http://dx.doi.org/10.4314/jpb.v8i2.12
Vol. 8 no. 2, pp. 141-146 (September 2011)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Hypoglycaemic effect of *Allium sativum* (aqueous extract) on normal and alloxan-induced hyperglycaemic Wistar rats

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Received 8th June 2011; Accepted 9th August 2011

Abstract
This study was carried out to evaluate the hypoglycaemic effect of aqueous extract of *Allium Sativum* (ASEt) in normal and alloxan induced hyperglycaemic rats. Hyperglycaemia was induced in the animals by intraperitoneal injection of alloxan monohydrate dissolved in sterile normal saline in a dose of 150mg/kg body weight. After 48 hours of the injection, rats with hyperglycaemia were used for the study. Blood samples were collected from the tail of the rats and blood glucose concentration was determined using a glucometer. The rats were divided into groups I – VI. Different concentrations of the extract per kilogram body weight were administered orally to different groups for seven consecutive days. Results showed that ASEt significantly ($P < 0.05$) lowered the fasting blood glucose dose dependently in alloxan induced hyperglycaemic rats, and in normoglycaemic rats that received 1000mg/kg/day of ASEt. However, *Allium sativum* showed no significant ($P > 0.05$) reduction in the fasting blood glucose of normoglycaemic rats that received 500mg/kg/day of ASEt.

Keywords: *Allium Sativum;* Hypoglycaemic activity; Diabetes mellitus

INTRODUCTION

Allium sativum (garlic) is a species in the onion family *Alliaceae*. Its close relatives include the onion, shallot, and leek. Garlic has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, 'hot' flavor that mellows and sweetens considerably with cooking (Gonzalvez *et al*, 2008). *Allium sativum* may also possess 'cancer-fighting' properties due to the presence of allylic sulfur compounds such as diallyl disulfide (DADs) which is believed to be an anticarcinogen (Khanum *et al*, 2004).

In 1858, Louis Pasteur observed garlics' antibacterial activity, and hence its use as an antiseptic to prevent gangrene during World War I and World War II (Ellen, 2005). More recently it has been found from a clinical trial that a mouthwash containing 2.5% fresh garlic shows good antimicrobial activity, although the majority of the participants reported an unpleasant taste and halitosis (Groppo *et al*, 2007).

In modern naturopathy, garlic is used as a treatment for intestinal worms and other intestinal parasites, both orally and as an anal suppository. Garlic cloves are used as a remedy for infections (especially chest

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ISSN 0189-8442

problems), digestive disorders, and fungal infections such as thrush (Jakubowski, 2003). Garlic has been reasonably and successfully used in the management of AIDS patients to (particularly to treat Cryptosporidiosis) in an uncontrolled study in China. It has also been used by at least one AIDS patient to treat toxoplasmosis with another protozoal disease (Jakubowski, 2003). When crushed, *Allium sativum* yields allicin, a powerful antibiotic and anti-fungal compound (phytoncide). It also contains alliin, ajoene, enzymes, vitamin B, minerals, and flavonoids (Lanzotti, 2006). Garlic can 'thin' the blood similar to the antiplatelet effect of aspirin (Ali *et al*, 1990).

Bleeding is a potentially serious side effect of garlic use, including bleeding after surgery and spontaneous bleeding (Chutani, and Bordia, 1981). Some degree of liver toxicity has been demonstrated in rats, particularly with ingesting large quantities of Garlic(Thomson, *et al*, 1998).

Diabetes mellitus is a syndrome characterized by disordered carbohydrate metabolism as well as fats and proteins and which is associated with inappropriately high glucose level (hyperglycaemia) directly resulting from either low levels of the hormone insulin or from abnormal resistance to insulin's effects coupled with inadequate levels of insulin secretion to compensate (Tierney, *et al*, 2002). The World Health Organization recognizes three main forms of diabetes mellitus: type 1, type 2, and gestational diabetes [occurring during pregnancy] (WHO,1999) which have similar signs, symptoms, and consequences, but different causes and population distributions. While, ultimately, all forms are due to the beta cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycemia, the causes are different (Rother, 2007).

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following (WHO, 1999):

- Fasting plasma glucose level at or above 126 mg/dL (7.0 mmol/l).
- Plasma glucose at or above 200 mg/dL (11.1 mmol/l) two hours after a 75 g oral glucose load as in a glucose tolerance test.
- Random plasma glucose at or above 200 mg/dL (11.1 mmol/l).

A positive result, in the absence of clinical symptoms of diabetes, should be confirmed by another of the above-listed methods on a different day. Most physicians prefer to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete.

Other plants with hypoglycaemic effects include Caraili (*Momordica charantia*), Aloe (*Aloe vera*), Ginseng (*Panax ginseng*), *Occimum grattisimum*, *Tinospora cordifolia*, *Bidens biternata*, *Securidaca longepedunculata*, *Morinda lucida* Benth, etc. An Indian study proved that *Tinospora cordifolia* has significant hypoglycaemic effect in alloxan-induced diabetic rats (Prince and Venugopal, 2001). The claim by Nigerian traditional herbal medicine practitioners that *Occimum grattisimum* leaves have antidiabetic properties was investigated by Egesie, *et al.*, (2006). The study showed that *Occimum grattisimum* has significant hypoglycaemic effect in streptozotocin-induced diabetic rats. In a related study, Enyikwola and Tshimba (1996), proved that *Bidens biternata* has a dose-dependent hypoglycaemic effect in normal rats.

EXPERIMENTAL

Collection of plant material: *Allium sativum* was procured from Katako market, Jos. Identified by Professor Akueshi, plant taxonomist in the Department of Botany, University of Jos, Jos. The air dried *Allium sativum* was pounded and filtered. The

powder was kept in airtight containers ready for use.

Preparation of extract: 60g of the dried powdered *Allium Sativum* was exhaustively extracted with ethanol in a soxhlet extractor at 58°C for 72 hours. The extract was dried to constant weight on a rotatory evaporator and the beaker content weighed. The residual extract was dissolved in saline and used in the study.

Experimental induction of diabetes in rats: Twelve week-old healthy male Wistar albino rats (weighing between 130g -200g) bred in the animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, were used in this study. The animals were fed with grower feeds (procured from Vital Feeds, Jos) and allowed water *ad libitum*.

The rats were injected with alloxan monohydrate (Sigma Aldrich, UK) dissolved in sterile normal saline in a dose of 150mg/kg body weight intraperitoneally (method of Prince and Venugopal, 2001). After 48 hours of the injection, rats that tested positive for diabetes (fasting blood glucose at or above 126mg/dL[7.0mmol/l]) were used in the study. Blood was collected from the tail of the rats and blood glucose was determined by glucometer which uses the 'One Touch Basic Blood Glucose Monitoring System' (Life Scan Inc., Milpitas, CA 95035, USA) (Adeneye *and Agbaje*, 2008).This system employs glucose oxidase method of glucose determination.

Treatment of animals: A total of 30 rats were used. Diabetes was induced in the rats 48 hours before drug administration. The rats were divided into six groups. In this study, 5 rats were used in each group. They were distributed into different cages and acclimatized for 24 hours. The groups were treated as follows:

Group I: non diabetic control group. Each animal was given 0.5ml of saline daily for seven days.

Group II: non diabetic rats given ASEt (500mg/kg body weight) daily for seven consecutive days.

Group III: non diabetic rats given ASEt (1000mg/kg body weight) daily for seven consecutive days

Group IV: (diabetic control) untreated diabetic rats given 0.5ml of saline daily for seven consecutive days.

Group V: diabetic rats given ASEt (500mg/kg body weight) in saline solution daily using an intragastric tube for seven consecutive days.

Group VI: diabetic rats given ASEt (1000mg/kg body weight) in saline solution daily for seven consecutive days.

Collection and analysis of samples: Blood samples were collected from the tail of the rats before and after seven consecutive days of treatment for analysis and estimation of their blood glucose levels using the glucometer.

Phytochemical screening: The crude aqueous and ethanol extracts were screened for its phytochemical constituents at the Pharmacognosy laboratory, University of Jos, using standard and characterization methods (Sofowora, 1982).

RESULTS

Effects of graded doses of ASEt on fasting blood glucose (FBG) in normoglycaemic and alloxan-induced hyperglycaemic rats.

Tables 1 and 2 show the effect of seven days oral administration of normal saline and graded doses of ASEt (500mg and 1000mg/kg) on normoglycaemic and alloxan-induced hyperglycaemic rats. As shown in the tables 1 and 2 below, intraperitoneal administration of alloxan (150mg/kg) induced significant ($P < 0.05$) hyperglycaemia in the rats in groups IV, V, and VI before the

commencement of treatment. The administration of 500mg/kg/day of ASEt to normoglycaemic rats (group II) for seven consecutive days did not cause any significant ($P > 0.05$) decrease in fasting blood glucose. However, daily administration of 1000mg/kg/day of ASEt to the normoglycaemic rats (group III) for seven consecutive days induced significant ($P < 0.05$) reduction in fasting blood glucose. Also daily oral administration of graded doses (500mg/kg and 1000mg/kg) of ASEt to hyperglycaemic rats (groups V and VI) caused significant ($P < 0.05$) dose dependent reduction in the blood glucose concentration when compared with group IV.

Using the Students't test, comparing the diabetic rats that received 500mg/kg/day (group V) and the diabetic rats that received 1000mg/kg/day (group VI) with the diabetic control (group IV), there has been significant reduction in their fasting blood glucose after seven consecutive days of oral administration of the ASEt with P values of < 0.05 and < 0.01 for groups V and VI respectively.

Comparing the fasting blood glucose of non diabetic rats (group III) that received 1000mg/kg/day of the ASEt, before and after seven consecutive days of oral administration, there has been significant fall in their fasting blood glucose with P value of < 0.05. However, no significant reduction in blood glucose concentration was noted in the group II rats (non diabetic that received 500mg/kg/day) before and after seven consecutive days of oral administration of the ASEt.

Table 1: Effect of oral administration of aqueous extract of *A. sativum* on blood glucose level of alloxan-induced hyperglycaemic rats.

Groups	Blood glucose concentration (mg/dl) n = 5	
	Day 0	Day 7
Diabetic control +0.5ml saline (IV)	207.67 ± 21.50*	253.33 ± 8.82
Diabetic + 500mg/kg ASEt (V)	216.67 ± 13.02	142.33 ± 14.66**
Diabetic + 1000mg/kg ASEt (VI)	229.33 ± 16.90	130.33 ± 5.61**

Mean ± SEM for five determinations. * $p < 0.05$ when compared with normoglycaemic control group.
** $p < 0.05$ when compared with hyperglycaemic control group.

Table 2: Effect of oral administration of aqueous extract of *A. sativum* on blood glucose level of normoglycaemic rats.

Groups	Blood glucose concentration (mg/dl) n = 5	
	Day 0	Day 7
Non diabetic control +0.5ml saline (IV)	62.67 ± 7.06	54.33 ± 3.38
Non diabetic + 500mg/kg ASEt (V)	73.67 ± 7.17	84.00 ± 7.37***
Non diabetic + 1000mg/kg ASEt (VI)	72.67 ± 2.60	52.00 ± 2.89*

Mean ± SEM for five determinations. * $p < 0.05$ when compared with normoglycaemic control group.
*** $p > 0.05$ when compared with normoglycaemic control group.

DISCUSSION

Various in vivo models (e.g. diazoxide, alloxan or streptozotocin – induced diabetic rats) are used in evaluating medicinal plants with potential hypoglycaemic potentials (Adeneye and Agbaje, 2008). In this study, diabetes mellitus was induced using intraperitoneal injection of alloxan at a dose of 150mg/kg of body weight. This dose established diabetes mellitus in the treated rats 48 hours after administration. Alloxan induces diabetes mellitus by selectively destroying the pancreatic beta cells which are involved in the synthesis, storage and release of insulin which is a peptide hormone that regulate carbohydrate, protein and lipid metabolism (Adeneye and Agbaje, 2008). Alloxan induces hyperglycaemia which is a basic parameter in the disease condition

Diabetes Mellitus. It may not exactly induce the disease condition Diabetes Mellitus.

In this study, diabetes was fully established as shown by the significant ($P < 0.05$) elevation in the fasting blood glucose concentrations in the groups IV, V and VI rats compared to the group I rats. Oral administration of ASEt (500mg – 1000mg/kg of body weight) for seven consecutive days significantly ($P < 0.05$) lowered the blood glucose of diabetic rats in groups V and VI in a dose related fashion when compared with group IV.

Also administration of 1000mg/kg/day for seven consecutive days to normal (non diabetic) rats produced significant ($P < 0.05$) reduction in their fasting blood glucose concentration. However, administration of 500mg/kg of ASEt to normal (non diabetic) rats for seven consecutive days did not produce any significant decrease in their blood glucose levels. The ASEt showed significant lowering activity in both diabetic and non diabetic rats. This observed hypoglycaemic activity is an indication that ASEt contains active constituents with potent hypoglycaemic property.

Phytochemical screening performed on the crude and ethanol extracts showed that *Allium sativum* contains Alkaloids, Steroids, Carbohydrates, Flavonoids, and Cardiac glycosides in significant amounts. *Allium sativum* is known to contain alliin, ajoene, enzymes, vitamin B, alkaloids, and flavonoids. Literature has equally shown the biological activity of alkaloids and flavonoids to include hypoglycaemia, hypolipidaemia, hypoazotaemia, hypotension among other biological effects (Oladele *et al*, 1995; Sudheesh *et al* 2005). The presence of alkaloids and flavonoids in garlic extract may be responsible for the hypoglycaemic effect recorded in this study. This study showed that the effective dose of ASEt for its hypoglycaemic effect to be felt is not less than 500mg/kg/day.

In alloxan induced diabetes mellitus, there is selective necrosis of the beta cells of the pancreas so that insulin production is totally or partially inhibited, depending on the concentration of the alloxan (Onyeche and Kolawole, 2005). The ASEt might be producing its hypoglycaemic effects via stimulation of insulin secretion from the islets of langerhans or increased peripheral utilization of glucose by the cells.

Conclusion. Results of this study show that ASEt has potent hypoglycaemic property in both normal and diabetic rats which may be mediated via increased secretion of insulin by the islets cells of langerhans or increased peripheral utilization of glucose by the cells. This implies that it will be useful in the management of type II diabetes mellitus. From the study it has been shown that concurrent administration of garlic extract with conventional oral hypoglycaemic agents may produce a synergistic effect that may be detrimental to the individual if not well monitored.

Acknowledgement. The authors are grateful to all the staff of Animal House and the Department of Pharmacognosy, University of Jos.

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