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Effect of ethanolic extract of *Allium sativum* bulbs on Streptozotocin induced diabetic rats

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ABSTRACT

The hypoglycemic properties of the ethanolic extract of the bulbs of Allium sativum were evaluated in normoglycemic rats in order to scientific validate its traditional therapeutic use with the doses of 500 mg/kg body weight. The ethanolic extract of A. sativum bulbs reduced the blood glucose level by 49 % after two weeks treatment of albino rats respectively. Oral administration of the ethanolic extract of A. sativum bulbs at dosed equivalent to 100, 250 and 500 mg/kg body weight produced significant (P<0.10) hypoglycemic effects in normal fasted animals after 7 days and 14 days respectively. The dose of extract reduced the blood concentration of the fasted normal rats from an initial mean value of 288.24 ± 7.48 at 0 day reduced blood glucose level 141.32 ± 10.61 at the end of 14 days. It is worthy to mentioned that animals treated with glibenclamide (500 µg/kg) showed a significant reduced in the blood glucose level.

Key Words: *Allium sativum*, normoglycemic, hypoglycemic, glibenclamide.

INTRODUCTION

Garlic is widely known to have beneficial effects on the cardiovascular system but some garlic compounds are chemical cousins to many of the compounds found for the antidiabetic activity. Diabetes has become a leading killer disease in recent years especially in India because Now a days, India in the Capital of Diabetes Mellitus. India has become 40.9 million diabetic people during the year of 2007 according to the International Diabetes Federation and WHO. Today, more than 246 million people in worldwide living with diabetes.

Common available garlic preparations in the form of garlic oil, garlic powder, pills and different extractions are widely used for therapeutic purposes, especially lowering blood pressure and improving lipid profile. Most of studies showed that garlic can reduce blood glucose levels in

diabetic mice, rats and rabbits. [1] Some prominent workers have reported that S-allyl cysteine sulphoxide, (allicin), a sulphar containing amino acid in garlic (200 mg/kg body weight), had a potential to reduce the diabetic condition in rats almost to the same extent as did glibenclamide and insulin. [2,3] Garlic in addition to its food value, have emerged as a potent hypocholesterolemic, antifungal and antibacterial agent. [4] Some Indian herbal plants like Allium sativum, Ocimum sanctum, Azadirachta indica, Momordica charantia not only possess hypoglycemic activity but some of them one antioxidant also.[5] Diabetes is a chronic disorder, which is caused by a lack of hormone insulin. Insulin is a hormone which acts as a key that opens the doors of the cells to allow glucose to enter. Insulin is produced from the β-cells of the pancreas in the body. Pancreas is the second largest endocrine gland, which consisted Islets of Langerhans and pancreatic tissue. The Islets of Langerhans is a endrocine part, contain three types of cells they helps in glucose metabolism; alpha cells which make glucagon; β-cells which produce insulin and delta cells which secrete somatostatin. Insulin and glucagon regulate blood glucose level causing almost all carbohydrate. Glucose is consumed as fuel by almost every type of body cells. Diabetes mellitus is a condition in which the pancreas no longer produces enough insulin or when cells stop responding to the insulin that is produced, so that glucose in the blood can not be absorbed into the cells of the body. The body will attempt to dilute the high level of glucose in the blood, a condition called hyperglycemia.

In traditional medicine, many plants are used either singly or in combination with other Ayurvedic herbs for the control of diabetes mellitus. India has rich diversity of medicinal plants. The supply base of 90 % herbal raw drugs used in the manufacture of Ayurveda, Siddha, Unani and Homoeopathy systems of medicine is largely from the wild. *Allium sativum Linn*, is a member of Lily family. Garlic is a cultivated annual herb, which is cultivated in mostly parts of India. Study discussed as a medicinal plants shows the plant has an antidiabetic effects on streptozotocin induced diabetic rats.

EXPERIMENTAL SECTION

Plant material:

Bulbs of *Allium sativum* plant collected from Vidisha and Bhopal District of Madhya Pradesh. The fresh bubs cloves of *A. sativum* were air dried at room temperature 25 ± 5°C. Proper identification of the plant was carried out by the Botany department of the S.S.L. Jain P.G. Collage, Vidisha and was also confirmed by standard book. A Voucher specimen is deposited in the herbarium record at Pest Control and Ayurvedic Drug Research Laboratory in the Department of Zoology, S.S.L. Jain Collage Vidisha (M.P.) India.

Animal materials:

Healthy adult mail albino rats *Rattus norvegicus* of wistar stain weighing between 250-300 gm were obtained from Bharat Animal House Jahagirabad, Bhopal (M.P.) India. Animals were maintained to standard laboratory condition, allowed to get acclimatized to a standard pellet diet, Golden feed Pvt. Ltd., New Delhi and water ad libitum. Room temperature maintained at 25 ± 5°C with 12 hour light and dark cycle. All animal experiments were conducted according to the ethical approved by Ministry of Environment & Forestry, Committee for the Purpose of Control and Supervision of Experiments on Animals and Institutional Animal Ethics Committee guidelines. (Approval No. 804/03/CA/CPCSEA)

Preparation of extract:

The air-dried cloves were grinded to powder about 40-60 mesh size. A known amount of powdered material 750 gm was used for extracted successively with solvent ethanol in a Soxhlet

apparatus until exhaustion. The extraction was done for 48 hours duration and up to 8 cycles of extraction of the solvent. The crude extracts thus obtained were filtered using Watmen filter paper No. 1 and the solvents were evaporated to dryness under reduced pressure in a 'Vacuum Evaporator' (RE-100) at 40°C. This extract is concentrated on a vacuum evaporator and it was found to deposit for chromatographic separation and bioassay. The concentrate crude drug gave 10.55 % yield with reference to EtOH. The crude was successively extracted with petroleum ether, chloroform, ethyl acetate and ethanol. The extracts were used for the present study as shown in Table-1.

Induced of Diabetes:

The animals were starved overnight then diabetes was induced by a single intraperitoneal injection of a freshly prepared STZ solution (50mg/kg body weight). Streptozotocin was dissolved in 0.1 M freshly prepared citrate buffer solution (pH 4.5). The animals were allowed to drink 5 % glucose solution overnight to overcome. After 5 days Streptozotocin administration, rats showing diabetes. Animal having blood sugar concentration between 250-350 mg were used for the experimental bioassay. The alcoholic extract of the *A. sativum* bulbs was administered orally at a concentration of 100, 250 & 500 mg/kg body weight/ rat/day for 14 days.

Experimental Design:

The animals were divided into four groups for the analysis of biochemical parameters. Each group has six animals.

Group I	*Normal control rats.*
Group II	*Diabetic control rats.*
Group III	*Diabetic rats treated with alcoholic extract 100 mg/ kg body weight/ day orally.*
Group IV	*Diabetic rats treated with alcoholic extract 250 mg/ kg body weight/ day orally.*
Group IV	*Diabetic rats treated with alcoholic extract 500 mg/ kg body weight/ day orally.*
Group V	*Diabetic rats treated with standard drug 500 µg/ kg body weight/ day orally.*

RESULTS AND DISCUSSION

In the present study ripened bulbs of *A. sativum* powdered material; when extracted on soxhlet apparatus gives maximum yield 10.55 % with ethanol extract and other extracts as shown in table (1). Ethanol extract was found to be effectives hence; it was use for experimental bioassay. The results maintained showed hypoglycemic at three different doses ranging from 100-500 mg/kg body weights. Maximum hypoglycemic activity observed at 500 mg dose, which was found 210.21 ± 15.18 and 141.32 ± 10.61 after 7 and 14 days intervals. Daily treatment of STZ-induced diabetic rats with crude extract of garlic 500 mg/kg bw orally for two weeks significantly lowered blood glucose level. These results indicate that garlic extract possesses a beneficial potential in reversing proteinuria in addition to reducing blood sugar in diabetic rats. The results when compared with student t-test give the level of significance at ($P<0.10$).

The present study was undertaken to study the antidiabetic activity of ethanolic extract of garlic in streptozotocin induced diabetic rats to check the significant effect on blood glucose in the treatment of diabetes. Mahesar [6] *et al.* (2010) have observed that Administration of alloxan (150 mg/kg) led to about 3- fold elevation of fasting blood glucose levels, which was maintained over a period of 4 weeks. One month of daily treatment with aqueous extract of garlic caused a significant fall in elevated blood glucose levels from 300 to 216 mg/dl (38.88%) in diabetic rabbit. The present study was undertaken to study the antidiabetic activity of ethanol extract of garlic in streptozotocin induced diabetic rats in order to check the blood sugar reduce 49 % also after 14 day the treatment. Garlic which is a common spice used in the kitchen in Indian house may have a diet role in the prevention and control of diabetes mellitus and cardiovascular

disease. Khan and Safdar [7] (2003) have observed the role of diet Nutrients, Spices and Natural Products in Diabetes Mellitus. Bever and Zahnd [8] (1979) have reported a list of plants which have orally hypoglycemic activity. Almost a decade later, Rehman and Zaman [9] (1989) published a list of several hundred species, which had antidiabetic properties. Hypoglycemic property of bitter gourd has been reported by Khana [10] (1985) and Satyavati *et.al*. [11] (1987). Stevinson and Ernis [12] (2000), Abdul Wahhab and Aly [13] (2003) and Kayam [14] *et al.* (2003) have reported common available garlic preparation in the form of garlic oil, garlic powder, pills and different extractions are widely used for therapeutic purposes especially lowering blood pressure and improving lipid profile.

Table (1) Percentage yield of *A. sativum* crude extract soxhleted by soxhlet apparatus.

Solvent Used in Soxhletion Method	Volume of Solvent (ml)	Weight of Powdered Materials (gm)	Weight of Extract after soxhlet apparatus (ml)	Weight of Extract (gm)	Yield Percentage of Extract
Petroleum Ether	600	750	740	63.45	8.46 %
Chloroform	600	750	688	46.08	6.14 %
Ethyl Acetate	600	750	432	32.94	4.39 %
Ethanol	600	750	846	79.18	10.55 %

Table (2) Showing Blood Sugar Lowering Effect of Ethanolic Extract of *Allium sativum*

Treatment Doses		Blood Glucose Level mg/100 ml		
		Initial Value	After 7 days	After 14 days
Normal Control	-	92.84 ± 4.14	94.76 ± 6.23	96.89 ± 10.17
Diabetic Untreated	-	288.46 ± 8.71	290.42 ± 14.18	296.55 ± 7.48
Diabetic + *A. sativum* Extract	100 mg/kg	289.02 ± 6.67	261.04 ± 9.28	209.17 ± 13.71
Diabetic + *A. sativum* Extract	250 mg/kg	290.88 ± 5.12	248.38 ± 6.31	205.24 ± 12.02
Diabetic + *A. sativum* Extract	500 mg/kg	288.24 ± 7.48	210.21 ± 15.18	* 141.32 ± 10.61
Diabetic + Glibenclamide	500 µg/kg	291.73 ± 8.43	207.23 ± 5.44	158.78 ± 5.44

*The values are mean ± SEM n = 6 animals in each groups. * P < 0.10 in comparison to corresponding value before treatment.*

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