

***In vitro* antidiabetic evaluation of *Allium sativum* L.**

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Abstract

Aqueous extracts of *Allium sativum* Linn. roots were studied at concentrations of 5, 10, 20 and 40g plant extract/L by an *in vitro* model to assess their effect on glucose diffusion in intestine. *Allium sativum* reduced glucose movement across the dialysis membrane up to 54% as compared to the control. In increasing concentration order, maximum inhibition was offered by 20, 40 g/L *Allium sativum*. Data analyses in terms of integrated areas under curves (AUCs) by trapezoidal rule revealed a decline in AUC_{glucose} by plant extracts. Extracts having 10, 20, 40 g/L *Allium sativum* concentrations significantly prevented glucose transfer but 5 g/L *Allium sativum* did not show any significant change in AUC_{glucose}. The antidiabetic effects of *Allium sativum* were found to be concentration-dependent. Further studies are warranted to clarify whether *in vitro* protocols can represent therapeutic potential by limiting postprandial glucose absorptions and for improving glycemic control in diabetic subjects. Results demonstrated the efficacy of *Allium sativum* from a new perspective that seem to act differently but synergistically to regulate glucose-homeostasis.

Key words: Diabetes mellitus, hyperglycemia, *Allium sativum* L

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1. Introduction

Diabetes mellitus a global epidemic, affects carbohydrates, fats and proteins metabolism. Due to morbidity and mortality caused by diabetes related complications, there is an escalating requisite for natural therapeutic management. The medicinal plants exhibit diverse antidiabetic mechanism owing to phenolics and flavonoids [1]. Mostly, phytoconstituents act on pancreas, elevate insulin secretion or action and thus trigger hypoglycemia. Another mode of antihyperglycemic action offered by these bioactive compounds is through inhibition of enzymes engaged in carbohydrate digestion [2, 3].

Among different antidiabetic plants, *Allium sativum* L. (garlic) is one of the main accepted herbs. Chemical analyses [4] demonstrated that *Allium sativum* L. is a main resource of sulfur holding compounds, predominantly S-alk(en)yl-L-cysteine sulphoxides (Cs), being alliin the foremost one. Volatiles such as alliin and lipid-soluble sulphur compounds such as diallyl disulphide, diallyl sulphide, ajoene, dithiins, diallyl trisulphide attributed typical odour, taste, as well as biological and therapeutic properties to *Allium sativum* L. It is used as an anti-diabetic mediator [3, 5-8]. *Allium sativum* L. (AS) possesses high antidiabetic, pharmacological and therapeutic properties. Alliin (S-allyl cysteine sulfoxide) is the major dynamic component, localized in garlic cloves and hypoglycemic activities are attributed to alliin [9-11].

AS lowers blood sugar level either by its capacity to excite the pancreas to boost insulin synthesis, or

positively affects insulin-receptors [12, 13]. Antioxidant properties of AS [14, 15] are responsible to alter the lipids markers and thrombogenic factors and may be valuable in cardiovascular syndrome, hyperlipidemic and hypertensive manifestations [16-18]. AS treatment reduced mortality rate, supported growth and increased the antioxidant activity in fish. Earlier studies [20, 21] recognized that spermiotoxicity, Cd-induced testicular oxidative damage and Cd-induced kidney damage were protected by AS. Thomson *et al.*, [22] and Singh and Singh, [23] reported role of AS against cardiovascular syndrome, hypertension, platelet aggregation, hyperlipidemia and blood fibrinolytic activity.

Despite extensive investigations reported in literature regarding medicinal attributes, role of AS in impeding intestinal glucose absorption has never been explored. Present study aimed to assess antidiabetic potential of AS in terms of hindering glucose diffusion through an *in vitro* model.

2. Material and Methods

2.1. Sample collection and Extract preparation

Allium sativum Linn. roots were collected from Botanical garden, University of Agriculture, Faisalabad, Pakistan. These were shade-dried, ground into a fine powder and stored at room temperature. Aqueous extracts were prepared by cold infusion. Briefly, 1 g powdered material was placed in 40 ml of boiling distilled water. It was allowed to infuse for 15 minutes and then filtered. The

volume was adjusted to 40 ml with distilled water, dried under vacuum and stored at -20 °C till further analysis. Before each assay, extracts were reconstructed with distilled water. Extract concentrations were expressed as g total plant material weight per liter water.

2.2. Antidiabetic Evaluation

Antidiabetic activity was determined by glucose entrapment potential of *Allium sativum* L. The concentration-dependent effects ($I_1:5$, $I_2:10$, $I_3:20$, $I_4:40$ g/L) of extracts to inhibit glucose diffusion into the external solution was investigated at set time intervals [24]. In a dialysis tube (6 cm-15 mm), 15 mL of a solution of glucose and NaCl (0.15 M) was introduced and the appearance of glucose in the external solution was measured. The sealed tube was placed in a centrifuge tube containing 45 mL of 0.15 M NaCl. The tube was placed on an orbital shaker at room temperature. Glucose concentrations were measured by glucose oxidase kit method. Incremental areas under the glucose curves (AUC) were calculated by trapezoidal rule.

All data were expressed as mean \pm SD and % age of triplicate measurement. Student's t-test was performed by Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL, USA) software (version 15.0) with level of significance set at $p < 0.05$.

3. Results and Discussion

A simple diffusion model was used to investigate glucose absorption inhibition by *Allium sativum* L. At the end of study period, glucose movement out of dialysis tube attained maximum concentration (18.2 mmol/L) in control. *Allium sativum* root extracts constrained glucose flow across the membrane and this effect was dose-dependent (Figure 1). Presence of 20 g/L AS decreased glucose movement across the membrane significantly and 12.4 mmol/L glucose levels observed represented 31.86% decrease in total glucose diffusion compared to control ($p < 0.05$). Similarly, 40 g/L ginger revealed 42.30% decrease in the glucose movement ($p < 0.05$) compared to control with 10.5 mmol/L external glucose concentrations. Extracts with 5 g/L (I_1) and

10 g/L (I_2) AS concentrations showed trivial potentials to block glucose diffusion. Averaged concluding glucose levels (mmol/L) in external solutions were 14.6 and 15.7 for I_2 and I_1 respectively. All the AS extracts inhibited glucose movement into external solution across dialysis membrane as compared to the control. These results demonstrated that AS can induce hypoglycemia by reducing intestinal glucose absorption.

Effect of AS to impede glucose diffusion out of the dialysis tube was also assessed in terms of integrated areas under curves (AUCs) for a period of 0-18 hours and calculated by trapezoidal rule (Table 1). As may be noted, plant extracts caused a decline in AUC_{glucose} and it was associated with increase in AS concentration. Experiment with 10, 20, 40 g/L AS concentrations significantly prevented glucose transfer but 5 g/L AS did not show any significant change in AUC_{glucose} .

The mechanisms by which plants can lower glucose involve the viscosity of different fibers in hampering diffusion of glucose and postponing absorption and digestion of carbohydrates [25,26]. High-fiber diets, especially of the soluble variety, and soluble fiber supplements may offer some improvement in carbohydrate metabolism and have other beneficial effects in diabetes mellitus [27].

In this paper, *Allium sativum* roots were used to investigate their glucose lowering mechanism *in vitro*. The results showed that AS lowered glucose levels significantly. While, the diffusion model had constant agitation, it may not reflect the actual intestinal environment. Moreover, study time period (18 hours) is not consistent with the time of glucose absorption in intestinal mucosa. Present results can be justified by various mechanisms. Firstly; plant fibers increase the viscosity and hinder diffusion of glucose, and secondly, fibers bind glucose and decrease the glucose transport across the membrane [25]. Although exact mechanisms by which AS exerted its hypoglycemic activity are unknown. However, the ability of AS fiber to retard absorption certainly has an important influence on carbohydrate metabolism [28].

Table 1. Effect of aqueous extracts of *A. sativum* on glucose diffusion

Sample	AUC (mmol/L glucose) ¹	% Change in AUC ²
Control (no extract)	186.0 \pm 2.71	-
Plant extract		
I_1 (5 g/L)	149.25 \pm 1.96	19.75%
I_2 (10 g/L)	134.91 \pm 2.45	27.46% *
I_3 (20 g/L)	111.60 \pm 6.36	40% *
I_4 (40 g/L)	85.35 \pm 4.22	54.11% *

Data are mean \pm SD or % for triplicate measurements.

* $p < 0.05$

¹AUC: (area under the curve) was calculated using total glucose diffusion during 18 h incubation period as described in the methods section.

² Change in AUC in comparison to control.

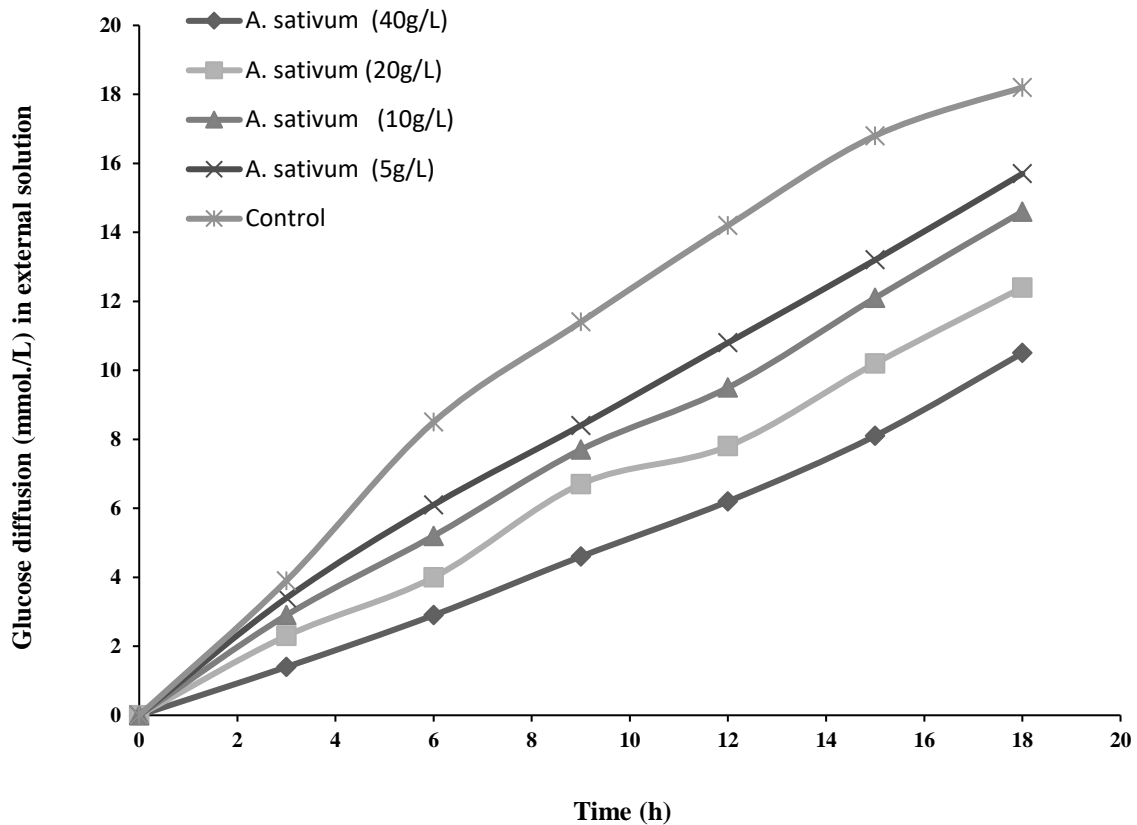


Figure 1. Effect of aqueous extracts of *A. sativum* on glucose diffusion.

Various polysaccharides delay absorption probably by impairing the access of luminal contents to the absorptive epithelium. In a similar experimental setting [29], the movement of glucose out of the dialysis tubing was increased by increasing the rate of contraction. Furthermore, guar gum prevented the rise in glucose uptake produced by increasing the rate of contraction. These effects suggest that guar probably reduces absorption by resisting the convective effects of intestinal contractions. Agrimony and avocado inhibited glucose diffusion (more than 60%), while elder and nettle extracts did not significantly decrease glucose diffusion [24]. Contrary to these results, Büyükbalci and El Nehir [30] stated that herbal teas did not inhibit glucose movement into external solution across dialysis membrane compared to control. Actually rates of glucose movement into external solution were higher than control for ten aqueous herbal tea extracts.

Further studies are warranted to clarify whether *in vitro* protocols can represent therapeutic potential by limiting postprandial glucose absorptions and for improving glycemic control in diabetic subjects.

4. Conclusion

Results demonstrated the efficacy of *Allium sativum* from a new perspective that seem to act differently but synergistically to regulate glucose-homeostasis.

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