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RESEARCH ARTICLE

Alliin obtained from leaf extract of garlic grown under *in situ* conditions possess higher therapeutic potency as analyzed in alloxan-induced diabetic rats

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Abstract

Context: Garlic, *Allium sativum* L. (Liliaceae), possesses high therapeutic and pharmacological properties. Hypoglycemic activity is attributed to alliin (*S*-allyl cysteine sulfoxide), the main active principle localized in garlic cloves.

Objective: To compare the production and therapeutic efficiency of alliin extracted from garlic leaves of plants grown under *ex situ* and *in situ* conditions.

Materials and methods: Alliin content of leaves was quantified and aqueous leaf extracts (from *ex situ* and *in situ* grown plants) were given to normal and alloxan-induced diabetic rats for five weeks.

Results: Alliin production noted ~50% enhancement in leaves from plants grown under *in situ* conditions. Serum glucose, triglycerides, total lipids, total cholesterol, low-density lipoprotein (LDL)-, and very low-density lipoprotein (VLDL)-cholesterol in diabetic rats treated with alliin produced from *in situ* grown plants noted significant reduction of ~54%, 15%, 14%, 20%, 24%, and 15%, while 35%, 14%, 10%, 12%, 17% and 11% reduction was noted in diabetic rats treated with alliin produced from *ex situ* grown plants in comparison with those administered with distilled water. High-density lipoprotein (HDL)-cholesterol did not show any significant change. Leaf extract of plants lowered serum enzyme levels (*alkaline phosphatase*, *aspartate aminotransferase*, and *alanine aminotransferase*) toward the norm better than glibenclamide. The histopathological alteration in pancreas caused by alloxan was also reduced by leaf extract.

Discussion and conclusion: These findings demonstrate leaf extract obtained from plants grown under *in situ* condition possess higher therapeutic efficiency in comparison with leaf extract obtained from plants grown under *ex situ* condition. Studies suggest that environmental factors influence production of alliin and its therapeutic potential.

Keywords: Alliin, *Allium sativum*, diabetes, Liliaceae

Introduction

Garlic possesses high therapeutic and pharmacological properties including antibiotic, antitumor, antithrombotic, hypocholesterolemic, fibrinolytic, hypertensive effects (Eidi et al., 2005; El-Demerdash et al., 2005; Thomson et al., 2007; Nasim et al., 2009a). The hypoglycemic activity of plant contributing to treatment of diabetes has been attributed to *S*-allyl cysteine sulfoxide, the main active principle localized in cloves (Jones

et al., 2007; Nasim et al., 2009b). Literature suggests that garlic administration decreased serum glucose and enhanced contractile response to phenylephrine, significantly improved impaired endothelium-dependent relaxations, and increased serum antioxidant levels in diabetic rats (Baluchnejadmojarad et al., 2003; Eidi et al., 2005; Drobiova et al., 2009). Moreover, garlic oil also showed hypoglycemic effect in diabetic patients, through increased insulin secretion as demonstrated

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in rats with streptozotocin-induced diabetes (Liu et al., 2006).

Application of plant cell culture technologies have improved production of plant secondary metabolites contributing to medicinal value of the plant (Schmeda-Hirschmanna et al., 2004; Nasim et al., 2010). *In vitro* cultures accelerate production of specific medicinal compounds at a rate similar or superior to that of intact plants by affecting the biosynthetic activity of cells. This can be controlled by regulating environmental factors, as well as artificial selection or induction of variant clones (Vanisree et al., 2004; Junaid et al., 2008). Enhanced production of secondary metabolites including increased alliin production has been reported in plants grown under *in situ* condition (Bhagyalakshmi et al., 2005).

The leaf is the primary site for synthesis of cysteine sulfoxide (alliin) from where it gets translocated to other plant parts such as the bulb (Hughes et al., 2005; Nasim et al., 2009b). Earlier studies showed higher metabolite content including alliin in garlic cloves, but production and therapeutic efficiency of alliin in leaves have not been checked. Therefore, the present study was carried with an aim to: (i) compare the alliin (metabolite) production in leaves of plants grown under different culture conditions, that is, *ex situ* and *in situ*; (ii) compare the therapeutic efficiency (antidiabetic effect) of leaf extract obtained from plants grown under the two different culture conditions; and (iii) compare the efficacy of leaf extract from plants grown under different conditions with the drug glibenclamide as standard reference.

Materials and methods

Plant material

Local Indian garlic, *Allium sativum* L. cv. Yamuna Safed (Liliaceae), procured from the National Horticultural Research and Development Foundation, Nasik, Maharashtra, was used for the present study. Plant identity was confirmed and voucher specimen IC-375117 was deposited at the National Bureau of Plant Genetic Resources, New Delhi, India (Nasim et al., 2010). Plants grown under natural conditions ($25 \pm 5^\circ\text{C}$) in a herbal garden were taken as *ex situ* grown plants. The plants were grown in earthenware pots having sandy loam soil (pH 7.2). The plants grown on agar medium (Murashige & Skoog, 1962), supplemented with 1 mg/L 6-benzylaminopurine (BAP) and 0.25 mg/L naphthalene acetic acid (NAA) at $25 \pm 2^\circ\text{C}$ in a culture room under a 16-h photoperiod provided by cool white fluorescent light (intensity 40 W/m^2) for 15 weeks, were taken as *in situ* grown plants (Nasim et al., 2009b). Alliin production was noted in leaves of 2-month-old plants and further investigations were carried out at the same time (data not shown).

Phytochemical analysis

For high-performance thin-layer chromatography (HPTLC) analysis, 1 g dry leaf powder was extracted

using 10 mL methanol–water (80:20, v/v) plus 0.05% formic acid (pH 3) following the modified method given by Arnault et al. (2003). An aliquot of 1 mL of 1 mg/L stock solution of alliin was taken and was further diluted with methanol to make the final volume to 10 mL (100 ng/mL). Chromatographic estimations were performed following Nasim et al. (2009b).

Animals

Healthy male albino rats (weight 200–300 g) procured from the Central Animal House facility, Jamia Hamdard were housed in clean cages under controlled environmental conditions ($22\text{--}24^\circ\text{C}$; relative humidity 40–60%) as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Induction of diabetes in rats

The induction of diabetes in rats was done following methods described previously (Mansour et al., 2002; Nasim et al., 2009a).

Preparation of garlic extracts and drug administration

For preparation of extract, 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 the 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. Aqueous extract obtained was stored at -20°C . Final dose administered corresponded to 0.5 g/kg body weight. The lower concentration was prepared according to the body weight of the rats by diluting the leaf extract with cold, sterile 0.9% saline (Nasim et al., 2009a). The present dose was decided on the basis of an earlier experiment that showed toxicity above the given value (data not shown).

Experimental design

In each experiment, 56 male rats were used. The rats were divided into seven groups (eight rats in each group). Treatment was given daily using intragastric tube for 5 weeks. Group I: Normal rats were administered 1 mL of sterile distilled water; Group II: Normal rats were administered 1 mL of aqueous leaf extract obtained from *ex situ* grown plants; Group III: Normal rats were administered with 1 mL of aqueous leaf extract obtained from *in situ* grown plants; Group IV: Alloxan-treated rats were administered with 1 mL distilled water; Group V: Alloxan-treated rats were administered with 1 mL aqueous leaf extract obtained from *ex situ* grown plants; Group VI: Alloxan-treated rats were administered with 1 mL aqueous leaf extract from *in situ* grown plants; Group VII: Alloxan-treated rats were administered glibenclamide orally (600 $\mu\text{g/kg}$ body weight) dissolved in 1 mL water (Eidi et al., 2005).

Separation of serum and biochemical estimation

The separation of serum was done as described previously (Nasim et al., 2009a). Serum collected was analyzed for enzyme levels immediately or within 24 h after storing at 4°C. Non-hemolyzed serum was taken. Serum glucose, lipid profile (total cholesterol, low-density lipoprotein (LDL)- and very low-density lipoprotein (VLDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, total lipids, triglycerides), and serum enzyme levels namely alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analyzed using assay kits (Span Diagnostic, Surat, India).

Histopathological studies

Rats from control and treated groups were perfused with 10% neutral formalin solution. The pancreas was removed immediately; paraffin sections (5- μ m thickness) were made and stained by hematoxylin-eosin (H&E) stain. The cell density, cell size, and cell injury in the islets of Langerhans in the pancreas of diabetic rats was compared with normal pancreatic cells. The sections were observed under light microscope and photographs were taken. Settings for histopathological studies were done as described by Luna (1960).

Statistical analysis

The data obtained from three independent experiments (three replicates each) were checked for the level of significance using Student's *t*-test and ANOVA variance analysis ($P \leq 0.05$). *P*-values under 0.05 were considered statistically significant (shown as *); *P*-values under 0.001 were considered highly significant (shown as ***); and those not significant are shown as NS.

Results and discussion

Leaves of plants grown under *in situ* condition show increased production of alliin in comparison with those raised under *ex situ* condition (Figures 1 and 2). HPTLC

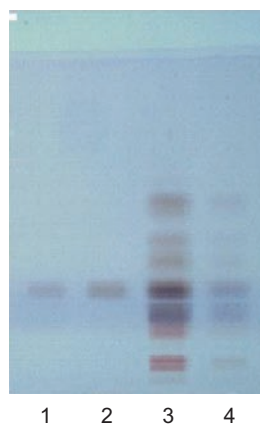


Figure 1. HPTLC fingerprint (alliin) obtained from leaf extract of *ex situ* and *in situ* raised plants. Track assignment: 1–2: Reference substance (alliin) with RF sample; 3: *in situ* leaves extract; 4: *ex situ* leaves extract.

analysis showed the prominent band (Lane 3) depicting higher alliin production in leaf samples taken from plants grown under *in situ* condition, although a light band (Lane 4) was observed in leaf samples obtained from plants grown under *ex situ* condition depicting comparatively low alliin production (Figure 1). Leaves from plants grown under *in situ* condition showed noted enhancement ~50% in alliin production (Figure 2). Alliin accumulation depends upon plant variety and environmental conditions in which the plants are grown (Nasim et al., 2009a, 2010). Controlled growth and environmental conditions including light and temperature factors affect production of carbohydrates, thus influencing the production of alliin (Nasim et al., 2010). Cell culture conditions have proved to be advantageous over conventional cultivation of plants because: (i) useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions and (ii) cultured cells would be free of microbes and insects (Schmeda-Hirschmanna et al., 2004; Vanisree et al., 2004; Nasim et al., 2010).

Alloxan-induced diabetic rats showed a decrease in liver and body weight as compared with control (Table 1). Administration of leaf extract did not affect body and liver weight in normal rats (Groups I, II, and III). The average body weight of diabetic control rats (Group IV) was significantly low as compared with the control rats (normal, Group I). The diabetic rats treated with aqueous leaf extract (dose of 0.5 g/kg body weight) from *ex situ* and *in situ* grown plants showed gain in mean body weight (Groups V and VI) as compared with diabetic control rats (Group IV). The

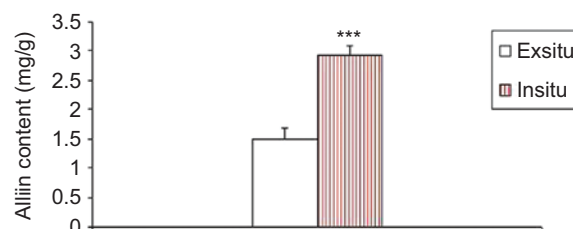


Figure 2. Alliin content of leaves from plants raised under *ex situ* and *in situ* conditions. Each value represents Mean \pm SEM. *** $P < 0.001$ denotes level of significance. *P*-values are highly significant at $P < 0.01$.

Table 1. Body and liver weight of normal and alloxan-induced diabetic rats under different treatments.

| Group | Body weight (g) | Liver weight (g) |
|---------------------------|--------------------------------|---------------------------------|
| I | 350 \pm 35.35 ^c | 9.59 \pm 1.35 ^c |
| II | 365 \pm 18.708 ^c | 10.17 \pm 0.812 ^c |
| III | 370 \pm 25.17 ^c | 10.00 \pm 0.53 ^c |
| IV | 220 \pm 35.35 ^a | 7.63 \pm 0.516 ^a |
| V | 250 \pm 28.28 ^{a,b} | 8.09 \pm 0.498 ^b |
| VI | 270 \pm 20.24 ^b | 8.40 \pm 0.384 ^{a,b} |
| VII | 240 \pm 9.69 ^{a,b} | 7.92 \pm 0.77 ^a |
| <i>P</i> _{ANOVA} | 0.0027 [*] | 0.1408 ^{NS} |

Each value represents mean \pm SEM of three independent experiments with three replicates each. Different letters in columns are significantly different at $P = 0.05$ level. **P*-values under 0.05 were considered statistically significant.

gain in body weight was significant in rats administered with leaf extract from plants grown under *in situ* conditions (Group VI). The body weight of diabetic rats administered with leaf extract from plants grown under *ex situ* and *in situ* conditions noted an increase of 13% and 22.7% (Groups V and VI) in comparison with diabetic control rats (Group IV). The body weight of diabetic rats also noted an increase after glibenclamide administration (Group VII). The mean liver weight of diabetic control rats (Group IV) was reduced considerably when compared with normal control rats (normal) (Group I). The administration of leaf extract from plants grown under *ex situ* and *in situ* conditions increased the mean liver weight in diabetic rats (Groups V and VI). Leaf extract from plants grown under *ex situ* and *in situ* conditions was effective in altering the liver weight of diabetic rats in comparison with glibenclamide (Table 1). The liver weight of diabetic rats administered glibenclamide (Group VII) showed a small gain, although rats administered leaf extract from plants grown in *ex situ* condition showed an increase in liver weight by 6%, and rats administered with leaf extract from plants grown in *in situ* condition showed an increase in liver weight by 10%. Alloxan acts as a cytotoxic agent that affects organs/metabolism that lead to significant decline in the growth rate (Khalil, 2004). The decrease in liver weight in the diabetic animals results from decrease in soluble protein content due to decrease in protein synthesis in cytosol (Shalini et al., 2004). The action of alloxan in the pancreas is preceded by its rapid uptake by the β cells that evoke a sudden rise in insulin secretion in the presence or absence of glucose (Szkudelski, 2001). Active metabolites present in the leaf extract possess antioxidant activity and thiol-containing proteins that possess hypoglycemic properties that protect functional organs (Rabinkov et al., 1998). Plants grown under *in situ* condition show high levels of sulfur compounds (alliin and its derivatives) in the leaf extract that might contribute to higher therapeutic potency.

The fundamental mechanism underlying hyperglycemia in diabetes involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) as well as decreased utilization of glucose by the tissue (Sheela & Augusti, 1992). A significant elevation (~75%) in serum glucose was observed in diabetic controls (Group IV) as compared with

normal rats (Group I). The administration of leaf extract from plants grown under *ex situ* and *in situ* conditions did not affect serum glucose levels in normal rats. The administration of leaf extract from plants grown under *ex situ* and *in situ* conditions and glibenclamide brought decline in serum glucose toward the normal values (Table 2), though results were significant in rats administered with leaf extract (plants grown in *ex situ* and *in situ* conditions) in comparison with rats treated with glibenclamide. Diabetic rats administered with aqueous leaf extract showed 35% and 50% reduction in serum glucose levels in rats administered with leaf extract (plants grown in *ex situ* and *in situ* conditions), while ~27% decrease was noted in glibenclamide-treated group (Table 2). Leaf extract obtained from plants grown under *in situ* condition was found to be effective in reducing serum glucose levels than *ex situ* grown plant. This is probably due to higher production of bioactive compounds, S-allyl cysteine sulfoxide, allicin, di(2-propenyl) disulfide, or 2-propenyl propyl disulfide, though leaf extract was more effective as compared with glibenclamide (Sangeetha & Quine, 2008). The mechanism of hypoglycemic action probably involves direct or indirect stimulation of insulin secretion (Eidi et al., 2005; Nasim et al., 2009a). It is suggested that disulfide compounds have the effect of sparing insulin from -SH inactivation by reacting with endogenous thiol-containing molecules such as cysteine, glutathione, and serum albumins (Eidi et al., 2005). It may be due to restoration of delayed insulin response or inhibition of intestinal absorption of glucose or increased secretion of insulin from existing β -cells or its release from bound insulin (Eidi et al., 2005).

The administration of aqueous leaf extract obtained from plants raised under different culture conditions affected the lipid profile of rats Table 2. The administration of leaf extract from plants grown under *ex situ* and *in situ* conditions did not affect level of total cholesterol, total lipids, triglycerides, and fractions of cholesterol (HDL-, LDL-, and VLDL-cholesterol) in normal rats (Groups I-III) (Table 2). Level of total cholesterol, total lipids, triglycerides, and fractions of cholesterol (LDL- and VLDL-cholesterol) were significantly higher in diabetic rats (Group IV) as compared with normal control rats (Group I). HDL-cholesterol did not exhibit any significant change. Aqueous leaf extract

Table 2. Serum glucose and lipid profile of normal and diabetic rats measured under different experimental conditions.

| Group | Serum glucose (mg/dL) | Serum lipid profile (mg/dL) | | | | | |
|---------------------------|-----------------------------|------------------------------|------------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | | Total cholesterol | Total lipids | Triglycerides | HDL-cholesterol | LDL-cholesterol | VLDL-cholesterol |
| I | 96.68 ± 8.85 ^e | 86.45 ± 5.16 ^d | 139.65 ± 8.42 ^d | 110.30 ± 3.40 ^c | 28.11 ± 2.67 ^b | 47.26 ± 3.77 ^{b,c} | 21.91 ± 2.75 ^c |
| II | 93.12 ± 4.72 ^e | 86.19 ± 4.4 ^d | 139.25 ± 5.80 ^d | 107.61 ± 4.08 ^c | 28.15 ± 3.34 ^b | 43.12 ± 4.44 ^c | 21.79 ± 2.92 ^c |
| III | 90.32 ± 5.39 ^e | 84.93 ± 3.07 ^d | 139.09 ± 5.67 ^d | 107.76 ± 4.61 ^c | 28.66 ± 2.73 ^b | 41.37 ± 5.76 ^d | 20.08 ± 1.75 ^c |
| IV | 392.59 ± 22.36 ^a | 119.40 ± 3.49 ^a | 185.92 ± 10.85 ^a | 141.47 ± 5.88 ^a | 23.02 ± 2.49 ^a | 58.64 ± 4.68 ^a | 27.81 ± 1.39 ^a |
| V | 254.63 ± 8.69 ^c | 104.47 ± 4.28 ^b | 166.31 ± 3.63 ^{b,c} | 121.40 ± 4.24 ^b | 25.77 ± 3.10 ^a | 48.35 ± 3.99 ^{b,c} | 24.31 ± 1.69 ^{a,b} |
| VI | 200.62 ± 8.89 ^d | 95.47 ± 6.28 ^c | 158.24 ± 4.85 ^c | 120.56 ± 4.17 ^b | 26.45 ± 4.01 ^{a,b} | 44.29 ± 3.66 ^c | 23.31 ± 1.44 ^b |
| VII | 285.32 ± 8.85 ^b | 111.63 ± 8.63 ^{a,b} | 169.56 ± 3.19 ^b | 126.27 ± 5.13 ^b | 24.31 ± 3.93 ^a | 52.40 ± 2.60 ^b | 25.25 ± 1.88 ^{a,b} |
| <i>P</i> _{ANOVA} | 0.0001*** | 0.0002*** | 0.0008*** | 0.0001*** | 0.8262 ^{NS} | 0.1334 ^{NS} | 0.2289 ^{NS} |

Each value represents mean ± SEM of three independent experiments with three replicates each. Different letters in columns are significantly different at *P* = 0.05 level. ****P*-values under 0.001 were considered highly significant. NS, not significant.

decreased the values of serum lipid profile (except HDL-cholesterol) in diabetic rats. Diabetic rats treated with leaf extract from *ex situ* and *in situ* grown plants showed better recovery in serum lipid profile than the glibenclamide drug; reduction was more significant in rats administered with leaf extract from plants grown under *in situ* condition. A significant reduction of total cholesterol (8.6%), total lipids (4.9%), triglycerides (~1%), LDL-cholesterol (8.4%), and VLDL-cholesterol (4.1%) was noted in rats treated with leaf extract obtained from plants grown under *in situ* condition in comparison with those treated with leaf extract obtained from plants grown under *ex situ* condition (Table 2). However, HDL-cholesterol value increased by about 6.5%. Epidemiological studies showed that supplementation of leaf extract decreases serum cholesterol, hepatic cholesterol, and/or triglyceride levels in animals (Yeh & Yeh, 1994), though the mechanism by which garlic does so is not fully elucidated. Studies have demonstrated that garlic-supplemented diets decreased activities of various lipogenic enzymes such as FAS, G6PDH, and the cholesterologenic enzyme, HMG-CoA reductase (Sheela & Augusti, 1995); therefore, it is speculated that the lipid-lowering effects of leaf extracts may result from decreased lipogenesis and cholesterologenesis.

Serum enzymes levels increased in diabetic rats (Group IV) as compared with normal rats (Group I) (Table 3). The administration of leaf extract from plants grown under *ex situ* and *in situ* conditions did not affect ALP, AST, and ALT activities in normal rats. The administration of leaf extract and glibenclamide brought the serum enzymes levels significantly toward the normal values in diabetic rats. Diabetic rats administered with garlic leaf extract

(both from *ex situ* and *in situ* grown plants) showed 8–11%, 14–26%, and 17–25% reduction in ALP, AST, and ALT activities in comparison with diabetic control (Table 3). The garlic leaf extract (*ex situ* and *in situ* conditions) was more effective in reducing the levels of enzymes in comparison with glibenclamide. The results were promising in rats administered with leaf extracts from plants grown under *in situ* condition as they possess higher levels of alliin. The decline in enzyme levels shows the normalizing effect of garlic leaf extract on hepatocellular damage and suppression of gluconeogenesis. The increase in enzyme levels is mainly due to leakage of enzymes into bloodstream as result of alloxan toxicity that leads to liver damage (El-Demerdash et al., 2005). The increase in enzyme levels in serum indicates liver destruction. After treatment with aqueous leaf extract (both from *in situ* and *ex situ* grown plants), enzyme levels were restored to normal by reducing their induction in diabetes.

Microscopic studies indicated reduction in cell size and cell injury in islets of Langerhans in pancreas of diabetic rats as compared with normal pancreatic cells observed in control (Figure 3A and 3B). Histopathological study confirmed destruction of β -cells in diabetic rats. Diabetic rats administered with leaf extract (both from *ex situ* and *in situ* grown plants) showed less cell damage and recovery of β -cells in pancreas (Figure 3C and 3D). The size and recovery/revival rates of β -cells were found to be better in rats administered with extract obtained from plants grown under *in situ* condition than normal rats (Figure 3D). However, higher recovery of pancreatic cells in rats treated with leaf extract from plants grown under *in situ* condition indicated high regenerative capacity (Abdel et al., 1997).

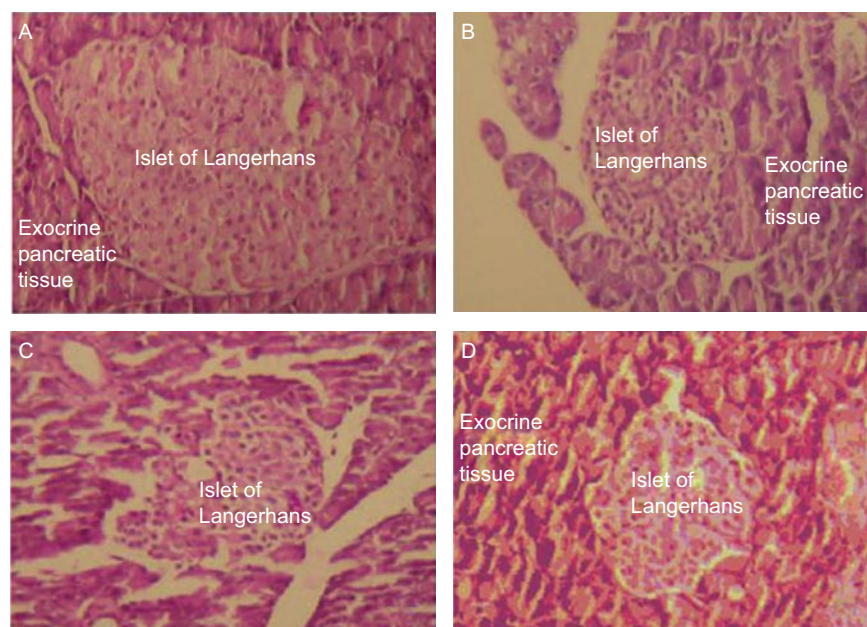


Figure 3. Light micrographs of rat pancreas under different conditions.

A Control rats showing normal pancreatic cells.

B Alloxan induced diabetic rats showing change in morphology of pancreatic cells.

C Diabetic rats treated with leaf extract from *ex situ* raised plants showing recovery of injured pancreatic cells

D Diabetic rats treated with leaf extract from *in situ* raised plants showing recovery of injured pancreatic cells.

Table 3. Serum enzyme levels of normal and diabetic rats measured under different conditions.

| Groups | Serum enzymes (IU/L) | | |
|--------|------------------------------|------------------------------|----------------------------|
| | ALP | AST | ALT |
| I | 104.98 ± 4.44 ^c | 75.94 ± 2.85 ^d | 51.76 ± 5.83 ^d |
| II | 102.91 ± 6.11 ^c | 77.12 ± 5.54 ^d | 50.78 ± 5.12 ^d |
| in | 101.31 ± 4.24 ^c | 73.63 ± 5.71 ^d | 48.90 ± 6.06 ^d |
| IV | 137.58 ± 9.47 ^a | 136.11 ± 5.6 ^a | 111.43 ± 7.30 ^a |
| V | 125.64 ± 4.25 ^{a,b} | 117.68 ± 5.42 ^b | 92.18 ± 7.07 ^c |
| VI | 121.23 ± 4.37 ^b | 100.40 ± 6.81 ^c | 83.73 ± 5.68 ^c |
| VII | 130.94 ± 4.14 ^{a,b} | 123.98 ± 4.69 ^{a,b} | 102.21 ± 6.55 ^b |
| PANOVA | 0.0002*** | 0.0001*** | 0.0001*** |

Each value represents mean ± SEM of three independent experiments with three replicates each. Different letters in columns are significantly different at $P = 0.05$ level. *** P -values under 0.001 were considered highly significant.

Conclusion

Conclusively, leaf extracts from *in situ* grown plants show higher production of alliin contributing to higher therapeutic potency (reducing serum glucose, serum enzyme levels, and revival of pancreatic cells) as compared with *ex situ* grown plants and drug glibenclamide. The studies suggest that environmental factors influence production and therapeutic potential of alliin. Further research is required to find the factors or mechanism responsible for enhancing the therapeutic value.

Declaration of interest

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