ANTIHYPERTENSIVE POTENTIAL OF THYMOQUINONE IN NORMAL AND L-NAME INDUCED HYPERTENSIVE RATS

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmacology)

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ABSTRACT

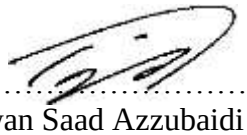
Introduction: Hypertension is one of the leading causes of death due to stroke and heart diseases, Nigella sativa “black cumin” seeds have been widely used in traditional medicine for diseases treatment including hypertension. Thymoquinone (TQ) is one of the major active constituents in its volatile oil. Objective of this study was to evaluate the antihypertensive potential of TQ and to investigate the underlying mechanisms of action. Method: In normotensive rats; mean arterial blood pressure (MAP) and heart rate (HR) was recorded using the non-invasive tail cuff technique. Dose-response relationship was obtained by using 3 TQ doses (2.5, 5 and 10 mg/kg) intraperitoneally to 3 different groups (n=5) of adult male Sprague-Dawley rats under pentobarbital anesthesia. MAP was then measured for other two animal groups pretreated either with atropine (P-at) or propranolol (P-pro) followed by 10 mg/kg TQ. Hypertension was induced in another group of animals (n=36) and control (n=6) by administration of L-Nitro-Arginine Methyl Ester (L-NAME) in their drinking water for four weeks. At the end of the induction period, rats were divided into six groups (n=8); TQ2.5+L-NAME, TQ5+L-NAME, TQ10+L-NAME, captopril+L-NAME, L-NAME only and control. MAP and HR were recorded by the tail cuff technique weekly for four weeks. Then animals were sacrificed, and blood was collected for determination of ACE activity and aldosterone concentration using ELISA. Lipid profile was assayed twice; at the end of the induction period and the end of the treatment period. Results: TQ produced a dose-dependent blood pressure lowering effect, where 2.5 5 and 10 mg/kg of TQ treatment decreased MAP by 8±1 mmHg, 12±3 and 29±3 mmHg, respectively. TQ-induced MAP reduction was significantly less in P-at than non-pretreated group. Conversely, TQ-induced MAP reduction in P-pro did not demonstrate a significant difference from the non-pretreated group. TQ reversed the established hypertension in TQ5 and TQ10 groups, and prevent further increase in MAP in TQ2.5 group. TQ antihypertensive activity was associated with a decrease in serum aldosterone concentration and an increase in ACE activity. TQ treatment lowered all the blood lipid profile parameters. Conclusion: This study confirms the dose-related hypotensive effect of TQ. The mechanism of TQ-induced hypotension involves at least in part activation of vascular muscarinic receptors, but not β-adrenergic receptors. The antihypertensive activity of TQ takes place through renin angiotensin aldosterone system.

خلاصة البحث

المقدمة: يعدّ إرتفاع ضغط الدم أحد المسببات الرئيسة للوفاة بسبب الجلطات وأمراض القلب. بذور الحبة السوداء (حبة البركة) استعملت على نطاق واسع في الطب التقليدي لمعالجة الأمراض ومنها إرتفاع ضغط الدم. الثيموكينون TQ هو أحد المواد الفعالة الرئيسية في زيتها الطيار. هدف الدراسة: تهدف هذه الدراسة إلى تقييم قدرة الثيموكينون على خفض ضغط الدم المرتفع، ودراسة الآليات التي يعمل من خلالها. الطريقة: في جرذان طبيعية ضغط الدم؛ تم قياس معدل ضغط الدم الشرياني (MAP) وضربات القلب (HR) باستخدام تقنية القياس عبر الذيل. تم الحصول على علاقة الجرعة–التأثير بعد دراسة 3 جرعات مختلفة من الثيموكينون (2.5 ، 5 ، 10) مغ\كغم بعد حقنها في منطقة البطن في ثلاث مجموعات مختلفة (ن=5) من ذكور جرذان SD. تم قياس معدل ضغط الدم الشرياني في مجموعتين أخريين عولجتا مسبقاً بأتروبين أو بروبرانولول ثم أتبع بثيموكينون 10 ملغ\كغم. استحدث مرض ضغط الدم في مجموعة أخرى من الجرذان (ن=36) بالإضافة لمجموعة السيطرة (ن=6) بإعطاء نيترو–أرجنين مثيل إستر (L-NAME) في مياه الشرب لمدة 4 أسابيع. في نهاية فترة إستحداث المرض تم تقسيم الجرذان إلى 6 مجموعات:L-NAME + 2.5TQمغ\كغم ، L-NAME + 5TQمغ\كغم ، L-NAME + 10TQ مغ\كغم ، L-NAME +كابتوبريل ، L-NAME فقط ، ومجموعة السيطرة. تم قياس ضغط الدم ومعدل ضربات القلب أسبوعياً خلال الأسابيع الأربعة التالية. وبعد ذلك تم قتل الجرذان والحصول على عينات الدم لتحيد فعالية أنزيم الأنجيوتنسين وتركيز الألدوستيرون باستعمال الإلايازا. دهون الدم تم قياسها مرتين، عند نهاية فترة استحداث المرض وعند نهاية فترة العلاج. النتائج: خفّض الثيموكينون ضغط الدم في المجموعات الثلاث ؛ حيث خفضت جرعة 2.5 ، 5 و 10 مغ\كغم ثيموكينون ضغط الدم 8±1 ، 12±3 و 29±3 مم زئبق على التوالي. العلاج المسبق بالأتروبين ثبّط بشكل ملحوظ قدرة الثيموكينون على خفض ضغط الدم باستعمال جرعة 10 مغ\كغم ، في حين لم يكن هناك أي فرق مقارنة مع الجرذان المعالجة مسبقاً بالبروبرانولول. جرعة 5 و 10 مغ\كغم ثيموكينون نجحت بمعاكسة الضغط المرتفع في الجرذان المصابة، في حين منعت جرعة 2.5 مغ\كغم ثيموكينون أي إرتفاع إضافي في ضغط الدم. فعالية الثيموكينون كخافض للضغط ترافقت مع إنخفاض في تركيز الإلدوستيرون وارتفاع في فعالية الأنزيم المحول للأنجيوتنسين. الثيموكينون خفض كل دهون الدم خلال فترة العلاج. الخلاصة: أكدت هذه الدراسة قدرة الثيموكينون على خفض ضغط الدم. والآلية لعمله تتضمن تنشيط مستقبلات المسكارين ولكن ليس مستقبلات بيتا الأدرينالية. قدرة الثيموكينون على خفض ضغط الدم المرتفع تمر من خلال نظام الرنين–أنجيوتنسين–ألدوستيرون.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion; it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Sciences.



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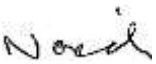

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DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Finally, this thesis is dedicated to the memory of my beloved father, and to my mother and family for their invaluable support throughout my life

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitor
ARBs	Angiotensin II receptor blockers
CAP	Captopril
CHOL	Total cholesterol
HDL	High density lipoprotein
HR	Heart rate
LDL	Low density lipoprotein
L-NAME	NG-nitro-L-arginine methyl ester
MAP	Mean arterial pressure
NC	Normal control
NO	Nitric oxide
NOSe	Nitric oxide synthase enzyme
RAAS	Renin angiotensin aldosterone system
TG	Triglyceride
TQ	Thymoquinone
TQ2.5	2.5 mg\kg Thymoquinone
TQ5	5 mg\kg Thymoquinone
TQ10	10 mg\kg Thymoquinone

CHAPTER ONE

INTRODUCTION

1.1 OVERVIEW

Up to date, all the available hypertension medications are symptomatic treatment, that aid in restoring the elevated blood pressure to prevent or delay the disease complication while no medications can cure hypertension yet.

Several mechanisms control blood pressure in our bodies. Some of these mechanisms are mediated through the autonomic nervous system in its two subdivisions; the Sympathetic Nervous System (SNS) and the Parasympathetic Nervous System (PNS).

Exploring the natural products can help finding natural active compounds to treat diseases including hypertension. The importance of investigating the mechanism of action of natural products is to give an idea of how these compounds interact with our body. Thus, it becomes possible to expect which patients will benefit more from its use. It can also lead to discovering newly involved mechanisms.

Hypertension extensively investigated in clinical and pre-clinical studies to understand the development of the disease as well as to test potential treatments. Several animal models of hypertension were developed. One of these models is the nitric oxide deficient model, more commonly known as L-NAME model, which is widely used to understand and study the antihypertensive potential of some natural products.

Juxtaglomerular apparatus secrets renin as a response to the decrease in blood pressure leading to activation of the renin-angiotensin-aldosterone system (RAAS).

Overexpression of this system is strongly believed to have a role in the pathogenesis of hypertension.

Angiotensin converting enzyme (ACE) plays an important role in maintaining blood pressure while inhibitors of this enzyme are widely used as antihypertensive drugs. Captopril, the Angiotensin converting enzyme inhibitor (ACEi) was used in this research based on the fact that blocking ACE will reverse the established hypertension by L-NAME.

Thymoquinone (TQ) is considered the main active constituent of Nigella sativa volatile oil, and one of the natural products that show a potential ability to cure diseases including hypertension. Lack of research to investigate the mechanism of TQ leads this research which aimed to evaluate the activity of TQ in reducing blood pressure and to examine possible mechanisms of action mediated though muscarinic, β adrenergic and renin-angiotensin-aldosterone system (RAAS).

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF HYPERTENSION

2.1.1 Definition

Hypertension is a chronic cardiovascular disease. One is considered hypertensive if average blood pressure readings are 140\90 mmHg or more depending on at least two readings after visiting the clinic two or more times (James et al., 2014).

2.1.2 Hypertension complications

Hypertension causes approximately fifty percent of total deaths from stroke and heart disease, one in three adults throughout the world has high blood pressure(WHO, 2012). High blood pressure readings are recognized as a risk factor for developing cardiovascular diseases, the chance of heart attack, stroke, heart failure and kidney diseases is increased while blood pressure stayed uncontrolled (Eckel et al., 2013).

2.1.3 Hypertension risk factors

Hypertension development is strongly related to age, where more than 50% of people aged 60-70 years old and around 75% of people older than 70 years old are hypertensive in US.(Burt et al., 1995; Go et al., 2014). In Malaysia, 27.8% of the population aged 15 years or older has hypertension, and for those aged 30 years or more hypertension incidence has elevated from 32.9% in 1996 to 40.5% in 2004. While prevalence among the elderly population was 74%. (Kiau et al., 2013; Rampal, Rampal, Azhar, & Rahman, 2008).

Many other factors aid in developing hypertension were described, such as obesity and overweight, high sodium diet, alcohol consumption and a sedentary lifestyle (Bazzano, Green, Harrison, & Reynolds, 2013).

2.1.4 Classification of hypertension

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) classified blood pressure as described in (Table 2.1), while they did not consider prehypertension as a disease, and no treatment is required for those people. But to delay the development of hypertension and its complications, lifestyle modifications are strongly advised. (Joint National Committee on Prevention, 2004). Table 2.1 provides a classification of BP for adults 18 years and older. The classification is based on the average of two or more properly measured, seated, BP readings on each of two or more office visits.

Table 2.1 The classification of BP for adults 18 years and older

Category	SBP/DBP (mmHg)
Optimal	<120/80
Prehypertension	120-139/80-89
Hypertension Stage 1	140-159/90-99
Hypertension Stage 2	160 or more/ 100 or more

2.1.5 Hypertension treatment

The aim of hypertension treatment is to decrease morbidity and mortality due to cardiovascular and renal complications. Fewer cardiovascular complications were reported after lowering SBP and DBP to < 140/90. The risk for complications has

been reduced when high blood pressure reduced as the following: Heart failure by 50%, myocardial infarction by 20% and strokes by 40%. (Trialists'Collaboration, 2000, 2003).

2.1.5.1 Non pharmacological treatment

Lifestyle modification can help to control hypertension, e.g. Weight loss, increasing the physical activity and a restriction of sodium intake. Where medications only apply to those who fail to retain normal blood pressure after these precautions (Brunton & Chabner, 2010).

2.1.5.2 Pharmacological treatment

Blood pressure is the result of cardiac output and the peripheral vascular resistance. Antihypertensive agents act by affecting either one or both of them. Cardiac output can be altered by either decreasing the contractility of the heart or by lowering the pressure to fill the ventricles. Peripheral vascular resistance can be reduced by relaxing the smooth muscle leading to vasodilatation or by modifying the action of the causative system, for example (the renin-angiotensin-aldosterone system, the sympathetic nervous system).

Several studies confirmed that hypertension was not controlled by a single therapy and require at least two medications from two different groups.(Black et al., 2003; Dahlöf et al., 2002; Gradman et al., 2013). Different classes of drugs are used to control hypertension; Table 2.2 summarizes these groups according to their mechanism of action.

Table 2.2 Summary of the antihypertensive groups and an example for each group

Group	Example
Thiazide diuretic	-Chlorothiazide -Chlorthalidone
Loop diuretic	-furosemide -Bumetanide
Pottassium sparing diuretic	-Amiloride -Triamterene
Aldosterone receptor blocker	Spironolactone
β blocker	-Propranolol -Atenolol
α blocker	-Prazosin -Terazosin
Central α2 agonist	clonidine
combined α and β blocker	Carvedilol
Angiotensin converting enzyme inhibitor	-Captopril -Enalapril
angiotensin II antagonist	-Losartan -Valsartan
Calcium channel blocker (nondihydropyridine)	-Diltiazem -Verapamil
Calcium channel blocker (dihydropyridine)	-Amlodipine -Nicardipine
Direct vasodilator	Minoxidil

2.2 SYMPATHETIC AND PARASYMPATHETIC NERVOUS SYSTEMS

The Autonomic Nervous System (ANS) has two parts:

1. The Sympathetic Nervous System (SNS).
2. The Parasympathetic Nervous System (PNS).

Acetylcholine is the preganglionic neurotransmitter for both systems and the postganglionic neurotransmitter in the majority of the parasympathetic division. Norepinephrine represents the major postganglionic neurotransmitter in the sympathetic nervous system.

2.2.1 Muscarinic receptors in cardiovascular system

Acetylcholine in small doses causes a vasodilation leading to a decrease blood pressure after systemic administration through an indirect mechanism that is accompanied by reflex tachycardia. The vasodilatation is mediated by vascular endothelial nitric oxide (Brunton & Chabner, 2010). Stimulation of muscarinic receptors, specifically M3, is believed to be the responsible for the vasodilation by administered acetylcholine (Khurana et al., 2004; Lamping, Wess, Cui, Nuno, & Faraci, 2004). Muscarinic receptors activation lead to nitric oxide-dependent vasodilation (Ignarro, 1999), while muscarinic receptors M2 mediate the effect of acetylcholine on the heart resulting in bradycardia, this effect seen with notably larger doses (Goin, Borda, Auger, Storino, & Sterin-Borda, 1999; Hilal-Dandan & Brunton, 2013).

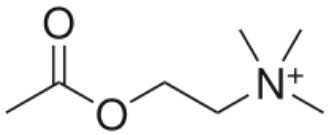

Figure 2.1 Acetylcholine chemical structure

2.2.2 Atropine as a muscarinic receptor blocker

Atropine is an anti-muscarinic agent that compete acetylcholine to block the muscarinic receptor. Atropine affects the cardiovascular system in two different ways according to the dose. Small doses tend to lower the heart rate (bradycardia) through prejunctional M1 receptor blockade while larger doses block M2 receptors located on the sinoatrial node thus increase the heart rate.(Clark, Finkel, & Rey, 2012). Atropine completely opposes the drop in blood pressure and the vasodilation caused by choline esters(Brunton & Chabner, 2010).

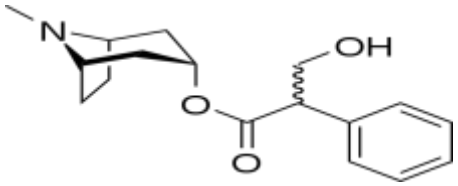

Figure 2.2 Atropine chemical structure

2.2.3 Beta adrenergic receptors

Beta adrenoceptors have been divided into β1, β2 and β3 subtypes according to their affinity for the agonist's epinephrine and norepinephrine, epinephrine has almost the same affinity towards β1 and β2 receptors, while norepinephrine has much more affinity towards β1 compared to β2. Adrenergic β3 receptors play a role in lipolysis (Fox, 2012). Table 2.3 summarizes some of the major effects of β adrenoceptor activation.

Table 2.3 The major effect of β adrenoceptor activation

β1 adrenoceptors	β2 adrenoceptors
Tachycardia	Vasodilation
Increase lipolysis	Decrease peripheral resistance
Increase myocardial contractility	Bronchodilation
Increase release of renin	Increase muscle and liver glycogenolysis
Increase AV conduction velocity	Increase release of glucagon
	Relax uterine smooth muscle

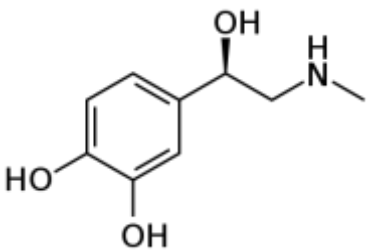

Figure 2.3 Epinephrine chemical structure

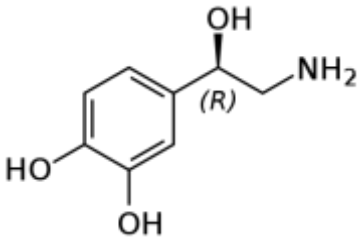

Figure 2.4 Norepinephrine chemical structure

2.2.4 Propranolol as a β adrenergic blocker

A non-selective β adrenergic receptor blocker that blocks both β1 and β2 adrenoceptors equally, and considered as the prototype for the β antagonist drugs.

On heart, propranolol possesses both negative inotropic (decrease myocardial contractility) and negative chronotropic (reduce heart rate) effect and decreases sinoatrial node and atrioventricular activity; the net impact is shown as bradycardia.

On the blood vessels, propranolol causes vasoconstriction due the fact that β2 adrenoceptors mediate vasodilation, this vasoconstriction is a reflex for the decrease in the cardiac output caused by blocking β1 adrenergic receptors located in the heart. Propranolol cannot decrease the blood pressure in normotensive individuals. Conversely, several mechanisms are involved in reducing blood pressure in hypertensive patients, cardiac output reduction is the main mechanism, whereas lowering renin secretion from the kidney, inhibiting the sympathetic outflow from central nervous system and minimizing the total peripheral resistance over long periods of treatments, have also roles in propranolol mechanism (Brunton & Chabner, 2010).

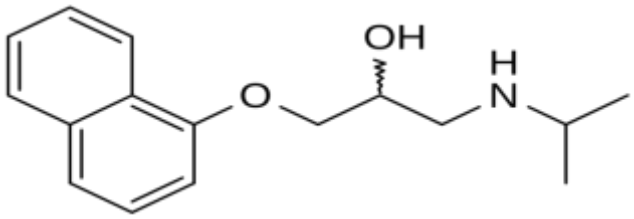

Figure 2.5 Propranolol chemical structure

2.3 OVERVIEW OF RENIN ANGIOTENSIN ALDOSTERONE SYSTEM (RAAS)

As a response to the decrease in blood pressure and blood flow to the renal artery, renin is secreted from the juxtaglomerular apparatus into the bloodstream. The plasma protein angiotensinogen is converted to angiotensin I by the action of renin enzyme. While angiotensin I reach the lung's capillaries, further modification takes place by angiotensin converting enzyme (ACE) to convert angiotensin I to angiotensin II. Angiotensin II is considered a potent vasoconstrictor (40 times more potent compared to norepinephrine) and cause the raising of the blood pressure by multiple mechanisms affecting the cardiovascular system and the kidneys. Angiotensin II is also believed to induce rapid and slow pressor responses on the cardiovascular system and leads to vascular and cardiac hypertrophy and remodeling. Briefly, the mechanism of action of angiotensin II involves a direct vasoconstriction (mediated via AT1 receptors) as well as increases aldosterone synthesis and release. Aldosterone synthesis and release is stimulated by the effect of angiotensin II on the zona glomerulosa of the adrenal cortex. This stimulation leads to sodium and water reabsorption and to excrete potassium through the action on the distal tubule and the collecting ducts in nephrons, ultimately increasing blood volume and pressure (Hilal-Dandan & Brunton, 2013).