A COMPARATIVE RESEARCH ON THE EFFECTS OF DIFFERENT ALLIUM SATIVUM BULBS EXTRACTS ON ALLOXAN INDUCED DIABETES MELLITUS

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Abstract

Objective: A comparative research study on the effects of different Allium sativum bulb extracts on alloxan-induced diabetes mellitus

The chloroform, acetone, ethanol, and aqueous extracts were prepared by macerating the bulbs of Allium sativum. The diabetic potential of the different extracts was evaluated by injecting Alloxan (150 mg/kg body weight) in a single dose into Wistar rats. The animals were divided into six groups, containing six rats in each group (non-diabetic group, diabetic group, and Allium sativum-treated groups). The extracts were administered orally at 100 mg/kg body weight to the diabetic rats for 14 days. The fasting blood glucose levels were measured, and histopathological studies were carried out. The administration of ethanolic, chloroform, aqueous, and acetone extracts of Allium sativum showed decreased necrotic lesions and perivascular edema compared to diabetic control rats. However, it was found to be greater in the aqueous extract. The current study concludes that administration of an aqueous extract of Allium sativum in rats attenuates fasting blood glucose significantly as compared to other extracts.

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1. Introduction

Diabetes mellitus is a metabolic condition characterized by persistent hyperglycemia and changes in the metabolism of carbohydrates, fats, and proteins as a result of deficiencies in insulin secretion, insulin action, or both. (1). In 2020, it was estimated that 463 million people have diabetes in the world. In current time, the numbers of diabetic patients are frequently increasing in rich as well as in developing countries (2). Insulin, insulin-secreting agents (sulfonylureas and glinides), α-glucosidase inhibitors and insulin sensitizers are the principal antidiabetic medications used in pharmacological therapies (thiazolidinediones and biguanides). Despite the prevalence of these medications, glucagon-like peptide 1 receptor (GLP-1R) agonists, sodium glucose co-transporter 2 (SGLT2) inhibitors, and dipeptidyl peptidase 4 (DPP-4) inhibitors are three new additions to anti-diabetic regimens. Antihyperglycemic medications, such as -glucosidase inhibitors, work through a variety of pharmacological mechanisms, including decreased hepatic glucose synthesis, increased insulin secretion by pancreatic beta cells, and increased insulin sensitivity (3).

The search for new natural anti-diabetic medications is ongoing since the use of synthetic drugs to treat diabetes mellitus has advanced significantly. According to estimates from the World Health Organization (WHO), traditional medicine is still used by 80% of the population in some underdeveloped nations. [4]. Several plants have been researched using various experimental techniques because of their anti-hyperglycemic properties in folk medicine. (5). Plants like Radix puerariae, Radix ginseng, Rhizoma anemarrhenae, a combination of the fruits, leaves, and root epidermis of Morus alba, Radix paeoniae and Radix paeoniae alba, Allium sativum bulb, and Gymnema sylvestre were found to contain more than one bioactive compound that showed significant results for improving blood glucose levels and also improved its associated hyperlipidemia and complications related to the diabetes (6.) The presence of apigenin, cirsimaritin, christinin-A, nordihydroguaiaretic acid, isorhamnetin, and isorhamnetin-3-O-rutinoside in plants has been reported to produce positive hypoglycemic effects. (7).

Secondary metabolites found in medicinal plants, such as flavonoids, terpenoids, alkaloids, and polysaccharides, have long been thought to have anti-diabetic properties. Human oxidative stress is a crucial factor in the development of diabetes and its associated complications. (8). It has been depicted that diabetic patient showed lowers levels of antioxidant status. The exact mechanism of action of oxidative stress in diabetic patient is still unknown but some proposed mechanism for diabetes included increase glycation of proteins, inactivation of some enzymes and some changes to the collagen basement membrane's structural properties (9).

It has been found that oxidative stress has significant effect in transporters of glucose and insulin receptor activity. It is identified that oxidative stress scavengers may have potential to possess hypoglycemic property in diabetics. Many plants having antioxidant activity showed have been screened for ability to scavenger of free radicals and are useful as protective agent against oxidative stress.

Allium sativum Linn., (Liliaceae) ripe bulbs found throughout the India, central Asia, southern Europe, and United States. The sulphur-containing chemicals alliin, allicin, ajoene, diallyl sulphide, diallyl disulfide, diallyl trisulfide, and vinyldithiines were identified in abundance in the bulbs of Allium sativum which has potential to treat thrombotic diseases , cancer, viral infections , fungal infections and microbial infections . Ajoene was found as an inhibitor of platelet aggregation induced by all known agonists which also a good inhibitor of tumour and antifungal property (11). It has been investigated that alliin, allyl cysteine, allyl disulfide, and allicin from Allium sativum possesses antioxidant activity and having a potential to protect against free radicals(10). Therefore , the current study is aimed at investigated the protective effect of different extracts of A. sativum on alloxan induced diabetes mellitus. In our present study, the aqueous Allium sativum extract has showed good results.

2. Materials and Methods

Plant Material and extracts preparation

The bulb of Allium sativum were purchased from the local market Nabha, India. The plant species was authenticated by Dr. Sunita Garg, Chief scientist, RHMD, CSIR-NISCAIR, Delhi and the specimen was submitted and preserved in the institute department for future reference (Voucher specimen number NISCAIR /RHMD /Consult/ 2013/ 2352-133). Allium sativum bulb were cleaned and shade dried. The dried bulbs was powdered in by electric grinder. Chloroform, Acetone, ethanol and aqueous extracts of Allium sativum were prepared by immersed individually in 500ml of each solvent for 72 hours with occasionally shaking. After 72 h, solvent was separated out from the powder with filtration and subjected to rotatory evaporation at 40°C. The

extracts were stored in air tight container at -10^0 C in incubator.

Animals and drugs

In this experimental work, wistar rats having weight (150-200g) of either sex were used. The animals were approved by institutional animal ethical committee via regd. No. 1616/Po/a/12/CPCSAE (Protocol No. IAEC/SSP/ 13/ PR-002). Animals were procured from the Animal house of Lala Lajpat Rai University, Hisar. Before beginning the experiment, they were housed (5 per cage) for two weeks to allow for acclimatization. Standard nutrition, temperature, relative humidity (55%) and light cycle (12 h light/12 h dark) were all freely available.Alloxan was purchased from SD fine-chem Limited, Mumbai. Glucose estimation kit and total cholesterol estimation kits were purchased from Avecon Healthcare, Parwanoo (H. P). All other chemicals were of AR grade. Alloxan was freshly dissolved in distilled water for intraperitoneal administration.

Induction of diabetes and experimental design

Diabetes was induced with alloxan (150mg/kg i.p) administration to Wistar rats. Rats were fasted overnight prior to the induction of diabetes. On the 3rd day after alloxan administration fasting blood glucose level was estimated. Animals more than 150mg/dl were considered as diabetic and used in the study. Thirty six rodents were randomly divided into six groups (5 diabetic and one healthy group) of six rats each. Group I: Non diabetic group- fresh animals were considered as non-diabetic group. In the remaining groups, diabetes was induced by single i.p injection of freshly prepared in a dose of 150mg/kg body weight. The FBS levels were estimated on the 3rd day for confirmation of hyperglycemia. All the treatments were started from 3rd day after the conformation of hyperglycemia. Group II: alloxan-induced diabetic control group and normal saline (0.9% NaCl) was given to diabetic rats orally daily from 3rd day to 14th day. Group III treated with chloroform A. sativum extract at a dose of 100 mg/kg. Group IV: treated with Acetone A. sativum extract at a dose of 100 mg/kg. Group V: treated with ethanol A. sativum extract at a dose of 100 mg/ kg. Group VI: treated with Aqueous A. sativum extract. All the treatment groups treated with their respective extracts from the 3rd day onwards up to 14th day.

Estimation and blood samples

Blood sample was collected on 14th day from retro-orbital sinus. Cold centrifugation at 3000 RPM for 15 minutes was done on blood sample. After the centrifugation plasma was separated and subjected to biochemical parameters estimation i.e. fasting blood glucose and total cholesterol.

Rat was sacrificed with the cervical dislocation method and dissection was done. Heart homogenate was used for assessing various biochemical assays i.e. Catalase (CAT) and thiobarbituric acid reactive substances (TBARS). The hearts were stored in 10% formalin for performing histopathological studies.

Biochemical assays

The heart was homogenized in 10% w/v ice cold 50mM potassium buffer (pH 7.4). The resulting homogenate was then centrifuged for 20 minutes at 40C at 2500 rpm.

Estimation of TBARs

Based on the reaction between MDA and TBA, thiobarbituric acid (TBA) reactive substances (TBARS) in plasma were used to measure the MDA level. Standard MDA solution (100 nmoles) in 5ml volume was processed along with test samples. To the 1ml of serum sample, 1.5ml of 0.8% of TBA was added. Finally, 5ml of distilled water were added to the mixture, which was then heated to 95°C for one hour. After cooling, 5ml of the 15:1 v/v mixture of n-butanol and pyridine were added along with 1ml of distilled water. The mixture was vortexed, and following centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer (upper layer) was measured using a distilled water blank and a Shimadzu UV-vis spectrophotometer at 532 nm. When TBA and MDA were allowed to interact aerobically, they created a colorful complex called the MDA-(TBA)2 complex, which was measured using a spectrophotometer.

Estimation of Fasting Blood Glucose Level

FBG levels was estimated with the help of spectrophotometer by using an enzymatic kit, (CDR medical industries ltd. Hyderabad, India).

Estimation of Total Cholesterol Level

Using common enzymatic colorimetric kits (Span diagnostics Ltd., India) total cholesterol was calculated.

Statistical Analysis

Results are expressed by using mean and standard deviation (SD) by one-way analysis of variance (ANOVA), followed by Tukey's multiple range test, was used to analyse the study's data at $P<0.005$, the level of significance was fixed.

3. Results

Phytochemical Screening of Different Extracts of Allium sativum

The presence of alkaloids, carbohydrates, saponin, fats, fixed oils, flavonoids, proteins and amino acids in the ethanol A. sativum extract was

revealed by phytochemical screening. The presence of carbohydrates, proteins and amino acids in in the chloroform A. sativum extract was revealed by phytochemical screening. The presence of alkaloids, carbohydrates, saponin, fats, fixed oils, proteins and amino acids in the acetone A. sativum extract was revealed by phytochemical screening. The presence of alkaloids, carbohydrates, saponin, fats, fixed oils, flavonoids, proteins and amino acid in the aqueous A. sativum extract was revealed by phytochemical screening.

Effect of different Allium sativum extracts on plasma blood glucose concentration in diabetic rats

Table 1 shows that alloxan treatment increase the FBG levels significantly ($p<0.05$) in the diabetic control as compared to the non-diabetic groups. The treatments with AqASE (aqueous A. sativum extract), Was found to decrease FBG, level significantly ($p<0.05$) as compared to diabetic control group. All the values are depicted **(Figure 1)**.

Effect of different Allium sativum extracts on plasma total cholesterol in diabetic rats

Table 1 shows that alloxan treatment increase the total cholesterol levels significantly ($p<0.05$) in the diabetic control as compared to the non-diabetic groups. The treatments with AqASE (aqueous A. sativum extract), Acetone A. sativum extract (AASE) and cholesterol A. sativum extract (CASE), was found to decrease FBG, level significantly ($p<0.05$) as compared to diabetic control group and values are in **Figure 2**.

Effect of different Allium sativum extracts on TBARS in diabetic rats

Table 1 shows that alloxan treatment increase the TBARS levels significantly ($p<0.05$) in the diabetic control as compared to the non-diabetic groups. The treatments with Ethanol A. sativum extract (EASE), Acetone A. sativum extract (AASE) and cholesterol A. sativum extract (CASE), was found to decrease TBARS level significantly ($p<0.05$) as compared to diabetic control group with values are represented in **Figure 3**.

Effect of different Allium sativum extracts on catalase in diabetic rats

It has found that alloxan treatment decrease the catalase levels significantly ($p<0.05$) in the diabetic control as compared to the non-diabetic groups. The treatments with Aqueous A. sativum extract (AqASE), Acetone A. sativum extract (AASE) and cholesterol A. sativum extract (CASE), was found to increase catalase level significantly ($p<0.05$) as compared to diabetic control group. All the values are depicted in **Figure 4**.

Table 1: Effect of Various Pharmacological Interventions on the Biochemical Parameters in the Diabetic Rats

Groups	FBG (mg/dl)	Total Cholesterol (mg/dl)	TBARS (nmoles/mg protein)	CAT (mols of H_2O_2/min/mg proteins)
Non diabetic control	88.33±3.88	163± 4.79	14.16667 ± 2.136976	1.365667 ± 0.317856
Diabetic control	160.833±6.33	309± 7.063	37.22 ± 4.22	0.35167± 0.155874
Chloroform A. sativum extract	101±8.43	246.833±12.13008	22.6667 ± 1.6322993	0.755± 0.109864
Acetone A. sativum extract	104.8333±6.210207	194±5.477	24.83333 ± 1.169045	0.441667± 0.135265
Ethanol A. sativum extract	94.1667±4.215052	232.2±9.98995	21.3333± 2.065591	1.17± 0.120996
Aqueous A. sativum extract	115±10.31504	186.1667±3.531	19.5± 1.0488809	0.535± 0.113974

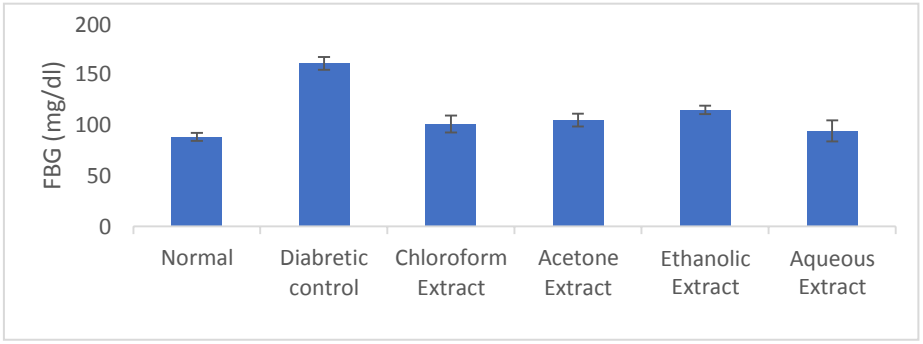

Figure 1. : Effect of different Allium sativum extract on fasting blood glucose level in diabetic rats on 14th day. (*p<0.005 significant vs diabetic control group)

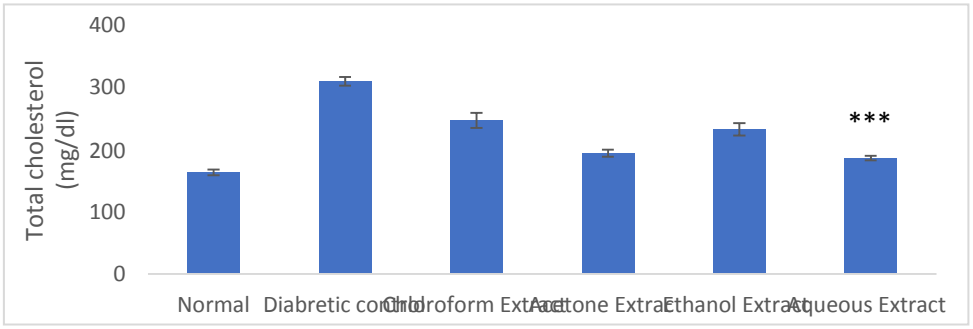

Figure 2. Effect of different Allium sativum extract on total cholesterol level in diabetic rats on 14th day. (*p <0.005 significant vs diabetic control group)

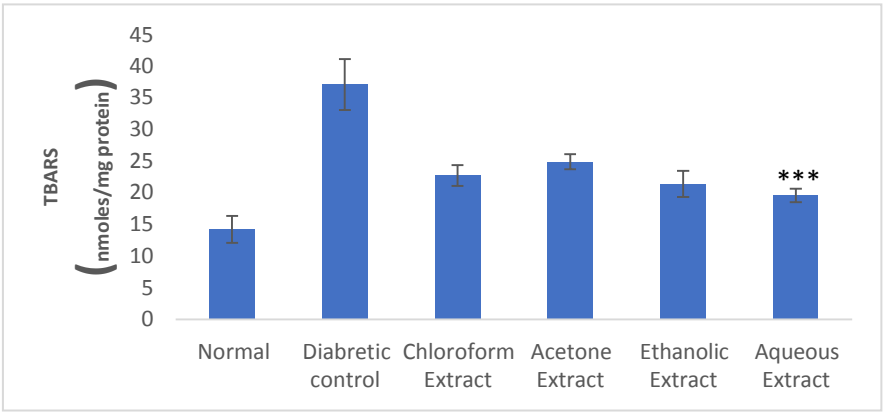

Figure 5. Effect of different Allium sativum extract on TBARS level in diabetic rats on 14th day. (*p <0.005 significant vs diabetic control group)

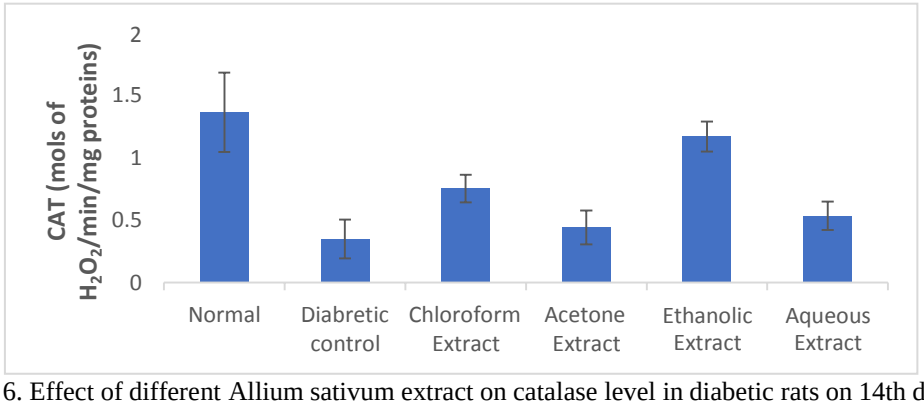

Figure 6. Effect of different Allium sativum extract on catalase level in diabetic rats on 14th day. (*p <0.005 significant vs diabetic control group

4. Discussion and conclusion

The benefits of natural herbs and spices as alternate forms of treatment for chronic diseases like DM have been the focus of substantial research over thepast two decades. Fresh garlic extract and several of its ingredients have been the subject of much research because they contain anti-diabetic and antioxidant qualities. *Allium sativum*, a bulb thatcan be purchased over the counter and has a recognised composition as well as acceptable ingestion and side effect characteristics, hasn't drawn much attention as a diabetic medication. The goal of present work was to investigate the ameliorative effect of different extracts from the bulb of *Allium sativum* in alloxan induced diabetes. Alloxan possess its diabetogenic actions when administered by intravenously, intraperitoneally or subcutaneously to the animal. Alloxan has been reported to induce persistent hyperglycemia by the generation of ROS(13). Alloxan has also been reported to induce cytotoxic effect on pancreatic cell due to generation of reactive oxygen species (oxidative stress) and peroxide (14). In the present study administration of alloxan developed rapid hyperglycemia. Treatment of diabetic rats with AqASE, EASE,CASE and AASE was found to decrease the FBG significantly as compared to the diabetic control rats. Dyslipidemia is also considered a major symptom and risk factor for the development of cardiovascular diseases in diabetes which is characterized by high serum levels of both triglycerides and cholesterol (15). In present study, elevated levels of total cholesterol is also observed in diabetic control as well as in diabetic vehicle groups. The Administration of AqASE, EASE,CASE and AASE was found to decrease the levels of total cholesterol significantly as compared to diabetic control group. In previous studies increased levels of ROS was observed in diabetic rats(16). It has been postulated that hyperglycemia leads to oxidation of glucose superoxide anion radicles generations (17).TBARS and catalase levels has been used to quantify the oxidative damage associated with oxidative stress. The treatment with AqASE, EASE,CASE and AASE was found to decrease the levels of TBARS significantly as compared to the diabetic control rats and level of catalase was found to increase with administration of AqASE, EASE,CASE and AASE. (18)

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