



Cymbopogon proximus Extract Decreases L-NAME-Induced Hypertension in Rats

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Accepted on: 21-04-2014; Finalized on: 30-06-2014.

ABSTRACT

Cymbopogon proximus (*C. proximus*) is a wild herb that is commonly used safely as an anti-spasmodic and diuretic agent and known as halfabar. In the current study, we aimed at exploring the potential antihypertensive effect of *C. proximus* on Nω-Nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats. Hypertension was induced in rats by L-NAME (40 mg/kg) given orally once daily for 4 weeks. The antihypertensive activities of the total methanolic extract, n-hexane, chloroform and methanol fractions of *C. proximus* (50 and 100 mg/kg) were studied using a non-invasive Rat Tail-Cuff Blood Pressure System. The total methanolic extract or n-hexane fractions caused a dose-dependent decrease in the blood pressure of hypertensive rats. On the other hand, chloroform and methanol fractions did not show any significant effect on blood pressure. All the used extracts did not show any appreciable hypotensive effect on normotensive animals in the tested dose levels compared to animals administered vehicle only. Total methanolic extract and n-hexane fraction caused a transient decrease in blood pressure of about 8% at 100 mg/kg dose after 2 weeks of treatment. According to the results *C. proximus* extracts possess a valuable antihypertensive activity which supports further development of the extract as a potential therapeutically useful natural antihypertensive agent.

Keywords: *Cymbopogon proximus*, L-NAME, antihypertensive, halfabar, Captopril.

INTRODUCTION

Cymbopogon proximus STAPF (Gramineae) is a wild herbaceous plant used as an effective renal antispasmodic and diuretic agent in the Egyptian folk medicine and known as "Halfabar"^{1,2}. The petroleum ether extract of *C. proximus* has potent and unique antispasmodic properties through its smooth muscle relaxant effect without abolishing the propulsive movement or expulsive effect on renal and ureteric calculi³⁻⁵.

Many biological activities of *C. proximus* have been reported^{3, 6, 7}. Bioactivity-assisted fractionation of the *C. proximus* extracts led to the isolation of an active sesquiterpene, proximadiol (cryptomeridiol) which was found to have antidiabetic activity^{3, 7}. In addition, *C. proximus* essential oil was found to possess a bronchodilator activity mediated via antagonizing both histamine and serotonin receptors⁷. Furthermore, it has a significant ganglionic blocking action and a mild anti-inflammatory activity⁷.

Plants from the genus *Cymbopogon* such as *Cymbopogon citratus* which contains the essential oil, citronellol possess antihypertensive properties⁸. Citronellol lowers blood pressure by a direct effect on the vascular smooth muscle leading to vasodilation⁸. The arterial blood pressure of anaesthetized rats measured directly via the carotid artery was decreased in when they administered the oil of *C. proximus* intraperitoneally without significant

changes in the heart rate⁹. However, these studies lack pharmacological assessment of the antihypertensive activity of *C. proximus* extracts in animals with established hypertension.

Thus, the main purpose of the present study was to investigate the antihypertensive effect of different *C. proximus* extracts in Nω-Nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats, a well-established, dependable screening model for antihypertensive agents¹⁰⁻¹³.

MATERIALS AND METHODS

Plant Material, Extraction and preparation of the extracts and fractions

The aerial parts of *Cymbopogon proximus* STAPF. (Halfabar) purchased from a local market in Taif, KSA. The air dried powdered aerial parts of *Cymbopogon proximus* (Halfabar, 100 g) were extracted with methanol under reflux conditions and the methanolic extract was evaporated under reduced pressure to give the dried total methanolic extract (7.0 g). Another part of *Cymbopogon proximus* (Halfabar, 2.0 kg) was extracted successively with n-hexane followed by chloroform and finally with methanol respectively and the solvents was evaporated under reduced pressure to afford dried n-hexane fraction (38.2 g), chloroform fraction (18.0 g) and methanol fraction (112.7 g) respectively. The obtained dried extract and fractions was freshly prepared as



suspensions in saline immediately before use for oral administration to study their antihypertensive activity at doses of 50 mg/kg and 100 mg/kg.

Study of the antihypertensive activity of *Cymbopogon proximus*

Animals

Adult male Wistar albino rats (170±20 g), were obtained from the Animal Center of King Fahd medical research center, King Abdul Aziz University, Jeddah, KSA. Animals were maintained under conventional laboratory conditions with free access to food (standard pellet diet) and water. The protocol of all the procedures relating to animal care and treatments was approved by the Research Ethical Committee of College of Pharmacy, Taif University, Taif, Saudi Arabia (Protocol for project number: 1-433-1911-2013/2014).

Drugs and chemicals

Extracts and fractions of *Cymbopogon proximus* was prepared as mentioned before⁶. L-NAME was purchased from Sigma-Aldrich (St. Louis, MO, USA). Proximadiol separated by preparative TLC from *C. proximus* after it was assessed quantitatively by colorimetric methods.

In addition, a 60 grams of Proximol® effervescent granules contains standardized proximadiol 8 mg /100 g granules in *C. proximus* extract together with hexamine and piperazine citrate⁶.

Measurement of blood pressure

A non-invasive tail cuff blood pressure system (CODA™ tail-cuff blood pressure system, Connecticut, USA) was used to measure rats blood pressure. The blood pressure of rats was measured daily over 14 days to adapt the rats to the restrainer and tail cuff measurement. The systolic and diastolic blood pressures were measured before and after drug administration. Blood pressure was measured 20 times and the mean value was used as the blood pressure measurement.

Effect of extract on hypertensive rats

Hypertension was induced in rats by L-NAME (40 mg/kg) given orally once daily for 4 weeks^{14, 15}. A significant increase in the blood pressure of the animals was observed after 4 weeks. Hypertensive rats were maintained on L-NAME daily treatment for one more week during which the animals received daily oral gavage of saline, 50 or 100 mg/kg body weight of the different extracts or a standard antihypertensive drug captopril. A normal control group of six animals received only saline for the entire experimental period. The blood pressure of all animals was measured as mentioned above.

Effect of extract on normotensive animals

The extract and fractions (50 or 100 mg/kg) were given daily by oral gavage for 2 weeks. A control group received only saline. The blood pressure of the rats was measured

after 1 and 2 weeks of treatment. The body weights of the animals were also recorded.

Statistical analysis

Results were presented as the mean ± SEM, and statistical comparisons were made using one-way analysis of variance (ANOVA), followed by Tukey–Kramer post hoc test for multiple comparisons. Differences were considered significant at **P* < 0.05 and ***P* < 0.01.

RESULTS

Effect of extract and fractions on hypertensive rats

Oral administration of L-NAME once daily caused a significant rise in blood pressure after 4 weeks of administration. The blood pressure continued to rise when L-NAME treatment was continued for one more week (Figure 1). Daily oral administration of the different extract, fractions or captopril at the indicated dose levels caused a variable decrease in blood pressure.

There was a dose-dependent significant decrease in blood pressure compared to control group after treatments with the total methanolic extract or *n*-hexane fraction, while chloroform and methanol fractions did not show significant effect on blood pressure at doses of 50 mg/kg and 100 mg/kg (Figure 1).

Effect of extract on normotensive animals

All the used extracts did not show any appreciable hypotensive effect on normotensive animals in the tested dose levels compared to animals taking vehicle only. Total methanolic extract and *n*-hexane fraction caused a small transient decrease in blood pressure of about 8% at 100 mg/kg dose after 2 weeks of treatment (Table 1).

Table 1: Effect of *Cymbopogon proximus* (halfabar) total extract and fractions on the blood pressure of normotensive rats

Treatment	Time	
	Week 1	Week 2
Control	124.3 ± 3.2	123.7 ± 2.2
Total methanolic extract (50 mg/kg)	122.5 ± 1.9	121.4 ± 2.1
Total methanolic extract (100 mg/kg)	119.1 ± 2.9	114.3 ± 2.7
<i>n</i> -hexane fraction (50 mg/kg)	123.3 ± 1.6	123.7 ± 2.4
<i>n</i> -hexane fraction (100 mg/kg)	121.3 ± 2.1	113.9 ± 1.1
Chloroform fraction (50 mg/kg)	125.2 ± 2.4	126.3 ± 2.0
Chloroform fraction (100 mg/kg)	125.9 ± 3.7	123.2 ± 2.5
Methanol fraction (50 mg/kg)	127.3 ± 1.1	126.3 ± 4.1
Methanol fraction (100 mg/kg)	123.2 ± 2.1	123.3 ± 3.1



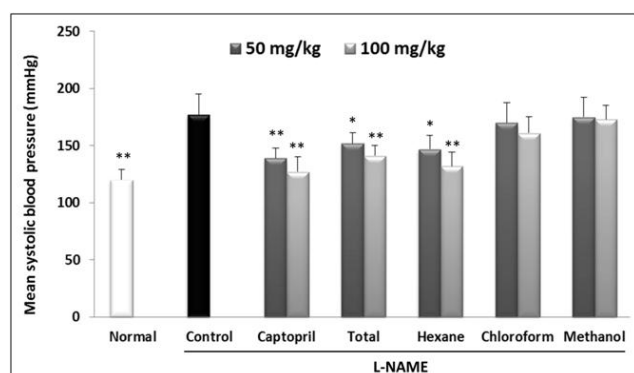


Figure 1: Effect of *Cymbopogon proximus* (halfbar) total extract and fractions on the blood pressure of L-NAME-induced hypertensive rats.

Total: total methanolic extract, *Hexane*: *n*-hexane fraction, *Chloroform*: chloroform fraction, *Methanol*: methanol fraction. Rats were treated with L-NAME for 1 week then given the different extract, fractions or saline (control) by oral gavage for a further week together with L-NAME. Columns, mean; bars, SEM (n = 6). Columns, mean; bars, S.D. (n = 6). **P* < 0.05, ***P* < 0.01 as compared to control hypertensive rats.

DISCUSSION

Hypertension is the major risk factor for many diseases such as stroke, heart diseases and renal failure^{16,17}. Many herbal remedies are used in folk medicine as effective antihypertensive agents. The aim of the present study was to evaluate the antihypertensive effects of *C. proximus* a wild herbaceous plant that is traditionally used as an effective renal antispasmodic and diuretic agent.

The results of the present study showed that *C. proximus* exhibited a dose-dependent significant decrease in blood pressure compared to control group after treatments with the total methanolic extract or *n*-hexane fraction on L-NAME induced hypertensive rats, while chloroform and methanol fractions did not show significant effect on blood pressure at doses of 50 mg/kg and 100 mg/kg. All the used extracts did not show any appreciable hypotensive effect on normotensive animals in the tested dose levels compared to animals taking vehicle only. Total methanolic extract and *n*-hexane fraction caused a small transient decrease in blood pressure of about 8% at 100 mg/kg dose after 2 weeks of treatment.

The arterial blood pressure of anaesthetized rats was decreased in a dose-dependent manner when they were administered the oil of *C. proximus* intraperitoneally without significant changes in the heart rate⁹. However, in our experiment we used the extraction procedure under reflux conditions which diminishes the role of the volatile oil and consequently, as proximadiol was found to form the major constituent of the total methanolic extract and *n*-hexane fraction, hence that the antihypertensive activity of *C. proximus* may be attributed to that particular compound. In addition, pretreatment of rats with the non-selective COX enzyme inhibitor, indomethacin blocked the observed hypotensive effect of *C. proximus* which indicated the possible involvement of

vasodilator prostaglandins in the oil-induced cardiovascular depressant effects⁹.

Since, *C. proximus* and its major component proximadiol possesses potent and unique antispasmodic properties via the relaxation of the smooth muscle fibers together with their diuretic effect⁵, the antihypertensive activity of *C. proximus* different extracts may be attributed to these activities. Moreover, the ability of the extracts to antagonize L-NAME-induced hypertension indicated the ability *C. proximus* to reverse the vascular changes involved in the L-NAME induced hypertension such as the blockade of nitric oxide production and the consequent inhibition of its vasodilator effect¹⁸.

Finally, *C. proximus* extract shows promising antihypertensive properties against the L-NAME model of hypertension. However, further studies are being carried out to challenge its antihypertensive activity in other models of experimental hypertension to elucidate the exact mechanism of action

In conclusion, the results of this study highlight the antihypertensive activity of different *C. proximus* extracts. The reported antihypertensive activity is mediated, at least in part, via its peripheral vasodilator and diuretic effects. These findings recommended *C. proximus* (*Halfbar*) extract as a promising antihypertensive natural drug.

Acknowledgment: The authors are grateful to The Research Projects Unit, Taif University, Saudi Arabia for the financial support of this study through the project number: (1-433-1911).

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Source of Support: Nil, Conflict of Interest: None.

