Original Article

Antihypertensive effect of allicin in dexamethasone-induced hypertensive rats

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ABSTRACT

Background: Glucocorticoid is among the most commonly prescribed medicine. Unfortunately, Excess glucocorticoid level leads hypertension in 80–90% patients. Garlic (Allium sativum) has been used since ancient times and even nowadays as a part of popular medicine for various ailments and physiological disorders. Hence this study was undertaken to investigate the antihypertensive activity of allicin in dexamethasone induced hypertension in wistar rats.

Methods: The animals were randomly divided into four groups comprising of six rats per group. Hypertension was induced by subcutaneous injection of dexamethasone (10 μg/rat/day) in hypertensive rats. Two hypertensive group animals were treated with nicorandil (6 mg/kg/day, po) and allicin (8 mg/kg/day, po) respectively for 8 weeks. While systolic blood pressure (SBP) was measured by the tail-cuff method weekly up to 8 weeks.

Results: Dexamethasone treatment resulted in significant increase in SBP while allicin treatment significantly decreases the SBP. Thus, this study confirmed that allicin treatment for 8 weeks partially reverse dexamethasone induced hypertension in rats. Allicin treatment also attenuated dexamethasone-induced anorexia and loss of total body weight.

Conclusion: This result suggests antihypertensive effects of allicin in dexamethasone induced hypertension. However, further studies are needed to explore the detailed mechanism of antihypertensive effect of allicin.

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1. Introduction

Glucocorticoid is among the most commonly prescribed medicines for asthma, rheumatological syndrome, eye disorder, skin disorder, organ transplant, glomerulopathies, malignancies, pain syndrome, and other conditions. 1,2 Glucocorticoids have potent anti-inflammatory and immunosuppressant activities. Unfortunately, long-term glucocorticoid

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therapy leads to hypertension and diabetic condition. Glucocorticoid treatment causes hypertension and abnormal glucose metabolism in 80–90% of patients having Cushing syndrome. In the current scenario, we are looking for herbal drugs to avoid such adverse effects. The usage of herbal and nutritional supplements is widespread all over the world. Now a large number of patients are using herbal drugs for cardiovascular diseases. Certain herbal drugs such as St John's wort, yohimbine, licorice, ephedra, garlic, etc. have been in use for many decades for the treatment of hypertension. However, the underlying mechanism of action of herbal drugs is not clearly understood. Most prominent among these herbs is the commonly used Indian traditional spice garlic, Allium sativum L., a member of the Alliaceae family, which has been used since ancient times and, even today, is a part of popular medicine for various ailments and physiological disorders. Fresh garlic extract contains the organosulfur compound allicin, which is considered to have various pharmacological activities, including, anti-thrombotic, anti-diabetic, anti-tumorigenic, antioxidant, anticarcinogenic, anti-atherosclerotic, and anti-hypertensive activities. Therefore, the aim of the present study was to explore the effects of allicin in the treatment of dexamethasone-induced hypertension in rats. Dexamethasone is the most potent synthetic glucocorticoid that has virtually pure glucocorticoid activity.

2. Methods

2.1. Animals

Wistar rats (150–200 g) of either sex were used in this study, and each experimental group included six animals. Animals bred in the animal house of Institute of Pharmaceutical Education and Research (Reg. No. 535/02/a/CPCSEA/Jun2002), Wardha (Maharashtra State), India. The rats were housed under standard laboratory conditions (22 ± 2 °C, 12-hour light/dark cycle) with free access to food (normal pellet diet) and water. The animals were treated in accordance with the Committee for the Purpose of Control And Supervision of Experiments on Animals CPCSEA guidelines. The experimental protocol was approved by the Institutional Animal Ethics Committee (approval number 10/200910).

2.2. Chemicals

Dexamethasone and nicorandil were procured from Zyduz Cadila Healthcare Ltd (Bangalore, India) and Medrech Saimirra Ltd (Chennai, India) respectively. All the other chemicals used for experimental purpose were of analytical grade.

2.3. Induction of hypertension

In the experimental rats, hypertension was induced by subcutaneous injection of dexamethasone (10 μg/kg/d) in the evening.

2.4. Preparation of aqueous extract of garlic

Garlic (A. sativum L) grown in Wardha was acquired from the market. Garlic bulbs were identified and authenticated by Dr Alka Chaturvedi, Post graduate Teaching Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, with a voucher specimen (No. 9803). Garlic bulbs were stored at 4 °C and used for analysis within 30 days. Allicin-containing garlic extract was prepared from 10 g of garlic cloves. The cloves were crushed with an electric vegetable crusher, and the juice was poured into a sterile centrifuge tube and centrifuged at 5000 rpm (3000 g) for 10 minutes in order to separate the majority of the pulp from the supernatant liquid. The supernatant garlic extract (allicin) was either used immediately for activity or stored at 4 °C; it was relatively stable during the experimental weeks. Accordingly, animals were administered with 8 mg allicin/kg body weight.

2.5. Analysis of allicin in fresh garlic extract

Allicin in garlic extract (10 μL, 20 μL, 30 μL, and 40 μL of the extract were used) was reacted with cysteine via the thiol-disulfide exchange reaction, and the remaining cysteine was subsequently determined by reaction with Ellman’s reagent 5,5’-dithiobis-(2-nitrobenzoic acid) to produce the 2-nitro-5-thiobenzoate anion. Absorbance was measured at a wavelength of 412 nm. One mole of thiosulfinate reacts with 2 mole cysteine, and since allicin makes up 60–80% of the thiosulfinates produced in garlic, multiplication of the total thiosulfinate content by a factor of 0.7 gives the approximate allicin content.

2.6. Experimental design

Animals were randomly divided into four groups, each consisting of six animals. Animals in group I (normal control rats) received 1 mL (po/d) 1% acacia gum suspension, group II (hypertensive control rats) received 1 mL (po/d) 1% acacia gum suspension, animals in group III (hypertensive rats) received nicorandil 1 mL (6 mg/kg/po/d) in 1% acacia gum suspension and group IV (hypertensive rats) received allicin 1 mL (8 mg/kg/po/d) in 1% acacia gum suspension during the course of the entire study (8 weeks).

2.7. Estimation of systolic blood pressure

Systolic blood pressure (SBP) measurements were recorded weekly by the same investigator, between 10 am and 12 noon, using the integrated BIOPAC -is an instrument use to measure blood pressure and NIBP-Non Invasive Blood Pressure 200A system. The animal is placed in the restrainer (animal holder) leaving the tail outside and adjusted to the position where the animal has limited movement. The restrainer is placed in the heating chamber and heated up to 32 °C. BSL PRO software is used for recording SBP. The basic software setup is done, and IR sensors are calibrated prior to starting the measurement. An IR sensor is then connected to the tail of the animal inside the restrainer. After the required setup and calibration of IR sensors, SBP was recorded.
2.8. **Estimation of body weight, food, and water intake**

Body weight, food, and water intake of each animal of every group were measured weekly, for 8 weeks.

2.9. **Statistical analysis**

Statistical analysis was carried out by two-way analysis of variance followed by Bonferroni post-tests. All values were expressed as mean ± standard error of the mean. All groups were compared with hypertensive control animals. A p value of <0.05 was considered to be statistically significant.

### 3. Results

3.1. **Quantification of allicin from fresh garlic aqueous extract**

Garlic extracts contained approximately 12.8 mg allicin/mL.

3.2. **Effect of allicin on SBP in hypertensive rats**

Table 1 shows that SBP (p<0.05) increased significantly in hypertensive control animals when compared with the normal control group. Allicin-treated animals were found to have significantly (p<0.05) decreased SBP when compared with hypertensive control group.

3.3. **Effect of allicin on body weight**

A significant decrease in body weight was recorded in all groups of dexamethasone-treated animals. Hence, all treated animals did not show any significant changes when compared with group II (hypertensive control) animals. However, body weight of group I (normal) animals significantly increased in the 7th (p<0.05) and 8th (p<0.001) weeks (Table 2) as compared with that of group II (hypertensive control) animals.

3.4. **Effect of allicin on food intake**

No significant changes in food intake were found in any group of animals until the 7th week, while in the 8th week a significant increase (p<0.05) in food intake was recorded in group I (normal) and group IV (allicin-treated) animals when compared with hypertensive control animals (Table 3).

### 4. Discussion

In the present study, we found that dexamethasone increased SBP in rats, and this hypertension was attenuated by allicin; this result showed the antihypertensive effect of allicin. Various studies have suggested that dexamethasone causes overproduction of reactive oxygen species such as superoxide, interacts with nitric oxide (NO) produced by the vascular endothelium—a vasodilator, causes NO-redox imbalance, and reduces its NO bioavailability, which may lead to hypertension.13,14,19–21 Further, dexamethasone can also block NO synthase gene expression at the transcriptional level.22 Thus, dexamethasone can act by several
Table 2 – Effect of allicin on body weight in hypertensive rats.

| Group(s) | Body weight (g) | 0th week | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | 7th week | 8th week |
|----------|----------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| I        | 160.33 ± 10.50 | 163.33 ± 12.80 | 165.83 ± 11.30 | 168.00 ± 9.70 | 170.17 ± 9.58 | 179.00 ± 8.70 | 193.50 ± 10.65 | 205.17 ± 13.80 | 220.67 ± 12.60 |
| II       | 165.83 ± 12.40 | 162.50 ± 10.50 | 159.00 ± 13.43 | 155.83 ± 11.75 | 152.16 ± 9.80 | 153.33 ± 10.60 | 151.66 ± 10.37 | 149.83 ± 11.45 | 147.33 ± 8.32 |
| III      | 164.67 ± 18.90 | 160.16 ± 16.55 | 156.66 ± 13.34 | 153.66 ± 15.87 | 151.83 ± 10.84 | 149.16 ± 9.60 | 150.83 ± 12.53 | 148.66 ± 11.55 | 148.16 ± 9.89 |
| IV       | 165.83 ± 17.70 | 163.16 ± 15.66 | 160.83 ± 12.74 | 158.66 ± 11.95 | 155.50 ± 14.75 | 153.00 ± 10.55 | 150.50 ± 9.50 | 147.33 ± 11.43 | 144.83 ± 9.30 |

Data are expressed as mean ± SEM (n = 6). Significance was determined by two-way ANOVA followed by Bonferroni post-tests. A p value of <0.05 was considered significant. All groups were compared with group II.

ANOVA, analysis of variance; group I, normal control animals; group II, hypertensive animals; group III, nicorandil-treated animals; group IV, allicin-treated animals; SEM, standard error of the mean.

* p < 0.05.
*** p < 0.001.

Table 3 – Effect of allicin on food intake in hypertensive rats.

| Group(s) | Food intake (g/d) | 0th week | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | 7th week | 8th week |
|----------|------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| I        | 14.00 ± 3.91     | 13.00 ± 1.79 | 11.50 ± 1.64 | 9.00 ± 2.60 | 12.00 ± 2.39 | 10.60 ± 2.06 | 13.80 ± 1.62 | 15.60 ± 1.60 | 17.30 ± 1.17 |
| II       | 12.30 ± 2.67     | 8.50 ± 1.48 | 9.00 ± 2.12 | 10.00 ± 2.77 | 6.50 ± 1.71 | 8.30 ± 1.83 | 11.40 ± 1.92 | 9.20 ± 1.51 | 7.80 ± 1.84 |
| III      | 15.30 ± 1.98     | 9.33 ± 1.57 | 9.00 ± 1.74 | 8.33 ± 2.09 | 10.50 ± 2.48 | 8.50 ± 3.43 | 11.20 ± 3.22 | 15.00 ± 4.37 | 13.66 ± 3.11 |
| IV       | 18.30 ± 17.70    | 12.60 ± 1.96 | 7.90 ± 1.55 | 8.00 ± 2.34 | 7.66 ± 1.68 | 11.33 ± 2.05 | 13.40 ± 1.47 | 15.70 ± 1.31 | 17.66 ± 2.80 |

Data are expressed as mean ± SEM (n = 6). Significance was determined by two-way ANOVA followed by Bonferroni post-tests. A p value of <0.05 was considered significant. All groups were compared with group II.

ANOVA, analysis of variance; group I, normal control animals; group II, hypertensive animals; group III, nicorandil-treated animals; group IV, allicin-treated animals; SEM, standard error of the mean.

* Shows the level of significance (p < 0.05).
pathways to deplete the supply of NO, consequently leading to vasoconstriction.\textsuperscript{23} Another possible explanation may be that dexamethasone increases the levels of enzymes such as angiotensin-converting enzyme, which might be responsible for a significant elevation in blood pressure.\textsuperscript{24,25} The hypertensive effect of dexamethasone can be reduced by elimination of the above risk factors via increases in NO level, angiotensin-converting enzyme inhibitor activity, and free radical-scavenging activity. It has already been established that antioxidants are effective in the treatment of dexamethasone-induced hypertension. In the present study, experimental animals of hypertensive groups, such as group I (normal) and group IV (allicin treated) animals, showed lower SBP (\(p < 0.05\)) when compared with group II (hypertensive control) rats (Table 1).

Various mechanisms for the antihypertensive effect of garlic have been reported, including reduction in vascular resistance and a subsequent fall in total peripheral resistance contributing substantially to the antihypertensive action by activating NO synthesis, the enzyme that produces NO.\textsuperscript{26,27} Further, Park et al\textsuperscript{28} reported that fermented garlic contains nitrite, which is converted to NO in the body, and increases protein kinase (PKG) and eNOS protein expressions in aortic tissues. This further leads to antihypertensive effects via the Soluble guanylyl cyclase (sGC)–Cyclic guanosine monophosphate cGMP–protein kinase pathway. In addition, garlic (allicin) has antioxidant property, which might be responsible for the antihypertensive effect of allicin by lowering the level of oxidative stress in hypertensive rats, consequently restoring NO–redox imbalance and increasing NO bioavailability.\textsuperscript{26,27} Recently, Hiramatsu et al\textsuperscript{29} reported that aged garlic extract increases the expression of nuclear factor erythroid 2-related factor 2, which is responsible for the antioxidant activity. They confirmed that aged garlic extract decreases oxidative stress and maintains cellular redox balance via the nuclear factor erythroid 2-related factor 2–antioxidant response element signaling pathway.

Further, garlic has potent vasorelaxant and K\textsuperscript+ channel opener activities, which lead to vasodilation, thus exerting an antihypertensive effect.\textsuperscript{5,6,11,30,31} This report was further supported by the finding that allicin selectively opens SUR2, a type of K\textsuperscript+ channel receptor, present in blood vessels, leading to dilation of blood vessels, which may be a possible mechanism for the antihypertensive activity of allicin.\textsuperscript{32} Other studies have also revealed that fresh garlic extract (allicin) has inhibitory activity against angiotensin 1 converting enzyme and diuretic activity, which may be involved in the antihypertensive action of garlic.\textsuperscript{10–12,28,33,34}

Previous evidence supports the theory that corticosteroids induce an imbalance between vasoconstriction and vasodilation, favoring vasoconstriction, resulting in hypertension.\textsuperscript{34} Corticosteroids seem to negatively affect the production of other vasodilatory substances, such as prostaglandin I\textsubscript{2} and prostaglandin, and to have a positive effect on vasoconstrictor prostaglandins such as thromboxane-B\textsubscript{2} in the vascular endothelium.\textsuperscript{35–37} Garlic causes more reduction in the synthesis of thromboxane B\textsubscript{2}, thereby reducing hypertension. Garlic was also found to inhibit endothelin-1–induced contraction.\textsuperscript{37,38} Recently, a randomized database search clinical trial reports suggested that garlic is an effective and safe therapeutic approach for the treatment of hypertension.\textsuperscript{39}

In addition, dexamethasone causes a decrease in body weight and food intake. The possible mechanism could be that dexamethasone increases leptin mRNA expression in the adipose tissue and induces long-lasting hyperleptinemia in rats; plasma leptin may play a role in dexamethasone-induced anorexia. Additionally, increased expression of monoamine oxidase A and 5-hydroxytryptamine (5-HT) reuptake transporter genes by repeated dexamethasone administration appears to be implicated in decreases of brain 5-HT levels in the hypothalamus.\textsuperscript{40,41} Synergistically low level of 5-HT and high plasma leptin level suppress food intake and increase energy expenditure, resulting in a loss of both fat and lean mass; this mechanism may be involved in the reduction of total body weight and food intake in dexamethasone-treated animals.

This study indicates that long-term treatment with allicin-containing garlic extract shows significant effect in reversing dexamethasone-induced SBP as well as improves body weight and food intake in dexamethasone-induced hypertension in rats. However, this study has some limitations; the molecular mechanism involved in antihypertensive effects needs to be explored. Moreover, it has already been established that allicin is the major compound of garlic, and all sulfoxides except cyanoallin are converted into allicin by enzymatic reactions.\textsuperscript{42} Hence, we need to evaluate cyanoallin-related activities. Further, it is well documented that allicin has a very short half-life, and it is difficult to maintain its long-term therapeutic activities. There are some other compounds such as stable active metabolite of allicin may be responsible for antihypertensive activity. Hence, further studies exploring the molecular mechanism and active molecule responsible for antihypertensive action are required.

Conflict of interest
The authors declare no conflict of interest.

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