ISSN: 0976-7126
Mohapatra & Jain, 14(4):25-31, 2023

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Antihypertensive activity of *Allium sativum* and *Limmonia acidissima*

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Article info

Received: 01/03/2023

Revised: 25/04/2023

Accepted: 20/04/2023

www.ijplsjournal.com

Abstract

Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat model leading to increased blood volume and hence increased blood pressure. SBP and DBP were increased persistently in Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat models as compared to normal rats and Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* revealed the presence of alkaloids, because of these compounds the plants shows significant antihypertensive activity. The present study revealed that Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* possessed profound antihypertensive activity. As expected Amlodipine shows significant effect against hypertensive rats. Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* (100mg/kg) showed good effect as compared to Amlodipine and Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* (200mg/kg) showed almost similar effect as Amlodipine.

Key Words: Antihypertensive, *Allium Sativum, Limmonia Acidissima,* Blood Pressure.

Introduction

The force exerted by the heart as it pumps blood via blood arteries is known as blood pressure. Each heartbeat change in blood pressure. Less than 130 millimetres of mercury is considered normal for systolic pressure. Less than 85 millimetres of mercury is considered normal diastolic pressure. 130/85 is the result of writing the systolic and diastolic pressures as two numbers, respectively. [1]

Exercise, intense emotions, chilly weather, and even eating a big meal can all cause blood pressure to increase. Nevertheless, blood pressure ought to drop to less than 130/85 and stay that way for the rest of the day. [2] 972 million individuals worldwide are affected by the complex aetiology condition known as hypertension. [3]

It has grown to be a significant global burden on public health and is a key danger factor for cardiovascular disease.[4] Therefore, managing blood pressure must be taken into account along with managing other concurrent cardiovascular risk factors. High rates of hypertension are common, and the number of prescriptions for antihypertensive medications is rising daily in correlation with other illnesses like diabetes, hypercholesterolemia, and cardiovascular disease. With increasing ageing, HTN and Diabetes Mellitus (DM) usually coexist. Patients with DM have an 8% higher rate of HTN than those without (approximately a 2:1 ratio). [5] According to the Helsinki Heart Study, 30% of NIDDM patients had HTN. Pharmacological utilization studies that access and analyzes the pharmacological therapy for HTN.

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Thus, the current study was done to determine the anti-hypertension medicine prescribing trends in hypertensive and diabetic hypertensive patients at Sri Ramachandra Hospital's General Medicine Department. This type of medical audit draws attention to how doctors are currently prescribing and aids in further patient health care improvement. [6]

A medium-sized deciduous tree called Limonia acidissimai is cultivated all over India. It is a slow-growing tree that can reach a height of 9 meters and is scented. It can be found all over India in arid, warm climates at elevations of up to 450 meters. On some of the zigzag twigs, the spines are axillary, small, straight, and 2–5 cm long. [7]

The leaves are 3 to 5 inches long, alternating, dark green, leathery, and deciduous. Often has tiny teeth, is blunt or notched at the tip, is studded with oil glands, and when crushed has a faint lemon aroma. Small, loose, terminal or lateral panicles contain numerous dull-red or greenish flowers that are in great abundance. The fruit is a berry, huge, globosely, round to oval, 2 to 5 inches broad, with a stiff, woody skin that is about 6 mm thick and is grayish-white and scurfy. The pulp is sticky dark, resinous, astringent, acidic, or sweetish, and contains white seeds throughout. A monotypic genus in the Rutaceae family is Feronia. There are two varieties, one having big, sweet fruits and the other having tiny, sour fruits. [8]

Allium sativum, a member of the Alliaceae family, is known for being a valuable spice and a well-liked treatment for a number of illnesses and physiological disorders. The leaves and cloves of garlic, a bulb that grows to a height of 25–70 cm and has hermaphrodite blooms, have long been utilised in traditional medicine. A common herbal medicine known as aged garlic extract (AGE), which has been shown to strengthen the immune system and possibly fend off cancer and cardiovascular disease, is made from aged garlic. Furthermore, garlic loses its potent flavour as it ages, so breath odour is not a concern. Both culinary and medical uses for garlic exist. The use in cooking includes a spicy flavour that significantly sweetens and mellows with cooking. In addition to helping to avoid heart disease, its medicinal benefits include the treatment of whooping cough, lung disease, stomach complaints and disorders brought on by childbirth, colds, sore eyes, and earaches. According to a study conducted in the Czech Republic, garlic oil, particularly dehydrated powder, may aid to lessen the buildup of cholesterol in animal vascular walls. [9]

Material and Methods

Drugs

Amlodipine, Normal saline were purchased from Khandelwal Chemicals

Procurement of the plant parts

The *allium sativum* and *limmonia acidissima* were purchased from market of Mhow (M.P.)

Procurement of the plant part

The accoutrements washed with the water and keep dried for 2 hr. Preparation of the Methanolic extracts using a soxhlet device, the fruits of *limmonia acidissima* and *allium sativum* were uprooted. The soxhlet detergent excerpts were made by soxhlet rooting 50g of dried powdered fruits from *limmonia acidissima* and *allium sativum* in 200 ml to 400 ml of detergents at 65°C. On a water bath, the excerpt was concentrated to 10 ml, also dried at room temperature. Different exce rpts' chance yields in each detergent were noted. [10]

Phytochemical evaluation

The phytochemical evaluation of Methanolic extract of the fruits of *limmonia acidissima* and *allium sativum* were carried out as per standard methods .The presence of flavonoids will determined by lead acetate test, tannins by acetic acid test, saponins by foam test, alkaloids by Dragendorff's test and steroids were determined.

Pharmacological Method

Animals

Wistar albino rats weighing between 260 and 270 g were purchased from the Swami Vivekanand College of Pharmacy's animal home in Indore, India. The animals were kept in cages with four animals per cage, given free access to food and water, and kept under constant conditions of temperature (23°C) and humidity (60%) with a 12-hour light/dark cycle (light on from 07.30–19.30 h). In line with the Principles of Laboratory Animal Care (NIH publication no. #85-23 amended 1985), animals were cared for and maintained.

Experimental Design

Total 24 rats have been separated into six groups of each rat:

Group I is separated for Control Drug that is Normal Saline 2ml/kg

Group II is separated for Standard Drug that is Amlodipine, 2mg/kg

Group III is separated for Test I Drug that is Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 100mg/kg

Group IV is separated for Test II Drug that is Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 200mg/kg.

Procedure

Male and female both sex rats were separated into four groups at random. Group I is separated for Control Drug that is Normal Saline 2ml/kg, Group II is separated for Standard Drug that is Amlodipine, 2mg/kg, Group III is separated for Test I Drug that is Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 100mg/kg and Group IV is separated for Test II Drug that is Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 200mg/kg. Each group of animals acted as its own control. Prior to extract administration, baseline heart rates and blood pressure were recorded at 0 hours. Non-invasive blood pressure equipment (NIBP) is used for the measuring equipment (IN125, AD Instruments, Sydney, Australia), blood pressure and heart rate of each of these groups were assessed from the tails of rats at 0, 2, 4, and 6 h after intraperitoneal injection of various doses of the extract. Each rat was then placed in an NIBP restrainer, the proper cuff with sensor mounted on its tail, and the temperature increased to between 33 and 35 °C. Using a Power Lab data collecting device and computer running Lab Chart 5.0 software (Ad Instruments, Sydney, Australia), the tail cuff (MLT 125/R) was inflated to a pressure that was significantly higher than the anticipated systolic blood pressure, or 200 mm Hg, then gently released during which the pulses were recorded. Diastolic blood pressure (DBP) was determined while systolic blood pressure (SBP), mean blood pressure (MBP), and heart rate were obtained immediately using pulse tracing.

Egg feed-induced hypertensive rat model

Both male and female Sprague Dawley rats were split into two groups (n = 5). For 21 days in a row, Group 1 was given an oral special prepared egg feed meal (12 egg yolks combined with 500 g of regular rat diet) in order to induce hypertension caused by cholesterol. Allium sativum and limmonia acidissima methanol extracts (100 and 200 mg/kg) were given to the animals in group 2 for the same amount of time along with egg feed diet. All animals were given saline ad libitum rather than tap water. Each of these groups had their blood pressure and heart rates monitored using the technique outlined above on weeks 0, 1, 2, and 3.

Glucose-induced hypertensive rat model

Both sexes of Sprague Dawley rats were randomly divided into two groups (n = 5). For 21 consecutive days, tap water was substituted in Group 1 with a 10% glucose solution. Animals in group 2 received 10% glucose solution for the same duration as well as 100 and 200 mg/kg of methanolic extracts of allium sativum and limmonia acidissima, respectively. Typical diet was used to feed the animals. These groups' blood pressure and heart rates were assessed using the aforementioned technique on weeks 0, 1, 2, and 3.

Statistical analysis

The data were presented as mean standard error of the mean (SEM), and Graph Pad Prism 5.0 was used for statistical analysis using the Student's t-test. At p 0.05, differences were deemed significant.

Results and Discussion

Table 1: Phytochemical analysis of *limmonia acidissima* and *allium sativum*.

S. No.	Test	Positive/ Negative
1.	Carbohydrate	+
2.	Terpenoids	-
3.	Flavone Glycoside	+
4.	Phenolic Compound	+

5.	Flavonoids	+
6.	Saponins	-
7.	Sterols	+
8.	Alkaloids	+

Note:- + = Present; -= Absent

Table 2: Antihypertensive activity of Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* on rats by using Egg feed-induced hypertensive rat model

Group	Treatment	Dose	Systolic Blood Pressure (mmHg) Before Drug Administration				Systolic Blood Pressure (mmHg) After Drug Administration			
			W1	W2	W3	W4	W1	W2	W3	W4
I	Control	2 ml/kg	210±3	218±4	236±2	248±3	215±3	220±4	232±2	249±3
II	Amlodipine	2 mg/kg	213±3	219±4	238±2	243±3	150±3	151±4	155±2	157±3
III	Test(Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 100mg/kg)	100 mg/kg	217±3	223±4	239±2	245±3	176±3	174±4	172±2	178±3
IV	Test(Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 200mg/kg)	200 mg/kg	214±3	221±4	237±2	250±3	164±3	167±4	160±2	162±3

The Values are shown in the mean±SEM, *$P < 0.05$, **$P < 0.01$ as all the values compare with the control group (it is followed by the one way ANOVA Dunnett's test).

Table 3: Antihypertensive activity of Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* on rats by using Glucose-induced hypertensive rat model

Group	Treatment	Dose	Systolic Blood Pressure (mmHg) Before Drug Administration				Systolic Blood Pressure (mmHg) After Drug Administration			
			W1	W2	W3	W4	W1	W2	W3	W4
I	Control	2 ml/kg	210±3	218±4	236±2	248±3	215±3	220±4	232±2	249±3
II	Amlodipine	2 mg/kg	213±3	219±4	238±2	243±3	157±3	153±4	154±2	156±3

ISSN: 0976-7126
Mohapatra & Jain, 14(4):25-31, 2023

III	Test I (Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 100mg/kg)	100 mg/kg	217±3	223±4	239±2	245±3	177±3	175±4	174±2	171±3
IV	Test II (Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*, 200mg/kg)	200 mg/kg	214±3	221±4	237±2	250±3	164±3	166±4	163±2	160±3

The Values are shown in the mean±SEM, *$P < 0.05$, **$P < 0.01$ as all the values compare with the control group (it is followed by the one way ANOVA Dunnett's test).

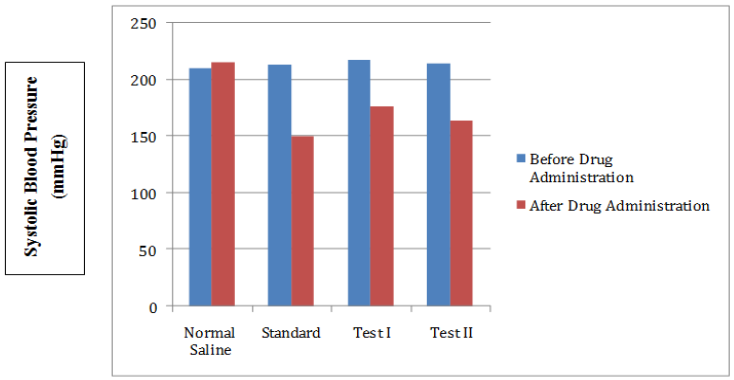

Fig. 1: Effects of Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* in the Egg feed-induced hypertensive rat model. The results are presented as means±S.E.M. ($n = 6$). Here are the following considerations shown: Systolic blood pressure. *$P < 0.05$, **$P < 0.01$, it has been compared with control-treated animals

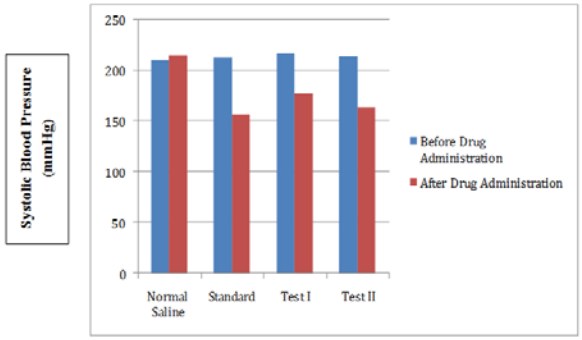

Fig 4.2 Effects of Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* in the Glucose-induced hypertensive rat model. Results are expressed as means±S.E.M. ($n = 6$). The following parameters are shown: Systolic blood pressure. *$P < 0.05$, **$P < 0.01$, compared with vehicle-treated animals

Summary
The Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* (200 mg/kg) showed significant Antihypertensive activity in Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat model. The plants (*allium sativum* and *limmonia acidissima*) containing carbohydrates, flavone glycosides, Phenolic compound, Flavonoids, Alkaloids and sterols were identified. Alkaloids may be responsible for the Antihypertensive activity of the plant. In the present study, we used Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat model of hypertension to evaluate the antihypertensive effects of the Methanolic extract of fruits of allium sativum and limmonia acidissim. Effects of 1, 2, 3 and 4 weeks of treatment with Methanolic extract of fruits of allium sativum and limmonia acidissima and Amlodipine on hypertensive animals. Hypertensive control group displayed a same result as before the drug administration. As expected Amlodipine shows significant effect against hypertensive rats. Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* (100mg/kg) showed good effect as compared to Amlodipine and Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* (200mg/kg) showed almost similar effect as Amlodipine.

Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat model leading to increased blood volume and hence increased blood pressure. SBP and DBP were increased persistently in Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat models as compared to normal rats and Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* revealed the presence of alkaloids, because of these compounds the plants shows significant antihypertensive activity. The present study revealed that Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* possessed profound antihypertensive activity.

Amlodipine decreased the elevated blood pressure. However, unlike many other plant extracts where an antihypertensive effect was accompanied, increase in the dose of Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* exerted decreased in blood pressure. In this study, Egg feed-induced hypertensive rat model and Glucose-induced hypertensive rat model were taken to measure of antihypertensive activity. The findings in this study suggest that the Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* possess Antihypertensive activity. The results have been obtained carefully from the controlled experiments model with laboratory animals. The statistical validity of the findings has been proven and they provide a scientific foundation for the uses of the biologically active ingredients of Methanolic extract of fruits of *allium sativum* and *limmonia acidissima* in antihypertensive activity for explain the clinical importance of the Methanolic extract of fruits of *allium sativum* and *limmonia acidissima*.

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Cite this article as:
Mohapatra S. and Jain S.K. (2023). Antihypertensive activity of *Allium sativum and Limmonia acidissima*. *Int. J. of Pharm. & Life Sci.*, 14(4): 25-31.

Source of Support: Nil
Conflict of Interest: Not declared
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