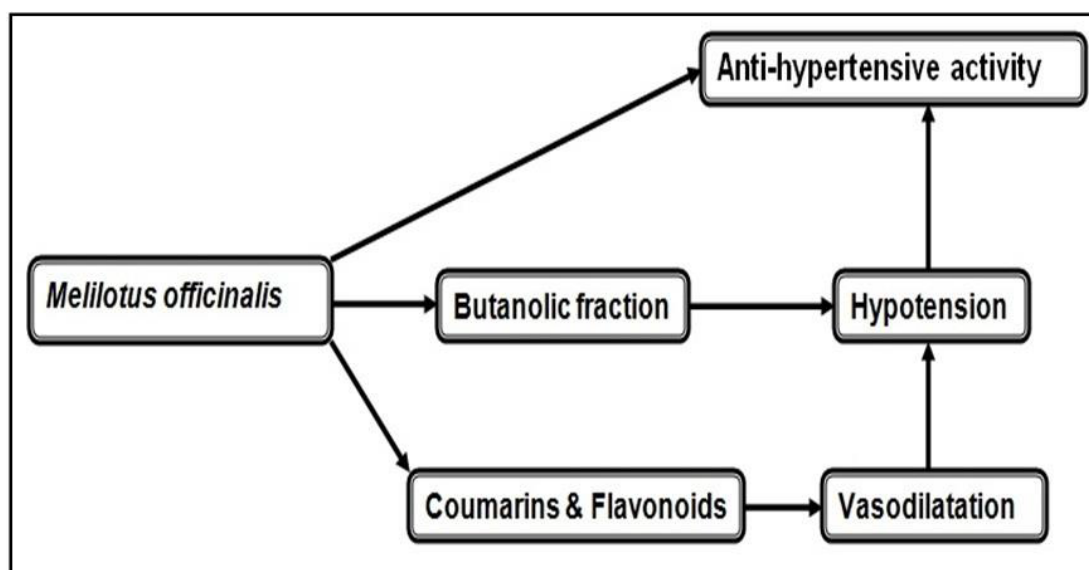


Figure.5. Proposed hypotensive mechanism of *Melilotus officinalis*

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