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Research Article
Ameliorative Effects of *Allium cepa* and *Allium sativum* on Diabetes Mellitus and Dyslipidemia in Alloxan-induced Diabetic *Rattus novergicus*

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Abstract
Background and Objectives: Dyslipidemia is a common complication of diabetes mellitus with a pathological link to cardiovascular diseases. The use of synthetic chemicals in therapy leads to unexpected toxic effects, hence, the need for the use of natural plants as therapeutic agents. The study was designed to investigate the natural therapeutic agents such as; *Allium cepa* and *Allium sativum* in the treatment of diabetes mellitus and dyslipidemia. **Materials and Methods:** A total of 68 rats were divided into 4 groups of 17 rats each. Group1 served as control and were fed with rat feed and distilled water only. Diabetes mellitus was induced in 51 rats by administering intraperitoneally 150 mg of alloxan per kg body weight (bw) of the rats. Diabetic rats were divided into groups II, III and IV which were fed with rat feed and distilled water. In addition, the three groups were fed with 1.00, 1.50 and 2.00 mg kg^{-1} between aqueous extract of *Allium cepa*, respectively. The feeding procedures for group II, III and IV were repeated with aqueous extract of *Allium sativum* replacing *Allium cepa*. Animals were fed for 4 weeks. Blood samples were collected from ocular median-cantus vein of the rats. Plasma was separated for analysis. Blood glucose and lipid profiles of the rats were determined by standard laboratory methods. **Results:** *Allium cepa* and *Allium sativum* aqueous extracts significantly ($p<0.05$) lowered the blood glucose, triacylglycerol, total cholesterol and low-density lipoprotein and significantly ($p<0.05$) increased the high-density lipoprotein when compared with the control. **Conclusion:** Aqueous extracts of *Allium cepa* and *Allium sativum* show ameliorative effects on diabetes.

Key words: *Allium cepa, Allium sativum*, diabetes mellitus, dyslipidemia, alloxan

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a group of chronic and heterogeneous diseases characterized by hyperglycemia and other lethal complications. Diabetes mellitus could arise as a result of the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced[1].

Three main types of diabetes mellitus exist. Type 1 diabetes mellitus results from the pancreas inability to produce enough insulin. Type 1 diabetes mellitus is characterized by loss of insulin-producing beta cells of the pancreatic islets, leading to insulin deficiency[2]. Type 1 diabetes mellitus was also referred to as "Insulin Dependent Diabetes Mellitus (IDDM)" or juvenile onset diabetes". However, type 1 diabetes can affect children or adults, but was traditionally termed "juvenile onset" because a majority of those suffering from type 1 diabetes were children. Sensitivity and responsiveness to insulin are usually normal in type 1 diabetes mellitus especially in the early stages of the diseases.

Type 2 diabetes mellitus; this form begins with insulin resistance. This condition is characterized by the inability or failure of body cells to respond to the presence of insulin properly. Lack of insulin may also develop as the disease progresses. A number of lifestyle factors are known to be important to the development and progress of type 2 diabetes mellitus including excessive body mass index.

Gestational diabetes is the third main type. It occurs when a pregnant woman without a previous history of diabetes develop high blood sugar level. Gestational diabetes resolves after the birth of the baby. Diabetes is often associated with both quantitative and qualitative abnormalities of lipoprotein that are responsible for increased incidence of microvascular and macrovascular complications. The chronic hyperglycemia of diabetes is also associated with oxidative stress and organ dysfunctions especially in the kidneys, nerves and eyes giving rise to diabetic nephropathy, diabetic neuropathy and diabetic retinopathy, respectively[3].

Dyslipidemia which involves raised triacylglycerol raised total cholesterol, raised Low Density Lipoprotein (LDL) and low High Density Lipoprotein (HDL) are common with patients with diabetes. Oxidative stress in diabetes mellitus has pathological link to cardiovascular diseases particularly the effects of oxidative stress on low density lipoprotein, inflammation status and hyper cholesterolemia[4]. The use of some synthetic chemicals in therapy, has led to many additional and unexpected toxic effects. Therefore, there is a global trend to exploit naturally occurring plants as alternative therapeutic agents for the treatment of some chronic diseases like diabetes mellitus.

Allium is a genus of some 500 species belonging to the family Liliaceae. *Allium cepa* (onions) and *Allium sativum* (garlic) have enjoyed wide reputations and usefulness as prophylactic and therapeutic agents. Several studies have connected the consumption of onions and garlic to the regulation of plasma lipid and blood glucose concentration[5].

The hypolipidemic and hypocholesterolemia activities of onions and garlic can be attributed to allicin and its derivatives. However, other non-sulphur components of garlic such as; the steroid saponins can also reduce serum cholesterol levels. Garlic and onions exert their hypolipidemic effects by limiting hepatic cholesterol biosynthesis, inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A(HMG-CoA) reductase and squalene mono-oxygenase enzymes[6].

The explanation for hypoglycemic action of onions and garlic is through their active components which contain mainly sulfur-containing compounds called allyl propyl disulfides. Allyl Propyl Disulfides (APDs) lower glucose levels by competing with insulin for insulin inactivating sites in the liver resulting in an increase of insulin[7].

Sulfur-containing compounds in garlic and onions could be volatile and non-volatile. The volatile compounds included alk(en)yl disulfides, while the non-volatile cysteine sulfoxides are S-methyl-L-cysteine sulfoxide, S-propyl-L-cysteine sulfoxide and S-allyl-L-cysteine sulfoxide[8]. The volatile and non-volatile active components of garlic and onions are critical in their anti-diabetic, anti-oxidant and anti-hyperlipidemic effects.

Therefore, the aim of this study was to investigate the ameliorative effects of *Allium cepa* and *Allium sativum* on diabetes mellitus and dyslipidemia in alloxan-induced diabetic *Rattus norvegicus*.

MATERIALS AND METHODS

Study area and period: The study was concluded during November, 2018-2019 in the Department of Medical Biochemistry, Faculty of Basic Medical Science, College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria.

Materials: Fresh *Allium cepa* (onions) and *Allium sativum* (garlic) used in the study were purchased from OrieOrba World Bank Assisted Market, Enugu state, Nigeria, Identification of plants was done at the Herbarium section, Department of Botany, University of Nigeria, Nsukka, Nigeria.

Table 1: Composition of animal feed

Feed composition	Percentage
Yellow corn	25
Wheat bran	26.2
Soybean meal	14
Molasses	3
$CaCl_2$	1
NaCl	0.4
Mixture of minerals	0.3
Vitamins	0.01 g kg^{-1} diet of vitamin E
Methionine	0.1

Animals tested: Sixty eight male albino rats weighing 150-250 g were supplied by the Animal House of the University of Nigeria Teaching Hospital Enugu, Nigeria. The rats were housed in clean, stainless steel cages under ambient temperature of 26°C with 12 h light/12 h dark cycle. The rats were fed with distilled water and standard feed (Cospa feed and flour mills limited, Enugu, Nigeria) *ad libitum*. The constituents of the animal feed are given in Table 1. Animal experiments were conducted in accordance with the guidelines described in U.K Animals (scientific procedures) Act, 1986 and associated guidelines.

Preparation of *Allium cepa* (onion) and *Allium sativum* (garlic) extracts: Aqueous *Allium cepa* and *Allium sativum* extracts were prepared[9]. About 60 g of onion was homogenized in 100 mL of cold distilled water. The resultant homogenized mixture was filtered three times through fine cloth. The mixture was centrifuged at 200xg for 10 min and the clear supernatant was collected. Based on the fact that 60 g/100 mL of onion was used initially, the concentration of the onion preparation was 600 mg mL^{-1}. The procedure was repeated for *Allium sativum* (garlic).

Induction of diabetes mellitus: Diabetes mellitus was induced in 51 rats by using the methods of Battu *et al.*[10]. A total of 150 mg of alloxan monohydrate per kg b.wt., of rat was administered intraperitoneally after overnight fast of 12 h to render the rats more vulnerable to diabetes. Rats with serum glucose concentrations exceeding 200 mg dL^{-1} were considered diabetic.

Animal treatment: The rats were fed with standard feed (Cospa feed). A total of 60 rats were divided into 4 groups of 17 rats per group. Group 1 served as the control and received no extract. Group II, III and IV received 1.00, 1.50 and 2.00 mg kg^{-1} b.wt., of the extracts, respectively using an intragastric tube for 4 weeks. The rats move carefully observed for any unhealthy development.

Samples collection: After 4 weeks from the day of experiment started, rats were fasted for 12 h. The blood samples collected from the ocular median-cantus vein of the rats by using sodium fluoride and plain capillary tubes. The collected blood samples were transferred to test tubes and allowed to clot and centrifuged at 3000 rpm for 5 min. Plasma and serum were separated for glucose and lipid analysis.

Biochemical measurements: The lipid profiles determination was done using kits manufactured by TECO diagnostics Lakeview, Anaheim CA, USA. Triacylglycerol was determined by the method of Burstein *et al.*[11]. Serum Total Cholesterol (TC) was determined by the method of Allain *et al.*[12]. The lipoproteins: High Density Lipoprotein (HDL) and Very Low-density Lipoproteins (VLDC) were precipitated using phosphotungstic acid and magnesium chloride. After centrifugation the supernatant contained the HDL-cholesterol fraction which was assayed for cholesterol[13]. The Low Density Lipoprotein Cholesterol (LDC-C) was estimated[14]. Glucose was estimated by the glucose oxidase method according to Randox Kit manufacturer's procedure (Randox Laboratories Ltd Ardmore, UK).

Statistical analysis: The data obtained were expressed as Mean±SD. The data was statistically analyzed using one-way Analysis of Variance (ANOVA) with Tukey's *post hoc* test to compare the levels of significance between the control and experimental groups. All statistical analysis was evaluated using SPSS version 20 software and Microsoft excel. The values of $p \leq 0.05$ were considered statistically significant.

RESULTS

The results of the effects of *Allium cepa* (onion) and *Allium sativum* (garlic) on lipid profile of alloxan-induced diabetic *Rattus novergicus* are given in Table 2. Data the effects of aqueous extracts of *Allium cepa* and *Allium sativum* on serum triacylglycerol of the rats in the different groups. Data shows the Mean±SD (x±SD) of the serum triacylglycerol in the control and groups II, III and IV. It is observed that aqueous extracts of *Allium cepa* and *Allium sativum* caused a significant ($p<0.05$) decrease in triacylglycerol in the rats.

Table 3 showed the effects of aqueous extracts of *Allium cepa* and *Allium sativum* on total cholesterol of the rats in the different groups. Data showed the Mean±SD (x±SD) of serum total cholesterol in the control and groups II, III and IV. It depicts the significant ($p<0.05$) decrease in the total cholesterol concentration of the rats by aqueous extracts of *Allium cepa* and *Allium sativum*.

Table 2: Effects of *Allium cepa* (onion) and *Allium sativum* (garlic) on lipid profile of alloxan-induced diabetic *Rattus novergicus*

	Triacylglycerol concentration (mg dL^{-1} x±SD)		
Groups	*Allium cepa*	*Allium sativum*	p-value
Group I (control): Animals fed with rat feed+distilled water only	273.20±3.00	272.10±2.50	
Group II: Animal fed with rat feed+distilled water+1.00 mg kg^{-1} b.wt., aqueous extract	268.20±4.00	235.30±1.00	p<0.05
Group III: Animal fed with rat feed+distilled water+1.50 mg kg^{-1} b.wt., aqueous extract	251.10±2.00	212.50±3.20	p<0.05
Group IV: Animal fed with rat feed+distilled water+2.00 mg kg^{-1} b.wt., aqueous extract	239.30±2.10	199.00±3.00	p<0.05

Table 3: Effects of aqueous extracts of *Allium cepa* and *Allium sativum* on total cholesterol of the rats in the different groups

	Total cholesterol (mg dL^{-1} x±SD)		
Groups	*Allium cepa*	*Allium sativum*	p-value
Group I (control): Animals fed with rat feed+distilled water only	317.70±3.00	318.75±2.00	
Group II: Animal fed with rat feed+distilled water+1.00 mg kg^{-1} b.wt., aqueous extract	264.00±1.00	235.30±4.00	p<0.05
Group III: Animal fed with rat feed+distilled water+1.50 mg kg^{-1} b.wt., aqueous extract	196.45±3.10	207.30±3.50	p<0.05
Group IV: Animal fed with rat feed+distilled water+2.00 mg kg^{-1} b.wt., aqueous extract	147.25±2.30	191.50±1.50	p<0.05

Table 4: Effects of aqueous extracts of *Allium cepa* and *Allium sativum* on High Density Lipoprotein (HDL) of the rats in the different groups

	High density lipoprotein (mg dL^{-1} x±SD)		
Groups	*Allium cepa*	*Allium sativum*	p-value
Group I (control): (Animals fed with rat feed+distilled water only)	41.32±4.00	39.38±1.10	
Group II: Animal fed with rat feed+distilled water+1.00 mg kg^{-1} b.wt., aqueous extract	63.40±2.90	53.10±1.00	p<0.05
Group III: Animal fed with rat feed+distilled water+1.50 mg kg^{-1} b.wt., aqueous extract	68.10±1.00	74.00±2.10	p<0.05
Group IV: Animal fed with rat feed+distilled water+2.00 mg kg^{-1} b.wt., aqueous extract	75.20±3.60	91.30±1.80	p<0.05

Table 5: Effects of aqueous extracts of *Allium cepa* and *Allium sativum* on low density lipoprotein of the rats in the different groups

	low density lipoprotein (mg dL^{-1} x±SD)		
Groups	*Allium cepa*	*Allium sativum*	p-value
Group I (control): Animals fed with rat feed+distilled water only	199.05±2.00	193.71±1.50	
Group II: Animal fed with rat feed+distilled water+1.00 mg kg^{-1} b.wt., aqueous extract	192.10±3.50	181.50±2.00	p<0.05
Group III: Animal fed with rat feed+distilled water+1.50 mg kg^{-1} b.wt., aqueous extract	175.30±3.00	153.81±1.50	p<0.05
Group IV: Animal fed with rat feed+distilled water+2.00 mg kg^{-1} b.wt., aqueous extract	164.10±1.00	134.20±2.10	p<0.05

Table 6: Effects of *Allium cepa* (onion) and *Allium sativum* (garlic) aqueous extract on fasting blood glucose concentration of alloxan induced diabetic *Rattus novergicus*

	Fasting blood glucose concentration (mg dL^{-1} x±SD)		
Groups	*Allium cepa*	*Allium sativum*	p-value
Group I (control): Animals fed with rat feed+distilled water only	76.25±2.10	78.27±1.00	
Group II: Animal fed with rat feed+distilled water+1.00 mg kg^{-1} b.wt., aqueous extract	272.40±5.00	270.60±3.00	p<0.05
Group III: Animal fed with rat feed+distilled water+1.50 mg kg^{-1} b.wt., aqueous extract	180.60±3.40	178.10±4.50	p<0.05
Group IV: Animal fed with rat feed+distilled water+2.00 mg kg^{-1} b.wt., aqueous extract	130.20±9.00	125.00±11.20	p<0.05

Table 4 showed the effects of aqueous extracts of *Allium cepa* and *Allium sativum* on High Density Lipoprotein (HDL) of the rats in the different groups. The data shows the Mean±SD (x±SD) of the high density hypoprotein in the control and groups II, III and IV. It shows a significant (p<0.05) increase in the high density lipoprotein caused by aqueous extract of *Allium cepa* and *Allium sativum*.

Table 5 depicted the effects of aqueous extracts of *Allium cepa* and *Allium sativum* on low density lipoprotein of the rats in the different groups. The data showed the Mean±SD (x±SD) of Low Density Lipoprotein (LDL) in the control and groups II, III and IV. Significant (p<0.05) decrease in the low density lipoprotein of the rats caused by aqueous extracts of *Allium cepa* and *Allium sativum* can be observed.

Table 6 showed the effects of *Allium cepa* (onion) and *Allium sativum* (garlic) aqueous extract on fasting blood glucose concentration of alloxan-induced diabetic *Rattus novergicus.* The data showed the Mean±SD (x±SD) of the fasting blood glucose in the control and groups II, III and IV. There is a significant decrease in the fasting blood glucose level (p<0.05) caused by the aqueous extracts of *Allium cepa* and *Allium sativum*.

DISCUSSION

In this study, aqueous extracts of garlic and onions separately reduced the blood glucose levels in alloxan induced diabetic rats. Another important finding of this study is that aqueous extracts of garlic and onions have antilipidemic effects.

Remarkable increase in the blood glucose levels in diabetes mellitus have been extensively reported in human and experimental animals[15].

Aqueous extracts of garlic and onions significantly reduced total serum cholesterol, Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and significantly increased High Density Lipoprotein (HDL) levels of the rats.

The hypoglycaemic effects of the aqueous extracts of onions and garlic found in this study is in agreement with the previous results of Babu and Srinivasan[16] and Sharma *et al.*[17]. Allyl propyl disulfide, an active component in onions could be responsible for the hypoglycaemic effects as reported by Andallu *et al.*[18].

Furthermore, the antilipidemic effects of aqueous extracts of onions and garlic found in this study is in line with the results of Efendy *et al.*[19] and Prasad *et al.*[20]. Significant reductions in the low density lipoprotein and triacylglycerol and serum total cholesterol by onions and garlic extracts[21] were of immense health benefits in forestalling atherosclerosis and other heart related problems.

The biochemical mechanism of the hypolipidemic and hypoglycaemic effects of *Allium cepa* and *Allium sativum* extracts are vague and not easy to comprehend.

It is suggested that more studies should be carried out to unravel the mechanism of the hypolipidemic and hypoglycaemic effects of *Allium cepa* and *Allium sativum*.

CONCLUSION

Present report demonstrates the ability of aqueous extract of onions and garlic to ameliorate diabetes mellitus by generating hypoglycaemic and hypolipidemic effects in alloxan induced diabetic *Rattus novergicus*. Aqueous extracts of onions and garlic were able to reduce blood glucose levels, serum total cholesterol and low density lipoprotein which are high in alloxan induced diabetic rats. This may provide a good rationale for adoption of dietary approach and use of natural plants like onions and garlic as alternative natural therapy for diabetes mellitus.

SIGNIFICANCE STATEMENT

This study discovers the antidiabetic and antilipidaemic capabilities of aqueous extracts of onions and garlic that can be beneficial for the management of diabetes mellitus and chemoprevention of atherosclerosis.

This study will help the researchers to uncover the critical areas of alternative therapy using natural plants that many researchers were not able to explore. Thus, a new theory on alternative therapy by using natural plants may be arrived at.

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