

## Cardiovascular Effects of Citrus Sinensis, Nigella Salivum, Astemizole versus Atenolol & Frusemide in Induced Hypertensive Rabbits

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**Abstract:** Hypertension is the most common cardiovascular disease with elevation of either systolic or diastolic arterial blood pressure. To investigate the possible cardiovascular effects of citrus sinensis, nigella sativum, astemizole in comparison with atenolol & frusemide in induced hypertensive rabbits & to anticipate their mechanism of action. Forty nine healthy domestic rabbits were involved in this study they were allocated to seven groups. Dexamethasone and hypertonic saline were given to the all groups to induce hypertension, after that to each group was given one of the followings citrussinensis, nigella sativum, alcohol, astemizole, atenolol and frusemide to examine their effect on the systolic and diastolic blood pressure, heart rate, blood flow and urine output. Citrus sinensis, nigella sativum, astemizole, atenolol & frusemide produced significant lowering effect to systolic and diastolic blood pressure. Astemizole and atenolol significantly decreased the heart rate. Citrus sinensis, nigella sativum, astemizole, atenolol & frusemide reduced blood flow significantly. The urine output significantly decreases by citrus sinensis, astemizole and frusemide with exception of nigella sativa and atenolol. Alcohol had no significant effect on all parameters. Citrus sinensis, nigella sativum have antihypertensive effect by different mechanisms at the doses mentioned and the results are promising.

**Keywords:** citrus sinensis, nigella sativum, hypertension, dexamethasone.

### INTRODUCTION

Hypertension is an elevation of either sustained systolic and diastolic arterial blood pressure to 140mmHg or 90mmHg respectively or more. It results from increased peripheral vascular muscle tone which leads to increased arteriolar resistance and reduced capacitance of the venous system [1]. Hypertension is usually occurs by multifactorial causes, but when there is no specific cause of hypertension can be found are said to be essential hypertension, which is concern with aging, race, genetic factors, stress and emotional factors, environmental and nutritional factors [ sodium intake] metabolic syndrome including [obesity, dyslipidemia, diabetes] cardiovascular diseases and consumption of alcohol and smoking [2]. The arterial blood pressure [BP] is directly proportional to cardiac output [CO] and peripheral vascular resistance [PVR].  $BP=CO \times PVR$  [3].

All antihypertensive drugs act by interfering with manual mechanisms; postural baro-reflex and renal response to decreased blood pressure include renin-angiotensin-aldosterone system [4]. It is obvious that dexamethasone with the help of hypertonic saline

solution in successive doses can induce hypertension [5].

A number of familiar medicinal herbs had been proved to have mild antihypertensive effect in patients with essential hypertension [6]. It is interest to explore the possible antihypertensive effect of citrus sinensis, nigella sativum, versus well known antihypertensive drugs with different mechanisms used as control in order to anticipate their mechanism of action through their effects on systolic and diastolic blood pressure, heart rate, blood flow and urine output.

Citrus sinensis is an over green tree with rounded crown cultivated on the Mediterranean and other subtropical regions. The medicinal parts are the fresh and dried peel and the oil that extracted from the peel. It is orange to orange –yellow in color. The chemical constituents are volatile oil and main components limonene, citronellal, sinesal, linalyl acetate flavonoids and furocoumarins can be used in anorexia, dyspepsia, antispasmodic and sedative [7].

*Nigella sativum* [black cumin] is an annual flowering plant. Native to south west asia which grows to height of 20-30cm with linear leaves, its capsule composed of 3-4 united follicles, each containing many seeds. It is used as a spice to flavor food its composition are volatile oil [negillone, thymoquinone, thymol, carvacol] negillin glycosides, saturated and unsaturated fatty acids beta-sitosterol, saponin, aminoacids etc [7].

*Negilla sativum* can be used in chronic brochial asthma [8]. In diabetes mellitus type 2 [9]. Antioxidant and antimutagenic [10]. In acute liver injury [11] and as antimicrobial in improvement of salmonellosis [12].

## MATERIALS AND METHODS

### Chemicals

All the chemicals used in present study were of analytic grade. Atenolol was supplied by medochem Ltd-cyprus. Frusemide sodium hydroxide by solvary pharmaceuticals-Germany. Dexamethasone sodium sulphate by medochem-cyprus. Astemizole by solvary pharmaceuticals-Germany. Ethanol alcohol 95% BDH chemicals Ltd poole England.

### Plant extraction

The tested medicinal herbs were purchased from well-known herbal shop were identified and authenticated by Iraqi national institute for herbs. The dried particles were carefully cleaned and powdered by electrical grinder and passed through sieve no.40 to remove debris. 10gm of well grinded citrus sinensis was

taken and mixed with 95% ethanol to 100ml by using electrical mixer for 15mins then the mixture was put in a hot plate magnetic stemmer for 48hr at temp of 45-50°C continuously. The solution was put in centrifugation of 1000 RPM for 30mins. The sediment was discarded and the supernatant was taken. The procedure was repeated 3 times to ensure the discharge of sediment, then supernatant was filtered by Seitz filter 0.45 um diameter pore. The supernatant was collected in dark container was concentrated by the use of rotatory evaporator and as the wanted volume [13].

Aqueous extract of nigella prepared by diluting one volume of the powder to 10 volume of water at temp. 80 °C in stoppered flask after shaking completely, thus the solution was allowed to stand for 10mins to be cold. The filtered to be used within 12hr [11].

### Animals

Forty nine healthy, domestic rabbits of both sexes, weighing 1000-1200gm were used in the present study. They were supplied by animal house of al-nahrain College of medicine. Animals were housed under good conditions at temp. 28°C in separated cages and were fed standard oxioid pellets and were given water ad-libitum. The rabbits were randomly allocated to seven groups [each group contain seven rabbits]. All the rabbits were given 2mg/kg dexamethasone I.P plus 2ml hypertonic saline three times daily for three successive doses until their BP became > 100/90mmHg. All the rabbits were given treatment at 9am for ten successive days.

Groups	Received / amount dose & strength	Routes of Administration	Frequency
Group-1	Distilled water/ 2ml	Intraperitoneal	
Group-2	Alcoholic extract of citrus sinensis/ 1mg/kg	Intraperitoneal	Three times daily
Group-3	Ethanol 95% / 0.5ml	Intraperitoneal	Three times daily
Group-4	Aqueous extract of nigella sativum	Intraperitoneal	Three times daily
Group-5	Astemizole / 0.3mg/kg	Orally	Once
Group-6	Atenolol / 1mg/kg	Intraperitoneal	Two times daily
Group-7	Frusemide / 1mg/kg	Intraperitoneal	Two times daily

The doses of citrus sinensis and nigella sativum and astemizole had been chosen by using many doses in pilot study. The animals have been anesthetized with pentobarbital in a dose of 30mg/kg I.P before administration the drugs in order to connect microphone transducer to measure BP [14]. This indirect method applied to the medical aspect of the thigh fixed to a constant tightness and joined to grass polygraph. Calibration of spikes with systolic BP. Baseline with diastolic BP.

Measuring urine output during the period of treatment which is beneficial to know mechanism of function. Urine output is collected to an accurate

graduated cylinder by polythene catheter inserted in to the urinary bladder while rabbits were anesthetized. The first urine before treatment is voided out of cylinder. Measuring blood flow by inserting carefully a small arterial blood flow probe and connecting the probe to the physiographer [3]. Calibration of physiographer so that blood flow can be measured ml/min.

### STATISTICAL ANALYSIS

All the obtained results were expressed as mean  $\pm$  SEM the differences in the means had analyzed by student' test using SPSS version 12. P. values are significant at < 0.05.

## RESULTS

**Table-1: Effect of alcoholic extract of citrus senensis. Alcohol, nigella sativum extract, astemizole, atenolol and frusemide on induced hypertensive rabbits**

Groups	Systolic BP mmHg	diastolic BP mmHg	Heart rate Beat /min	Blood flow Ml/min	Urine output ml
Dexamethasone + hypertonic saline	140±7.56	99.67±4.55	277.83±7.93	30±3.41	29.83±4.07
Citrus sinensis extract	<sup>x</sup> 110±8.23	<sup>x</sup> 70±4.98	260±9.23	<sup>x</sup> 33.93±3.11	<sup>x</sup> 41.83±3.66
Alcohol 95%	137.83±3.92	95±5.1	267.83±8.04	34±4.15	32±3.22
Nigella sativum extract	<sup>x</sup> 114.83±3.97	<sup>x</sup> 80.33±3.78	270.17±3.43	<sup>x</sup> 33.57±1.97	30±3.9
Astemizole	150.17±9.43	97.83±3.46	<sup>x</sup> 221.86±2.97	31.83±3.6	<sup>x</sup> 35.75±2.61
Atenolol	<sup>x</sup> 99.66±4.53	<sup>x</sup> 60.17±6.53	<sup>x</sup> 125±8.13	<sup>x</sup> 21.17±6.18	28.17±5.08
Frusemide	<sup>x</sup> 109.83±6.68	<sup>x</sup> 79.67±3.78	274.83±8.08	<sup>x</sup> 33.5±2.88	<sup>x</sup> 60.17±4.36

<sup>x</sup>p<0.05

The successive doses of dexamethasone can induce hypertension to the rabbits of all groups when given I.P with help of hypertonic saline. All the groups were given different treatment except group 1 which received distilled water only and consider as control. All the herbs extract and drugs reduced significantly both systolic and diastolic blood pressure except alcohol and astemizole groups. Atenolol is the drug that reduced the heart rate significantly more than others from 277.83±7.93 to 125±8.13 followed by astemizole to 221.86±2.97. All the blood flow of citrus sinensis, nigella sativum, atenolol and frusemide are significantly changed from 30±3.41 versus 33.93±3.11, 33.57±1.99, 21.17±6.18 and 33.5±2.88 respectively. The urine output of citrus sinensis, astemizole, and frusemide are significantly increased from 29.83±4.07 to 41.83±3.66, 38.45±2.61 and 60.17±4.36 respectively. Alcohol was used in group 3 as control to exclude its effect on alcoholic extract of citrus sinensis and has no effect on all the parameters.

## DISCUSSION

The normal blood pressure of the rabbits about 110/75mmHg [14], but administration of dexamethasone to the rabbits intraperitoneally with hypertonic saline solution in multiple doses can significantly increase the BP to < 130/90 mmHg which is similar to essential hypertension. The method of induction BP is the same like others [16].

Both atenolol and frusemide are antihypertensive drugs. The first related to  $\beta$ -adrenoreceptor blocking drugs (group 6) while the other is related to loop diuretics (group 7), both drugs acting by different mechanisms in lowering BP, they are used as control in this study. Atenolol is a  $\beta$ 1-selective antagonist that is devoid of sympathomimetic activity and has a negative inotropic and chronotropic effects, decreasing BP mainly through reducing cardiac output and suppression of renin release and acting on the CNS [16]. Atenolol decrease the blood flow significantly as a result of the lowering cardiac output which exerted by the drug and in having minimal  $\beta$ 2-adrenoceptor

blocking effect. Lastly urine output was not significantly changed by the drug. Frusemide is a diuretic which is mostly effective on hemodynamic disturbances. It was found to be significantly effective in lowering systolic and diastolic BP and significantly effective in increasing blood flow [17] which is related to decrease peripheral vascular resistance. Urine output is significantly increased, but there was no significant effect on heart rate [18].

The alcoholic extract of citrus sinensis was found to be significantly effective on both systolic and diastolic BP (group 2). It significantly increased blood flow due to vasodilation of flavonoids which is present in its composition [6], this will decrease the PVR and by this mechanism can reduce BP. The significant increase in the urine output is another mechanism that can lower BP through diuresis which acts in reducing blood volume and cardiac output [19]. The diuretic effect might be partly due to vasodilating effect by increasing blood flow to kidney [20].

Extract of citrus sinensis was more effective than frusemide in lowering diastolic BP but was equal in lowering systolic BP at the same time the extract was less effective than atenolol in lowering systolic and diastolic BP.

Since the vehicle moiety of the extract of citrus sinensis is alcohol (group 3), it is used at the same dose of concentration to exclude any effect of alcohol on systolic and diastolic BP and other parameters. Alcohol can cause hypotension by affecting the vasomotor center and cardiac depression. This effect is noticed only at high or toxic doses [21]. The aqueous extract of nigella sativum was found significantly effective in lowering both systolic and diastolic BP and increased blood flow which can be explained by vasodilation [increase radius of the vessel] and the urine output significantly (group5), but without affecting the heart.

The results of nigella sativum are consistent to the results of others [22] who proved a significant

hypotensive effect of nigella and also had a diuretic effect, but this is not found in our study because we use nigella sativum extract while the authors used instead of that dichloromethane extract of nigella sativum seed and they use different dosage and concentrations.

Atemizole is highly selective H1-receptor antagonist (group 5) which is free from sedative effect. Although it lowered the systolic and diastolic BP [a little bit] but this effect was insignificant but also lower the heart rate significantly, this effect possibly related to antagonizing histamine effect on the heart [23] also astemizole increase urine output significantly.

## CONCLUSION

The results of this study confirm that alcohol extract of citrus sinensis and extract of nigella sativum are effective as antihypertensive agents at the doses mentioned and the results are promising.

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