

The Effect of Ethanolic Extract of *Melilotus officinalis* Flowers on Bleeding Time in Male and Female Rabbits

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#### Abstract

The present study aims to examining the effect of the ethanolic extract of *Melilotus officinalis* flowers on bleeding time ,WBC and RBC count .Total number in males and females adult rabbit eighteen, (9 males and 9females ) are randomly divided into three equal groups (3 males and 3females per group). The first (control) is drenched normal saline while the second and third groups are drenched the ethanolic extracted (20 and 50mg/ml/kg of B.W) for 24 hours. The results reveal significant increase in bleeding time in third group followed by the second group compared with the control while RBC and WBC count record no significant differences .On the other hand the gender had no significant effect on the studied parameters.

**Key Words : Melilotus officinal , Ethanolic Extract**

### INTRODUCTION

*Melilotus officinalis* L, Lam, (M.o) is ageneses of herbs including 20-25 species, that are widely distributed all over the world, the main species used for medical purposes contain *melilotus officinalis* L, Lam, *Melilotus desr*, *Melilotus suaveolens* Lebed and other. Most of these species used as herbal medicine to treat inflammation and infection in throat and alimentary system in china (Sichtuan Ganzi Institute for Drug ,1995).

In literatures, there are a few reports on *melilotus*, it was documented that it used to reduce spasm (Zoltan and Foldi,1970), its coumarinic extract have effects on lymph edema (Pastura & etc,1999) and its polysaccharides have immunocorrecting; anti- anemia and

adoptogenic effect (Podkolzin & etc,1996).

Number of studies has tried to screen out potential candidate activity of anti coagulants and anti platelets agents; some herb has been identified with potent anti coagulant and anti platelet activity (Dong &etc,1998).

Although anti coagulant effect of *Melilotus officinalis* was reported (Smith & Gorz,1965), but need for more investigation, especially for local species of this herb.

The aim of this study is to investigate in vivo the effect of local species of the herb on bleeding time and other hematological parameters of male and female rabbits.

## Material and Methods

1 –Animals: 18 local adult domestic rabbits (8 -12) month of age, and (1.2 -1.8) Kg body weight were used .Those rabbits were kept in animal house at  $25\pm 3^{\circ}\text{C}$  and 12/12 light /dark cycles.

The animal fed on standard diet {corn40%, wheat15%, barley 13%, soybean 28% and protein 0.5%, and vitamin/ minerals 0.5%} and water , which were given ad libitum. The rabbits were given anti –coccidiosis (Amprollium) through the drinking water (1gm /liter) for two weeks as prophylaxis dose; the animals were left for one month in their cages for adaptation.

2 – Preparation of *Melilotus officinalis* extraction:

Fifty grams of dry flowers of *Melilotus officinalis* was collected from local field, and kept for air dried for more than on week, then were refluxed with 200 milliliter ( ethanol 70% ) for 12 hours by soxhlate and then filtered by using buchner funnel and filter paper (wattman No.185). The solvent was dried and concentrated by using rotary evaporator at  $50^{\circ}\text{C}$ . The final dryness was done by leaving residue in room temperature. The resultant extract (2.6 grams) , which was deep greenish and viscous liquid. The extract was kept in dark glass container at  $4^{\circ}\text{C}$ .

3 –Experiment design:

The eighteen rabbits from both gender ( 9 male , 9 female ) are divided equally and randomly into three groups, each one 6 rabbits has both genders ( 3 male, 3 female )

,the first group used as control group , and given a single dose of normal saline ( 1ml ), while the other two groups treated with specific dose ( 20 mg/ml/kg, and 50 mg/ml/kg) body weight , using stomach tube for oral administration .

The injury induced by lancet in to ear vein at different time using Dukes test, ( 1 hour , 3 hour , 6 hour , and 12 hours) for 24 hours. To determine the bleeding time and collection the blood for erythrocytes counts and white blood counts.

4 –Statistical analyses :

All determinations are subjected to calculation of mean and SD for the outcome variable, statistical analyses are performed by using SAS(2001) and using CRD (Complete Randomize Design) and using Duncan test Statistically significance level was set at ( $p < 0.05$ ) (Duncan ,1955)

## Results and Discussion

The effect of *Melilotus officinalis* flower extract on bleeding time:-

The dose of 20m/kg body weight did not show prolong in bleeding time during all the period of the experiment. While the dose of 50 mg/kg body weight showed significant increase the bleeding time in compares with zero time, and other times of experiment (tables -1a & 1b).

The results show the dose of 50mg/kg caused significant increase in bleeding time at 6 hours compared with dose of 20 mg/kg , while at 12 hours post the administration exhibited significantly prolongation ( $p < 0.05$ ) in bleeding time compared with 20 mg/kg .

In general one can notice from the results above that the animals which were given 50mg/kg showed gradually increase in coagulation time till reach to the maximum at the first 12 hours and then gradually decrease with time.

The effect of the extract of both doses on the gender, there was no significant differences as in (table - 2).

On erythrocyte count:-

As shown in( table -3), there was a significant decrease ( $p<0.05$ ) in erythrocyte count between the animal that received 50mg/kg, compared with the control group.

On white blood cells count:-

As shown in( table -4) the significant increase ( $p<0.05$ ) was brought out at 18 hours, and 24 hours between the animal administered with extract compared with control group.

Table (1- a) The effect of different doses of *Melilotus officinalis* (M.o) on the mean ( $\pm$  S.E) of coagulation time of adult rabbits (n=6)

treatment	0 time	1 hour	6hour	12 hour
Control group	1.35 $\pm$ 0.45	2.01 $\pm$ 0.47	2.1 $\pm$ 0.46	2.05 $\pm$ 0.43
20 mg/kg of M.o	1.4 $\pm$ 0.46	2.16 $\pm$ 0.48	2.3 $\pm$ 0.5	2.03 $\pm$ 0.63
50 mg/kg of M.o	1.53 $\pm$ 0.32	2.57 $\pm$ 0.31	2.75 $\pm$ 0.24	2.78 $\pm$ 0.24

Small letter refer to a significant differences between groups ( $p<0.05$ ). Capital letter refers to significant differences between times of same group.

Table (1- b) The effect of *Melilotus officinalis* (M .o) on the mean ( $\pm$ S.E) of coagulation time of adult rabbits, on second day; (n =6 )

treatment	0 time	1 hour	6hour	12 hour
Control group (N.S)	1.46 $\pm$ 0.43	2.04 $\pm$ 0.42	2.12 $\pm$ 0.23	2.21 $\pm$ 0.44
20 mg/kg of M.o	2.02 $\pm$ 0.37	2.15 $\pm$ 0.36	2.16 $\pm$ 0.41	2.11 $\pm$ 0.43
50mg/kg of M.o	2.66 $\pm$ 0.33	0.322.55 $\pm$	2.12 $\pm$ 0.31	2.02 $\pm$ 0.22

Small letter refer to a significant differences between groups ( $p<0.05$ ). Capital letter refers to significant differences between times of same group.

Table (2) The effect of *Melilotus officinalis* extract 50mg/kg, on the mean ( $\pm$  S.E) of coagulation time in males and females rabbits.

Treatment with 50mg/kg M.o	0 time	1 hour	3 hours	6 hours	12hours
Male	1.95 $\pm$ 0.43	2.05 $\pm$ 0.31	2.35 $\pm$ 0.26	2.9 $\pm$ 0.34	2.4 $\pm$ 0.38
Female	1.90 $\pm$ 0.41	1.94 $\pm$ 0.28	2.64 $\pm$ 0.28	3.0 $\pm$ 0.28	2.3 $\pm$ 0.32

Table (3) The effect of *Melilotus officinalis* on erythrocyte count of rabbits (n=6)

Treatment	O time	6 hour	12 hour	24 hour
Control group	595±12.88	604.3±1.3	601.4±1.3	2.4±13.8
20 mg/kg of M.o	470±41.3	518.1±38.13	538.4±37.14	532.7±36.9
50 mg/kg of M.o	432.2±30.87	461.2±25.7	501.6±32.7	499.6±14.7

Table (4) The effect of *Melilotus officinalis* on leukocyte count (WBC) on rabbits (n=6)

Treatment	O time	6 hour	12 hour	24 hour
Control	7.34±0.49	7.29±0.43	7.4±0.44	7.3.8±0.43
20 mg/kg of M.o	8.2±0.71	8.9±0.51	9.1±3.8	9.2±0.41
50 mg/kg of M.o	7.9±0.44	9.02±0.46	9.4±0.46	8.9±0.34

The *Melilotus officinalis* L, Lam; (M.o), or sweet clover proved to be nutrition when used as fresh fodder or as hay, (Farm Chemicals hand book, 1994); also brought a fetal disease in which the cattle developed sever; spontaneous bleeding (Mike,1990).

Most of the reports and study talk about the toxicity of M.O on large animals (cattle), and for more than one month pasture (Lake ,1999). In this study using the extract of the flower of ( M .o) in two doses and administered to small animal (rabbits) .we notes a changing in bleeding time through out the time, of 1 hour, 3 hours, 6hours, and 12 hours after single oral dose of the extract and the toxicity either by intra muscular or intera peritoneal of the extract , in this study , we investigate the effect of single oral dose of ethanol extract of M.o simulate the acute dose which represent the actual life situation in

which the general public take these herbal supplement, using animal model .The result shows the effect started after 3 hours and reach the peak at 12 hours, when using the higher dose (50mg/kg, body weight) , this due to the effect of M.o on gastrointestinal tract of the rabbits causes transitory and repetitive inhibition of motility and decrease of gastro intestinal muscle tone by melilotus extract may greatly contribute to facilitate G.I.t transit of contents (Emanula & Llarria,2006) . Our explanation for the increasing in bleeding time due to the effect of M.o on vitamin K, since M.o inhibit vitamin K epoxide reductase ,which catalyses an essential reaction in the vitamin K-dependent path way. For erythrocyte and white blood count increasing, may be due to its polysaccharides have immunocorrecting, anti- anemia and adoptogenic effect (Podkolzin &etc,1996). Finally we look forward

to repeat the experiment, with other blood parameter and for long g period.

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## تأثير المستخلص الكحولي لأزهار نبات الحندقوق في زمن النزف وعدد كريات الدم البيضاء والحمراء في ذكور وإناث الأرانب

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### الخلاصة

هدفت التجربة الحالية دراسة تأثير تجريع جرعة مفردة من المستخلص الكحولي لأزهار نبات الحندقوق *Melilotus officinalis* في زمن النزف المستحدث تجريبيا وإعداد كريات الدم الحمراء والبيضاء في ذكور وإناث الأرانب البالغة . تم تقسيم 18 أرنبًا بالغًا (9 ذكور و 9 إناث) على ثلاث مجموعات ضمت الواحدة منها (3 ذكور و 3 إناث) جرعت الأولى (السيطرة) المحلول الفسيولوجي وجرعت الثانية والثالثة محلولًا يحتوي على المستخلص الكحولي لأزهار نبات الحندقوق بجرعة 20 50 ملغم

24 / /

أظهرت النتائج زيادة معنوية في زمن النزف لحيوانات المجموعة الثالثة تلتها المجموعة الثانية بالمقارنة مع السيطرة في حين لم يظهر التحليل الإحصائي أية فروقات معنوية في أعداد كريات الدم الحمراء والبيضاء . أظهرت معنوية بين الجنسين.

الكلمات المفتاحية : نبات الحندقوق ، المستخلص الكحولي