



## 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 dis-

order, skin disorder, organ transplant, glomerulopathies, malignancies, pain syndrome, and other conditions.<sup>1,2</sup> 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.<sup>2,3</sup> 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.<sup>4</sup> 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.<sup>5-7</sup> Fresh garlic extract contains the organosulfur compound allicin, which is considered to have various pharmacological activities, including, antithrombotic, antidiabetic, antitumorigenetic, antioxidant, anticarcinogenic, antiatherosclerotic, and antihypertensive activities.<sup>5,8-12</sup> 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.<sup>1</sup>

## 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/Jan2002), Wardha (Maharashtra State), India. The rats were housed under standard laboratory conditions ( $22 \pm 2^\circ\text{C}$ , 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 Zydus Cadila Healthcare Ltd (Bangalore, India) and Medreich 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 ( $10 \mu\text{g}/\text{kg}/\text{d}$ ) in the evening.<sup>13,14</sup>

### 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  $4^\circ\text{C}$  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  $4^\circ\text{C}$ ; it was relatively stable during the experimental weeks. Accordingly, animals were administered with 8 mg allicin/kg body weight.<sup>15,16</sup>

### 2.5. Analysis of allicin in fresh garlic extract

Allicin in garlic extract (10  $\mu\text{L}$ , 20  $\mu\text{L}$ , 30  $\mu\text{L}$ , and 40  $\mu\text{L}$  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 thiosulfinate produced in garlic, multiplication of the total thiosulfinate content by a factor of 0.7 gives the approximate allicin content.<sup>15</sup>

### 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 ( $6 \text{ mg}/\text{kg}/\text{po}/\text{d}$ ) in 1% acacia gum suspension,<sup>17,18</sup> and group IV (hypertensive rats) received allicin 1 mL ( $8 \text{ mg}/\text{kg}/\text{po}/\text{d}$ ) in 1% acacia gum suspension<sup>16</sup> 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  $32^\circ\text{C}$ . 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.

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