

HYPOTENSIVE EFFECTS AND TOXICOLOGICAL PROFILE OF MULBERRY TREE ROOT BARKS

Aydın ERENMEMİŞOĞLU*, Hüseyin BEYDAĞI**, Fariborz BEHFEROOZ***, Hüseyin ÜSTÜN****, Mekin TANKER*****, Kadirhan SUNGUROĞLU*****

Anahtar Terimler: *Morus alba L, Morus nigra L, hipotansif etki*

Key Words: *Morus alba L, Morus nigra L, hypotensive effect*

SUMMARY

The root bark of the mulberry tree has been used in Chinese Herbal Medicine. Some hypotensive compounds have been isolated from the root barks of mulberry tree. Hypotensive effects of mulberry root barks (*Morus alba L* and *Morus nigra L*) were investigated in this study.

Morus alba L root bark extract lowered the blood pressure of dogs in a dose-dependent manner. Hypotensive response was accompanied by a slight bradycardia. They produced slightly falls in blood pressure and heart rate which generally lasted less than 3 min. Whereas, *Morus nigra L* root bark extract was found ineffective on blood pressure. In addition, subacute and chronic toxicity studies were performed in mice by *Morus alba L* root bark extract. Drug was administered at dosages of 50 and 200 mg/kg by daily single subcutaneous injection. The treatment lasted 3 weeks for subacute and 3 months for chronic examination. Animals showed no abnormal change attributable to the drug.

ÖZET

Dut Ağacı Kök Kabuğunun Hipotansif Etkisi ve Toksikolojik Profilinin İncelenmesi

Dut ağacı kök kabuğu geleneksel Çin tıbbında kullanılan bir maddedir ve hipotansif etkisi olduğu kabul edilen bazı belişikler izole edilmiştir. Bu çalışmada iki tür dut ağacının (*Morus alba L* ve *Morus nigra L*) kök kabuğu ekstraterinin hipotansif etkisi araştırılmıştır.

Morus alba L kök kabuğu ekstresi köpeklerde kan basıncını doza bağlı olarak düşürmüş ve beraberinde hafif bir bradikardi meydana getirmiştir. Bu etki üç dakika kadar devam etmiştir. Oysaki, *Morus nigra L* kök kabuğu ekstresi ile herhangi bir etki görülmemiştir.

* Erciyes Üniversitesi Tıp Fakültesi Farmakoloji ABD.Yrd.Doç.Dr.

** Gaziantep Üniversitesi Tıp Fakültesi Fizyoloji ABD.Yrd.Doç.Dr.

*** Ankara Üniversitesi Eczacılık Fakültesi Farmakognozi ABD.Ecz.Dr.

**** İnönü Üniversitesi Tıp Fakültesi Patoloji ABD.Doç.Dr.

***** Ankara Üniversitesi Eczacılık Fakültesi Farmakognozi ABD.Prof.Dr.

***** Ankara Üniversitesi Tıp Fakültesi Biyokimya ABD.Doç.Dr.

Morus alba L kök kabuğu ekstresi ile farelerde subakut ve kronik toksisite çalışması da gerçekleştirilmiştir. Drog günde tek doz olarak 50 ve 200 mg/kg her gün subkütan enjekte edilmiştir. Subakut toksisite için 3 hafta, kronik toksisite için 3 ay boyunca enjeksiyonlara devam edilmiştir. Deneyler sonunda hayvanlarda droga atfedilebilecek herhangi bir değişiklik saptanamamıştır.

INTRODUCTION

The root bark of mulberry tree, has been used medicinally since olden times in oriental countries and Turkey(1). It is said in Chinese Medicine that *Morus alba* L has antipyretic, antitussive, expectorant, diuretic and laxative activities(1). Hypoglycemic activity of *M.alba* L root barks was reported(2) and some hypotensive compounds(Kuwanons G,H,M and mulberofurans) have been isolated from the root bark of mulberry tree(3,4). In this study, hypotensive effects of mulberry tree root barks (*Morus alba* L and *Morus nigra* L) and toxicological profile of *Morus alba* L root bark extract were investigated.

MATERIALS AND METHODS

Extraction procedure

Mulberry tree root barks (*M.alba* L and *M.nigra* L) were collected from Turkey in August 1991 and dried at room temperature. 1 kg root barks were powdered from each variety. Then, they were extracted with 3 L.Methanol 7 times (5) by means of a Soxhlet apparatus during 20 h and the extracts were evaporated in vacuo and desiccated. These total extracts were used in our experiments.

Experimental procedure

Hypotensive Effect:Mongrel dogs of either sex weighing between 10 and 18 kg were anesthetized with thiopental sodium, 25 mg/kg iv. Anesthesia was maintained by supplementary injections of the barbiturate as needed. Cannulas were inserted in a femoral artery and vein and in the trachea for blood pressure recording, drug administration and artificial ventilation respectively(6). Blood pressure was recorded with a Harvard 50-8952 transducer and a Harvard 50-8622 recorder. Hypotensive effects of *M.alba* L or *M.nigra* L root barks extracts were investigated in the experiments. Animals were divided into three groups(control, *M.alba* L and *M.nigra* L root barks extracts). There were seven dogs for each group. Drugs were dissolved in saline and neutralized by sodium carbonate solution. *M.alba* L or *M.nigra* L extracts were administered at the dose of 5,10, 20 and 40 mg/kg intravenously(cumulative). Control animals received saline only.

Toxicological Studies:In this part of study, Swiss albino mice from both sexes were used. They were fed on a standard lab diet and allowed to drink tap water ad libitum. The animals were divided into six groups (two controls and four test groups). Each group contained equal number of both sexes. *Morus alba* L root extract dissolved as above described and injected daily single subcutaneous

injection at dosages of 50 and 200 mg/kg. The control group received saline only by the same route. The treatment lasted 3 weeks for subacute and 3 months for chronic examination. The animals were observed daily for the signs of toxicity and behavioral changes.

At the end of the study, from the overnight fasted mice; blood samples were obtained by decapitation and plasma was separated. The biochemical examinations (alkalen phosphatase, bilirubine, AST, ALT, creatinine, glucose, urea-N, cholesterol, total protein, albumin, Na⁺, K⁺ and Cl⁻) were performed on plasma samples using autoanalyzer. In addition, hematological parameters were also investigated by routine methods.

After the section (total 60 animals) the following organs were preserved in 10 % formalin and routine histopathological examination was performed; heart, lungs, liver, kidneys and brain. Slides were examined under light microscope.

Data Analysis

Results were given as means values \pm SEM. Comparison were done using t test for paried (hypotensive effect) or unpaired observations (toxicity studies) and level of significance was set at 0.05.

RESULTS

Hypotensive Effect:Initial blood pressure and heart rate values for experimental groups of the first series are shown in table 1. There were no statistically significant difference between two groups.

M.alba L root bark extract lowered the blood pressure of dogs in a dose-dependent manner. Hypotensive response was accompanied by a slight bradycardia. They produced slightly falls in blood pressure and heart rate which generally lasted less than 3 min.

1. M.alba L root bark extract lowered the blood pressure at the doses of 10, 20 and 40 mg/kg ($p < 0.05$, 0.05 and 0.01 respectively). It has no effect on blood pressure less than 10 mg/kg ($p > 0.05$). In addition, heart rate was decreased slightly at these doses ($p < 0.05$). These results have been presented in figure 1.

2. M.nigra L root bark extract was ineffective on blood pressure nor heart rate at the doses of 5, 10, 20 and 40 mg/kg ($p > 0.05$).

Toxicological Studies:Subacute and chronic toxicity studies in mice were uneventful and on pathologic changes attributable to the drug evident. Biochemical or hematological parameters were not different from control in extract administered groups ($p > 0.05$).

Any drug-related changes were not observed in mice. Results of biochemical and hematological examinations have been presented in table 1 and 2.

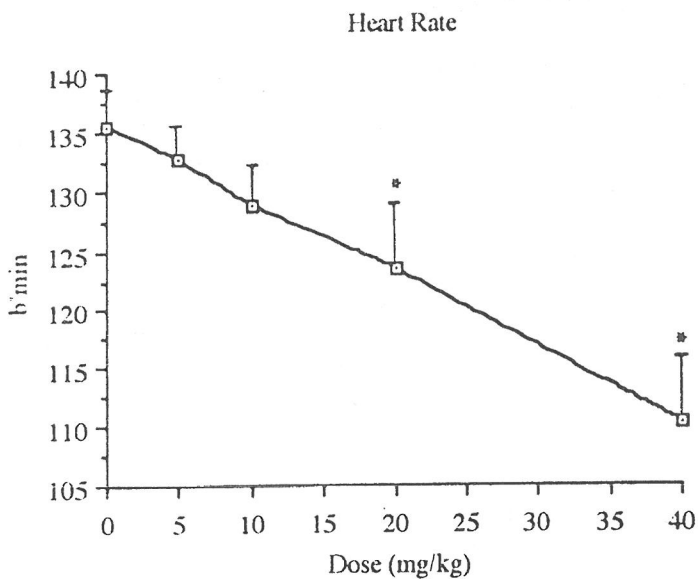
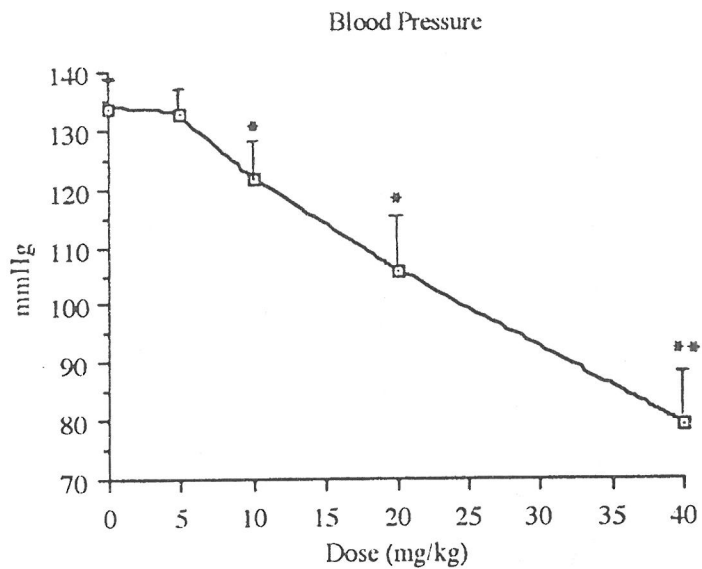


Figure 1: Effect of *Morus alba* L root bark extract on blood pressure and heart rate (bars represent SE) $n=7$, *: $p<0.05$, **: $p<0.01$

GROUPS	GLUCOSE (mg/dl)	UREA-N (mg/dl)	CHOL:STEROL (mg/dl)	CREATININE (mg/dl)	Na ⁺ (mM/L)	K ⁺ (mM/L)	Cl ⁻ (mM/L)	BILIRUBINE (mg/dl)	AST (U/L)	ALT (U/L)	ALK PHOS. (U/L)	T.PROTEIN (g/dl)	ALBUMIN (g/dl)
Controls S	82.17±8.3	17.5±3.5	34.3±5.7	0.97±0.15	147±2.5	5.3±1.04	107.5±1.6	0.55±0.15	25.1±5.3	14.7±4.7	15.5±7.7	6.1±1.4	3.5±1.5
Control C	88.27±10.5	19.2±7	44.5±9.9	0.56±0.25	146.2±3.2	5.05±1.12	106.8±3.7	0.81±0.4	32.3±5.8	13±7.1	19±5.9	5.9±0.7	4.1±0.8
Extract S (50 mg/kg)	91.17±9.75	16.5±3.5	39.17±8.7	0.75±0.16	141±5.3	4.9±1.08	110±3.8	0.67±0.24	34±9.1	15±8.9	17±7.9	5.5±0.87	4.7±0.7
Extract C (50 mg/kg)	79.99±8.21	15.3±2.7	45.6±6.7	0.87±0.38	145.4±4.5	5.77±1.13	107.8±3.4	0.79±0.37	37.6±8.4	16.6±7.6	13.3±6.3	5.2±1.1	4.4±1.2
Extract S (200 mg/kg)	85.16±7.9	18.3±5.3	37.7±8.1	0.65±0.2	142.4±4.5	5.5±0.95	107.3±2.3	0.49±0.1	35.3±8.3	17.4±7.4	15.7±3.6	5.3±0.8	4.4±0.5
Extract C (200 mg/kg)	90.1±7.5	18.8±3.7	47.7±7.9	0.9±0.4	144±3.5	4.9±0.5	108.8±2.4	0.55±0.2	29.9±8.5	19.5±7.5	20.4±5.6	5.8±0.6	4.5±0.6

Table 1: Biochemical parameters of mice (mean±SE). n=10 for each group. p<0.05. S:Subacute, C:Chronic.

	Hemoglobin (g 100 ml)	WBC ($10^3/\text{mm}^3$)	RBC ($10^6/\text{mm}^3$)	Hematocrit (Vol %)
Control S	11.1 ± 1.1	13.5 ± 2.5	11.7 ± 2.2	46.7 ± 5.11
Control C	13.2 ± 2.7	14.4 ± 2.2	10.8 ± 1.9	45.6 ± 4.4
Extract S (50 mg/kg)	12.7 ± 1.8	13.5 ± 3.1	12.6 ± 3.3	43.7 ± 3.4
Extract C (50 mg/kg)	13.3 ± 1.4	12.9 ± 2.1	11.2 ± 2.7	47.9 ± 4.5
Extract S (200 mg/kg)	12 ± 1.6	13.8 ± 2.4	9.9 ± 2.5	46.6 ± 3.5
Extract C (200 mg/kg)	12.1 ± 1.1	14.1 ± 3.1	13.2 ± 2.2	44.6 ± 2.5

Table 2: Hematological parameters of mice (mean ± SE). n=10 for each group. p > 0.05. S: Subacute, C: Chronic.

DISCUSSION

According to our results *M. alba* L root barks extract has a transient hypotensive effect. In addition, it has produced slight bradycardia. These results were harmonious with literature (1,4). Whereas, *M. nigra* L root bark extract did not have the same effects and we could not find any knowledge in literature on this topic. On the other hand, it has also been thought that, this effect was not related to the barbiturate anesthesia because it was not any significant change on *M. nigra* L extract administered dogs.

Some investigations have demonstrated that *M. alba* L root barks contain some hypotensive compounds and the most important of them is Kuwanon G (5,7). Kuwanon G and H have been found effective on arterial blood pressure in rabbits (4). Whereas, Kuwanon G and others (Kuwanons H, M and mulberofurans) are very low quantity in *M. nigra* L root bark when compared with *M. alba* L (5). In our opinion, this condition may clarify the ineffectiveness of *M. nigra* L root bark extract on blood pressure but, further studies are necessary to explain the hypotensive mechanism on mulberry tree root barks.

On the other hand, toxicity studies have demonstrated that, *M. alba* L root extract has not any toxic effect on mice during subacute or chronic period. Biochemical or hematological parameters are not different from control in the extract

administered groups ($p > 0.05$) and these data were include physiologic range (8,9). In addition, there were no pathologic changes on organs of mice. On the other hand, Yamateke et al have reported in an acute toxicity study that, *M.alba* L root bark extract caused no death in mice 5 g/kg intravenously(1). This result has demonstrated that, *M.alba* L root bark extract has not own an acute toxicity and our results are also harmonicus with this report. In conclusion, *M.alba* L root bark extract has a transient hypotensive effect on dogs and no toxic effect was observed in mice but *M.nigra* L root bark extract was found ineffective on blood pressure.

REFERENCES

- 1- Yamateke Y., Shimabata M., Nogai M.:Pharmacological studies on root bark of mulberry tree (*Morus alba* L) *Jpn J Pharmacol* 26, 461-468, 1976.
- 2- Hikino H., Mizuno T., Oshima Y., et al.:Isolation and hypoglycemic activity of Moran A, a glycoprotein of *Morus alba* root barks. *Planta Med* 2, 159-160, 1985.
- 3- Nomura T., Fukai T., Momose Y., et al.:Hypotensive constituents of the root bark of mulberry tree (*Morus alba* L) and the mechanism of their actions. Third Symposium of the Development and Application of Naturally Occuring Drug Materials. Symposium paper, p.13 Tokyo, Japan, 1980.
- 4- Nomura T.:Phenolic compounds of the mulberry tree and the related plants. In:Progress in chemistry of organic natural products. Herz V, Grisebach H, Kirby GW, Tamm CH(eds). Wiem Springer Verlag, New York, 1988.
- 5- Behferooz F.:*Morus alba* L ve *Morus nigra* L üzerinde farmakognozik araştırma. Ankara University Medical Sciences Institute. Ankara, Türkiye. 1993 (unpublished thesis with English abstract).
- 6- Vidrio H., and Magos G.Pharmacology of *Casimiora Edulis*; II.Cardiovascular effects in the anesthetized dog. *Planta Med* 57, 217-220, 1991.
- 7- Nomura T., and Fukai T.:Kuwanon G, a new flavone derivative from the root barks of the cultivated mulberry tree. *Chem Pharmacol Bull* 28, 2548-2552, 1980.
- 8- Schalm OW.:Veterinary hematology, p.307, Lea and Febiger, Philadelphia, 1971.
- 9- Canadian Council on Animal Care. Guide to the Care and use of experimental animals. 2 vols.Ottawa, Ont;CCAC, 1984.