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Sulphur treatment alters the therapeutic potency of alliin obtained from garlic leaf extract

S.A. Nasim [a,*], B. Dhir [b], F. Samar [a], K. Rashmi [a], Mahmooduzzafar [a], A. Mujib [a]

[a] Environmental Biotechnology Laboratory, Department of Botany, Hamdard University, New Delhi 110 062, India
[b] Department of Environmental Biology, University of Delhi, Delhi 110 007, India

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ABSTRACT

The therapeutic potency of garlic leaf extract obtained from normal and sulphur treated plants was compared. Alliin, the active compound of garlic leaf extract showed ~32% increase in yield under sulphur treated conditions. Alliin obtained from leaf extract of plants brought a significant reduction in serum glucose, triglycerides, total lipids, total cholesterol, LDL- and VLDL-cholesterol levels than glibenclamide in alloxan-induced diabetic rats. Alliin from sulphur treated plants was more effective in comparison to that obtained from plants raised in normal conditions. Serum glucose levels showed significant reduction of 50% in rats administered with leaf extract from sulphur treated plants in comparison to 37% noted in rats administered with leaf extract from normal plants. No alteration in HDL-cholesterol was noted. Similarly, alliin obtained from leaf extract of plants lowered the serum enzyme (ALP, AST and ALT) levels towards normal than glibenclamide. The reduction in serum enzyme levels was significant in rats administered with leaf extract of plants raised under sulphur treated conditions in comparison to that raised under normal conditions. The present findings suggest that leaf extract from sulphur treated garlic possess more antidiabetic potential and hence show more therapeutic potency in comparison to extract obtained from normal plants.

1. Introduction

The consumption of traditional plants have increased worldwide mainly because of their effectiveness, fewer side effects and relatively low cost (Venkatesh et al., 2003). Garlic (*Allium sativum* L., Liliaceae) is a common flavoring agent used since ancient times. The importance of garlic in treating diabetes is well documented in literature. Pioneering works by many researchers proved that garlic possess potent hypoglycemic activity (Roman-Ramos et al., 1995; Kasuga et al., 1999). The potency is linked to the organosulphur compounds, particularly cysteine sulfoxides and thiosulfinates. Among them, S-allyl-L-cysteine sulfoxide (alliin) which is derived from dipeptide γ-glutamyl-S-allylcysteine (GLUAICS) accumulates in high concentration in garlic (Block, 1985; Block et al., 1993) and accounts for hypoglycemic effect (Sheela and Augusti, 1992a).

Sulphur is one of the essential nutrients required for plant growth (Ahmad and Abdin, 2000) and forms an important component of sulphur compounds including amino acids (Scherer, 2001). Increased sulphur supply affect growth and development of crop plants (Ahmad and Abdin, 2000; Scherer, 2001). Earlier studies revealed that increased sulphur supply improve crop yield and alter concentration of metabolites such as glucosinolates in the plants (Scherer, 2001; Lošák and Wiśniowska-Kielian, 2006; Bloem et al., 2007). Though extensive literature is available focusing the impact of increased sulphur supply on crop plants but studies related to effects on medicinal plants and their properties are scanty. Therefore, present investigations were carried out with an aim: (i) to study and compare the alliin (metabolite) production in leaves of normal and sulphur supplied plants; (ii) compare the therapeutic efficiency (antidiabetic effect) of alliin extracted from leaves of plants raised in normal and sulphur rich conditions and (iii) compare the efficiency of alliin from sulphur supplied plants with that of standard drug glibenclamide (standard reference).

2. Materials and methods

2.1. Plant material

Local Indian garlic (*A. sativum* L. cv Yamuna safed) procured from the National Horticultural Research and Development Foundation, Nasik (Maharashtra) was used in the present study.

2.2. Experimental set up for growing garlic plants in normal and sulphur supplied conditions

Garlic plants were raised under natural conditions in 14 in. earthen pots having 10 kg of varying soil. The soil was sandy loam, with pH 7.2, and deficient in S (0.01%). For sulphur treatment, gypsum ($CaSO_4$) @40 mg/kg was supplied after

* Corresponding author. Tel.: +91 9990306151; fax: +91 11 6088874.
E-mail address: nasimdu@gmail.com (S.A. Nasim).

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2 weeks of planting at a single basal dose. The dose was decided as per the earlier studies (Arnault et al., 2003). Ten pots of each treatment (normal and sulphur treated) were arranged in north–south direction in randomized design for uniform light conditions and were suitably irrigated to provide possible uniform soil moisture conditions. The experiment was repeated for three consecutive years. The samples were analyzed for single developmental stage i.e. full emergence of leaves and/or before development of bulb (i.e. 5 weeks after planting). Healthy leaves were sampled and samples were immediately shock-frozen in liquid nitrogen and subsequently freeze-dried to prevent the decomposition of alliin.

2.3. Phytochemical analysis

2.3.1. Leaf powder extraction

For HPTLC analysis, 1 g dry leaf powder was extracted at room temperature by using 10 ml methanol–water (80:20, v/v) plus 0.05% formic acid (pH 3) following method described previously by Arnault et al. (2003).

2.3.2. Preparation of dilution of standard alliin

For HPTLC analysis, 1 ml of 1 mg l^{-1} stock solution of alliin was taken and was further diluted with methanol to make the final volume to 10 ml (100 ng/ml) (Kanaki and Rajani, 2005).

2.3.3. Chromatographic conditions

The samples were spotted in the form of bands (width 3 mm) with a Camag microlitre syringe on pre-coated silica gel 60 F_{254} aluminum sheets (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag linomat V (Switzerland). A constant application rate of 80 nl/s was employed and space between two bands was 5.2 mm. Linear ascending development was carried out in twin through glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of chromatogram run was 90 mm. Subsequent to the development, TLC figures were dried in air current with the help of drier. Densitometry scanning was performed on Camag TLC Scanner IV in the absorbance mode at 550 nm after spraying with 2% ninhydrin and drying at 100 °C for 10 min. The source of radiation utilized was tungsten lamp (Kanaki and Rajani, 2005). The slit dimension was kept at 4 mm × 0.1 mm, and 20 mm/s scanning speed was employed. The other details of methodology include:

Plate: HPTLC silica gel (Merck).
Solvent: Butanol:propanol:acetic acid:H_2O (3:1:1:1).
Detection: Spray with 2% Ninhydrin followed by heating.

2.4. Animals

Healthy male albino wistar rats weighing between 200 and 300 g procured from the Central Animal House facility, Jamia Hamdard were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India). Rats were housed in clean cages under controlled environmental conditions (temperature: 22–24 °C; relative humidity 40–60%). In the present study, 56 male wistar rats were used. They were divided into seven groups on the basis of different treatments given to them (details Section 2.7.).

2.5. Experimental induction of diabetes in rats

Rats were starved overnight. Each rat received an intra peritoneal injection of alloxan monohydrate in freshly prepared sodium-acetate buffer (0.15 M, pH 4.5) (Mansour et al., 2002). The dose of alloxan was 20 mg/100 g body weight in a volume of 0.10–0.15 ml. The same volume of acetate buffer was given to each control rat. From next day, a single injection of two units of protamine–Zn insulin prepared in normal saline was given to each alloxan treated rat for 6 days. This decreased the mortality of the animals. Controls were given the same volume of normal saline instead of insulin.

2.6. Preparation of garlic extracts and drug administration

Healthy leaves were collected from plants grown under normal and sulphur rich conditions. 50 g leaves were homogenized in 75 ml of sterilized 0.9% saline solution in ice cold conditions. The homogenized mixture was filtered three times through cheesecloth. The filtrate was centrifuged at 2000*g* for 10 min and volume of clear supernatant was made up to 100 ml with normal saline solution. The concentration of leaf preparation was found to be 500 mg/ml (0.5 g/ml), on the basis of the leaf weight. The aqueous extract obtained was stored at 20 °C. Final dose administered corresponded to 0.5 g kg^{-1} body weight. The lower concentration was prepared according to body weight of rats by diluting leaf extract with cold, sterile 0.9% saline.

The present dose was decided on the basis of earlier experiment that showed toxicity above given value (data not shown).

2.7. Experimental design

In the present study, 56 male wistar rats were used in each experiment. Rats were divided into seven groups with 8 rats in each group.

Group I: Normal rats that were administered 1 ml of sterile distilled water using intragastric tube for 5 weeks.
Group II: Normal rats that were administered 1 ml of aqueous leaf extract, (NLE) daily using intragastric tube for 5 weeks.
Group III: Normal rats that were administered 1 ml of aqueous leaf extract obtained from sulphur treated plants (SLE), daily using intragastric tube for 5 weeks.
Group IV: Alloxan treated rats that were administered 1 ml distilled water daily using intragastric tube for 5 weeks.
Group V: Alloxan treated rats that were administered 1 ml aqueous leaf extract (NLE) obtained from normal plants, daily using intragastric tube for 5 weeks.
Group VI: Alloxan treated rats that were administered 1 ml aqueous extract from sulphur treated plants, (SLE) daily using intragastric tube for 5 weeks.
Group VII: Alloxan treated rats that were administered glibenclamide orally (600 μg/kg body wt) dissolved in 1 ml water daily using intragastric tube for 5 weeks (Eidi et al., 2005).

2.8. Collection of blood

At the end of the treatment regimen, blood was withdrawn from the orbital sinuses after starving the animal overnight under ether anesthesia.

2.9. Separation of serum

Blood was collected in a sterile centrifuge tube and left undisturbed at 37 °C for 1 h till the formation of clot. The serum was aspirated using a sterile pipette after centrifugation at 3000 rpm for 15 min. Serum collected was analyzed for enzyme levels immediately or within 24 h after storing at 0 to 4 °C. *Precaution*: Non-haemolysed serum was taken.

2.10. Biochemical estimations

Serum glucose, lipid profile (total cholesterol, LDL- and VLDL- cholesterol, HDL-cholesterol, total lipids, triglycerides) and serum enzyme levels (ALP, AST and ALT) were analyzed using assay kits (Span Diagnostic Ltd., Surat, India).

2.11. Statistical analysis

Final data was expressed as mean ± SEM. Significance between the groups was estimated using Student's *t*-test and ANOVA variance analysis. The criterion for statistical significance was $p < 0.05$.

3. Results

3.1. HPTLC analysis of alliin

Alliin content of leaves from sulphur treated plants was significantly higher than that obtained from leaves of plants grown under normal conditions ($F(1,4) = 46.90$, $p < 0.001$, ANOVA) (Figs. 1 and 2).

3.2. Effect on serum glucose

In diabetic rats, administration of leaf extract from plants grown under normal and sulphur supplied conditions decreased the serum glucose significantly ($F(6, 28) = 606.85$, $p < 0.001$, ANOVA) in comparison to those administered with glibenclamide (Fig. 3). The reduction in serum glucose was more significant in rats administered with leaf extract from sulphur treated plants in comparison to those administered with leaf extract from normal plants.

3.3. Effect on serum lipid profile

The effect of aqueous leaf extract obtained plants raised under normal and sulphur supplied conditions on lipid profile was examined (Table 1). The levels of total cholesterol, total lipids, triglycer-