Kocatepe Tıp Dergisi
The Medical Journal of Kocatepe
8: 55-58 / Ocak 2007
Afyon Kocatepe Üniversitesi

The Use Of "Mad Honey" As An Antihypertensive Agent In Rats - A Preliminary Study

Sıçanda Antihipertansif Olarak Deli Bal Kullanımı

Nuray OZTASAN[1], Ahmet SONGUR[2]

Afyon Kocatepe University School of Medicine, Department of Physiology[1] and Anatomy[2], Afyonkarahisar, TURKEY

ÖZET: ***Giriş:*** Deli Bal (Mad Honey), Türkiye'de özellikle Doğu Karadeniz dağlarında yetişen *Rhododendron ponticum* bitkisinin çiçeklerinden elde edilen bir bal çeşididir. Bu balın fazla tüketilmesi, içeriğindeki grayanotoksinlerden dolayı toksik etkiler meydana getirmektedir. Fakat Doğu Karadeniz halkı tarafından az miktarlarda tüketilerek bazı hastalıkların geleneksel tedavisinde, özellikle hipertansiyon için kullanılmaktadır. Çalışmamızda biz de, sıçanda deli balın antihipertansif etkisini araştırmayı planladık.

Gereç ve Yöntem: N^{ω}-nitro-L-arginine methyl ester (L-NAME) ile deneysel hipertansiyon oluşturulan ratların kan basıncı ve kalp hızına ait kayıtlar Acquisition Sistem (Powerlab 8SP®) ile yapıldı. Sonrasında, bu deneklere 14 gün boyunca deli bal (10 ve 50 mg/kg doz) intragastrik yolla verildi ve ölçümler devam edildi.

Bulgular: L-NAME ile hipertansiyon oluşturulan sıçanlarda deli balın kan basıncı ve kalp hızını azalttığı görüldü.

Sonuç: Deli bal antihipertansif etkisini, içeriğinde bulunan grayanotoksinler tarafından parasempatik sinir sistemini uyararak göstermektedir. Sonuçlarımız, deli balın antihipertansif etkisinden, dozu belirlendikten sonra, yararlanılabileceğini göstermektedir.

Anahtar Kelimeler: Deli bal, hipertansiyon

ABSTRACT: ***Background:*** Mad honey (*Deli bal* in Turkish) is obtained from *Rhododendron ponticum* which grows extensively on the mountains of Eastern Black Sea Region in Turkey. Excessive intake of this honey causes intoxication due to its grayanotoxins, hence the name 'mad honey'. 'Mad honey' is used in traditional medicine by Eastern Black Sea people and is recommended for the cure of certain disease, especially hypertension. In this study, we aimed to investigate the antihypertensive effects of 'mad honey' in hypertensive rats.

Methods: The blood pressure and heart rate of N^{ω}-nitro-L-arginine methyl ester (L-NAME) induced hypertensive rats were recorded by a data acquisition system (Powerlab 8SP®). After this, 'mad honey' (10 and 50 mg/kg dosage) was administered for 14 days by gavage feeding, at the end of which the measurements were repeated.

Results: It was found that the 'mad honey' treatment decreased the blood pressure and heart rate in L-NAME induced hypertensive rats.

Conclusion: It is speculated that the grayanotoxins of 'mad honey' decrease the blood pressure by stimulating the parasympathetic nervous system. On the basis of this effect, we hypothesize that mad honey may be used as an antihypertensive agent in hypertensive patients.

Key Words: Mad honey, hypertension.

INTRODUCTION

Hypertension, which affects 30% of adults, is the one of the most widespread clinical problems in the world (1). It is a multifactorial, polygenic disease that involves complex interactions between genetically determined homeostatic control mechanisms and environmental factors (2). Clinical and experimental studies also indicate that oxidative stress contributes to the development of hypertension in human beings (1) and animals (3-5). Hypertension produced by nitric oxide (NO) synthesis inhibition with N^{ω}-nitro-L-arginine methyl ester (L-NAME) is associated with increased oxidative stress. It is also known that inactivation of NO inhibits its vasodilatory and natriuretic actions (4,6,7).

Deli bal (mad honey) is generally obtained from *Rhododendron ponticum* which grows extensively on the mountains of Eastern Black Sea Region in Turkey as well as in Japan, Nepal, Brazil and some parts of North America and Europe. Its grayanotoxins, produced from the nectar of Rhododendron Ponticum flowers, are constituents of 'mad honey' (8-11). When taken in excess, the grayanotoxins in this honey causes intoxication symptoms such as hypotension, bradycardia, cardiac arrhythmias, vomiting, sweating, weakness, dulled vision, mild paralysis and convulsion by stimulating central and parasympathetic nervous system

Corresponding Author; Yrd. Doç Dr. Nuray ÖZTAŞAN
Afyon Kocatepe Üniversitesi Fizyoloji Anabilim Dalı.
AFYONKARAHİSAR
Tel: +90 2722167901 Faks: +90 272 2142067
(E-posta: nurayoztasan@hotmail.com)

(8,12,13). The grayanotoxins especially cause cardiotoxicity by stimulating the M_2 muscarinic receptors. Grayanotoxin I, also known as andromedotoxin, acetylandromedatol and rhodotoxin, is responsible for these symptoms (8,9). But these symptoms are generally not fatal and respond to i.v. saline infusion containing atropine and last for within 24 hours (9). Nevertheless, 'mad honey' is used in traditional medicine by Eastern Black Sea people, and low doses of it are recommended in the cure of some diseases such as hypertension and diabetes mellitus.

Pharmacological drugs, when used over recommended doses, may cause toxic effects. Therefore, the positive effects of alternative therapies such as the use of 'mad honey' for the treatment of hypertension should be considered. There are several reports in literature that focus on the toxic effect of 'mad honey', however, there is no data regarding its beneficial effects on blood pressure.

In this study, we report our preliminary findings on the effects of the low doses of 'mad honey' on blood pressure and heart rate in L-NAME induced hypertensive rats.

MATERIALS AND METHODS

Animals and procedure of treatment

The experimental protocol was approved by the Ethical Committee of Afyon Kocatepe University on Animal Experimentation. Additionally, principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Adult male albino Wistar rats (weighing 230±20 g) were obtained from the Biomedical Research Unit of the Institute of Health Sciences, Selcuk University, Konya. After an acclimatization period of 1 week, the rats were assigned by computer randomization to 3 different groups. Group I, Group II and Group III were control (not hypertensive and not treated), hypertensive rats treated with 10 mg/kg 'mad honey' and hypertensive rats treated with 50 mg/kg 'mad honey', respectively.

Hypertension treatment

The hypertension in rats was generated with L-NAME (SIGMA, St. Louis, MO, USA). The compound was dissolved in tap water (75 mg/L) and included in the drinking water administered to the rats over a period of 21 days. The L-NAME content was renewed by replacing the tap water every day (5-7).

Blood pressure and heart rate of rats were measured by tail-cuff procedure (pneumatic transducer) by a data acquisition system (Powerlab® 8SP). Blood pressure measurements were taken at the same time each day. Rats were anesthetized with ketamine plus xylasine throughout the processes.

Administration of 'mad honey'

The 'mad honey' was dissolved in distilled water and administered to the L-NAME induced hypertensive rats at the dose of 10 mg/kg in Group II rats and 50 mg/kg in group III rats by gavage feeding for 14 days at the same time each day (11). Blood pressure and heart rate of rats were measured by method described above on day 7 and day 14.

Rats were kept in plastic cages during the treatment and restless. The cages were cleaned regularly and water (tap water) and food (Institutes' stock diet) was replaced every day. The rats were checked daily and body weights were recorded every week and the end of the study.

During the study, the temperature of the room air were maintained at 25±2 ^{0}C, the humidity at 40-50%, and light was 12 h day/12 h night cycle. Food and water were provided ad libitum during the treatment and rest of the time.

Statistical Analyses

Values expressed as mean ± standard deviation (SD). Statistical analysis was performed on a personal computer using SPSS for Windows software. One-way analysis of variance (ANOVA) was used to compare continues variables between the each groups followed by Tukey HSD Test. $P<0.05$ was considered as statistically significant.

RESULTS

The results are summarized in Tables I and II. Table I shows the levels of the blood pressure before hypertension induction (column A), after hypertension induction (column B) and after 'mad honey' administration in hypertensive rats (column C). The data presented in Table I indicates that blood pressure was significantly decreased in Group II and Group III rats after 'mad honey' administration, compared with before 'mad honey' administration. The decrease in blood pressure in Group III rats was more than group II rats. The heart rate measurements of the three groups of rats are shown in Table II. We observed that the heart rate results were decreased after Mad Honey administration.

Table I: *Comparison of the blood pressure levels of rats in control (Group I), L-NAME induced hypertensive rats treated with 10 mg kg 'mad honey' (Group II) and hypertensive rats treated with 50 mg kg 'mad honey' (Group III). The levels of blood pressure represent mean ± SD.*

Groups	Blood pressure (mmHg)			P values		
	HT (A)*	HT1 (B)*	HT2 (C)*	A-B	A-C	B-C
I (n:6)	99.8 ± 10.8	100.2 ± 9.8	102.2 ± 3.2	NS	NS	NS
II (n:7)	113.6 ± 5.7	172.7 ± 9.9	160.9 ± 8.7	0.000	0.000	0.039
III(n:8)	104.1 ± 9.4	162.3 ± 4.5	117.5 ± 9.9	0.000	0.011	0.000

NS: Not significant (p>0.05).
*HT – Before hypertension induction, HT1 - after hypertension induction, HT2- Mad honey treated hypertensive rats.

Table II: *Comparison of heart rate levels of rats in groups I, II and III (see Table I for explanation of abbreviations). The levels of heart rate represent mean ± SD.*

Groups	Heart Rate (beats/min)			P values		
	HT (A)	HT1 (B)	HT2 (C)	A-B	A-C	B-C
I (n:6)	441.3 ± 22.9	433.7 ± 18.5	439.7 ± 20.5	NS	NS	NS
II (n:7)	452.7 ± 61.5	495.6 ± 63.7	428.9 ± 47.9	NS	NS	0.046
III(n:8)	411.5 ± 28.7	478.0 ± 45.3	402.1 ± 44.8	0.003	NS	0.001

NS: Not significant (p>0.05).

DISCUSSION

Most of the studies found in literature, focus on the toxic effect of 'mad honey'. Onat *et al.* reported that the major toxic effects of high doses of 'mad honey' (1 and 5 g/kg i.p.) in rats were dose-dependent hypotension, sinus bradycardia and respiratory depression. Bradycardia and hypotension are also the characteristic effects of 'mad honey' in humans. Since the extract was not bradycardic in vagotomised animals, Onat *et al.* asserted that the grayanotoxins in 'mad honey' affect the central nervous system and its cardiodepressant effect is thought to be mediated via stimulation of the vagus nerve (8,9). In another study, the same group reported that atropine sulfate improved 'mad honey' induced bradycardia and bradypnea, while AF-DX 116, which is a selective M_2-muscarinic receptor antagonist, restored only the heart rate. These finding suggest that M_2-muscarinic receptors are involved in the cardiotoxicity of grayanotoxins (10).

In this study, we found that 'mad honey' caused significant decreases in blood pressure and heart rate in L-NAME induced hypertensive rats in a dose dependent manner. We believe that these effects may be due to the grayanotoxins in 'mad honey'. 'Mad honey' is traditionally used as alternative medicine by Eastern Black Sea people and in low doses is recommended for the cure of diseases such as hypertension and diabetes mellitus. The ingestion of grayanotoxins through the use of leaves, flowers and nectar of Rhododendrons in tea or cigarettes is also used for the relief of arthritis (8).

With growing concerns over the potential toxic effects of over use of pharmacological drugs, the use of alternative natural medicines has been gaining prominence. In this regard the positive effects of low doses of 'mad honey' on hypertension are of importance.

We believe that the preliminary results reported in our study will encourage future studies on the use of 'mad honey' as an alternative therapy. The beneficial use of small doses of 'mad honey' is a testament for the proverb "All medicines are poisons; the difference between them is only their doses". Further studies are needed to determine the contents, evaluate the beneficial dosage and clarify the exact mechanism of the effects of 'mad honey' on hypertension.

Acknowledgment: This study was supported by grant from Research Fund of Kocatepe University (BAPK, Project no: 031.TIP.04).

REFERENCES

1. Galley HF, Thornton J, Howdle PD, Walker BE, Webster NR. Combination oral antioxidant supplementation reduces blood pressure. Clin Sci. 1997; 92:361–365.
2. Lerman LO, Chade AR, Sica V, Napoli C. Animal models of hypertension: an overview. J Lab Clin Med. 2005; 146:160-173.
3. Vaziri ND, Wang XQ, Oveisi F, Rad B. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. Hypertension. 2000; 36:142–146.

4. Meng S, Cason GW, Gannon AW, Racusen LC, Manning RD. Oxidative stress in Dahl salt-sensitive hypertension. Hypertension. 2003; 41:1346–1352.

5. Sainza J, Wangensteena R,Rodriguez Gómez I, Moreno JM, Chamorro V, Osuna A, Bueno P, Vargas F. Antioxidant enzymes and effects of tempol on the development of hypertension induced by nitric oxide inhibition. Am J Hypertens. 2005; 18;871-877.

6. Duarte J, Jiménez R, O'Valle F, Galisteo M, Pérez-Palencia R, Vargas F, Pérez-Vizcaino F, Zarzuelo A, Tamargo J. Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. J Hypertens. 2002; 20:1843–1854.

7. Tsukahara H, Hiraoka M, Kobata R, Hata I, Ohshima Y, Jiang MZ, Noiri E, Mayumi M. Increased oxidative stress in rats with chronic nitric oxide depletion: measurement of urinary 8-hydroxy-2′-deoxyguanosine excretion. Redox Rep. 2000; 5:23–28.

8. Onat FY, Yegen BC, Lawrence R, Oktay A, Oktay S. Mad honey poisoning in man and rat. Rev Environ Health. 1991; 9: 3-9.

9. Onat FY, Yegen BC, Lawrence R, Oktay A, Oktay S. Sites of action of grayanotoxins in mad honey in rats. J. Appl. Toxicol. 1991; 11:199-201.

10. Onat FY, Kurtel H, Yegen BC, Oktay A and Oktay S. Atropine and AF-DX 116 in mad honey intoxication. Eur. J. Pharmacol. 1990; 183:575-577.

11. Oztasan N, Altinkaynak K, Akay F, Gocer F, Dane S. Effects of mad honey on blood glucose and lipid levels in rats with streptozocin-induced diabetes. Turk J Vet Anim Sci. 2005; 29:1093-1096.

12. Gossinger H, Hruby K, Haubenstock A, Pohl A, Davogg S. Cardiac arrytmias in a patient with grayanotoxin-honey poisoning. Vet. Hum. Toxicol. 1983; 25:328-329.

13. Biberoglu S, Biberoglu K, Komsuoglu B. Mad Honey. J. Am. Med. Assoc. 1988; 259:1943-1945.