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PREVENTIVE EFFECT OF *Allium sativum* ON ALLOXAN INDUCED DIABETIC RAT

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ABSTRACT

This study was carried out to evaluate the possible protective effects of garlic extract on alloxan-induced diabetic rats. The rats were divided into four groups: group 1 serves as diabetic control; group 2 served as normal control; group 3 received garlic extract seven days before alloxan induction and 14 days after the induction while the last group received normal saline before induction and garlic extract after induction. The blood glucose levels of the rats were determined before the treatment and at regular interval till the end of the study. The result obtained showed that pre-administration of garlic extract before alloxan induction prevents the elevation of blood glucose in alloxan induced rats.

Keyword: diabetic rat, *Allium sativum*, alloxan, preventive, diabetes, pre-administration.

INTRODUCTION

The human population appears to be in the midst of an epidemic of diabetes and this disease is gradually becoming a real problem of public health in developing countries where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable in these poor areas. The greatest increase in prevalence is however expected to occur in Asia and Africa, where more patients will likely be found by 2030. Although there is a paucity of data on the prevalence of diabetes in Nigeria and other African countries, available data suggested that diabetes was emerging as a major health problem in Africa (Mbanya *et al*., 1996). The prevalence of diabetes in Nigeria was estimated to be between 1.4 to 2.7% of the population (Mbanya *et al*., 1996; Erasmus *et al*., 1988; Ngumah, 1995; Bakari *et al*., 1999) and over 90% of these are non-insulin dependent diabetes mellitus (Ohwworiole *et al*., 1999).

Despite major investigation into understanding the pathophysiology and treatment of diabetes mellitus, it has continued to be a major health problem worldwide (Osinubi *et al*., 2006). The possibility of its management by the oral administration of hypoglycaemic agents has stimulated great research interest over the years. Though different types of oral hypoglycaemic agents are available along with insulin for the management of diabetes mellitus, there is increased demand by patients for the use of herbal preparations with hypoglycaemic activity (Eisenberg *et al*., 1998). The growing public interest and awareness of herbal medicine have led the pharmaceutical industry and biomedical researchers to pay more attention to medicinal plants (Day, 1998). The current shift to the use of herbal preparations may therefore be due to presumed effectiveness, relatively low cost, presumed less side effects and low toxicity even though the biologically active constituents may be often unknown (Osinubi *et al*., 2006). Because the cost of administrating modern anti diabetic drugs is beyond the reach of most people in the low income group and those living in the rural areas, the use of plants for the treatment of common diseases such as diabetes are very common. Herbal medicine therefore can solve the economic problem of the poor. Investigators have consistently found that several plant products showed unique hypoglycaemic activities in diabetic animal model (Kusano and Abe, 2000) and Nigeria is blessed with many of these medicinal plants which have been used for the treatment of various diseases.

In humans, the preventive effect of garlic is not well documented. Most reports have shown a significant effect of garlic on blood glucose of normal healthy individuals but not in the prevention of diabetes in people with tendency to develop diabetes later in life. Thus the role of garlic in prevention of diabetes is yet to be confirmed (Habib *et al*., 2005).

The aim of this study was to investigate the efficacy of aqueous extract of raw garlic in preventing elevation of serum glucose levels in the alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant Material: The *Allium sativum* used for the experiment were purchased from Masaka market Nasarawa state, Nigeria. The plants were identified to species level at the Herbarium Unit, Department of Biology Bingham University, Karu, Nasarawa state, Nigeria.

Animal model

Adult white wistar rats (R. norvegicus) weighing 190 to 280g bred in the animal house of College of Medicine, Bingham University, Karu, Nasarawa state, were used for the study. They were fed ad labium with water and feed (Guinea feed). They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage for 21day in the animal house of College of Medicine, Bingham University Karu, Nasarawa state. All

animals were maintained under the standard laboratory condition for temperature (26± 20°C) and light (12 hours day length) and were allowed free access to feed and water.

Extract preparation

The garlic bulbs were peeled on crushed ice. Then 50 g of the peeled garlic was cut into small pieces and homogenized in 70 ml of cold, sterile 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 30 second bursts for a total of 10 minutes. The homogenized mixture was filtered 3 times through cheesecloth, the filtrate was centrifuged at 2000 RCF for 10 minutes and the clear supernatant was diluted to 100 ml with normal saline. The concentration of this garlic preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50 g/100 ml). The aqueous extract of garlic was stored in small aliquots at -20°C until use.

Induction of diabetes mellitus

Two grams of crystalline powdered alloxan monohydrate (sigma) was dissolved in 50 mls of normal saline to yield a concentration of 40mg/ml. 150 mg/kg body weight of alloxan per rat was administered intraperitoneally after overnight fast (access to only water) to rats in groups: 2, 3 and 4. The serum glucose levels were determined after 72 hours.

Experimental design

The study was carried out for three weeks. For baseline data, blood glucose levels were determined before the plant extract treatments of the animals (initials), blood was drawn from all the animals' tails and allowed to clot. Immediately, the clotted blood was centrifuged at 3500 RPM for 30minutes. The serum was separated and analysis for glucose concentration. The animals were then randomly divided into four groups (4).

Group 1: the normal control group was administered daily with 1ml saline through stomach intubation for the treatment period.

Group 2: the control diabetic group, was administered daily with 1ml saline through stomach intubation for seven days before a single intraperitoreal injection of alloxan (150mg/kg bwt of rat) was administered and subsequently daily with 1ml saline was administered daily through stomach intubation for the treatment period.

Group 3: the pre-and post alloxan-induced garlic-treated group was administered daily with 1ml garlic extract (500 mg/kg bwt) through stomach intubation for seven days before a single intraperitoreal injection of alloxan (150mg/kg bwt) was administered and subsequently daily with 1ml garlic extract (500 mg/kg bwt) was administered daily through stomach intubation for the treatment period.

Group 4: was administered daily with 1ml saline through direct stomach intubation for seven days before a single intraperitoreal injection of alloxan (150mg/kg bwt) was administered and subsequently 1ml garlic extract(500 mg/kg bwt) was administered daily through direct stomach intubation for the treatment period.

Body weight of all treated rats (2 groups), normal control and diabetic control groups were taken before and during the treatment by electronic balance. Blood glucose level of all treated rats (2 groups), normal control and diabetic control groups were taken. At the end of the experiment, the rats were sacrificed under sodium pentobarbitone anaesthesia. (Nafisa, 2007)

Assays

Blood glucose level determination:

The blood glucose in a protein free serum was determined as described by Sood (Sood, 1999).

Statistical analysis

The data are expressed as mean ± SD. Readings within a group were compared using the one-way ANOVA analysis and readings between groups were compared using the Independent sample test. Statistical analysis was performed using SPSS (Version 17). A level of $p < 0.05$ was considered to be significant.

RESULTS

Table-1. Effect of *Allium sativum* extract on serum glucose levels in diabetic rats.

Groups	Treatment	Day 0 (before induction)	Day 3 (72hours after induction)	Day 7	Day 14
1	The normal control (mg/dl)	73.3 ± 5.0^a	68.8 ± 6.8^a	63.0 ± 3.7^a	73.3 ± 5.4^a
2	Control diabetic group (mg/dl)	63.0 ± 3.7^a	268.0 ± 5.1^c	269.8 ± 7.3^c	287.0 ± 10.2^c
3	Pre-and post alloxan-induced garlic-treated group (mg/dl)	71.0 ± 2.4^a	$67.0\pm1.7.2^a$	70 ± 1.6^a	68.0 ± 2.2^a
4	Post alloxan-induced garlic-treated group (mg/dl)	66.0 ± 3.9^a	251.3 ± 10.3^c	176.3 ± 7.5^b	90.8 ± 6.5^a

Values given represent the Mean ±SD of 4 observations
Values with different superscript are significantly different at $p< 0.05$

Table-2. Percentage change in the body weight of the treated rats.

Group	Treatment	DAY 0 (g)	DAY 14 (g)	% Change in body weight
1	The normal control	265.28±5.25	266.36±5.17	0.41
2	Control diabetic group	226.06±12.13	216.80±12.23	- 4.10
3	Pre-and post alloxan-induced garlic-treated group	200.18±5.72	225.90±5.88	+12.84
4	Post alloxan-induced garlic-treated group	197.66±9.31	208.86±9.27	+ 5.66.

Values given represent the Mean± SD of 4 observations

DISCUSSIONS

Earlier studies on the hypoglycemic activity of garlic have showed variable results (Augusti and Sheela, 1996; Sheela and Augusti, 1992; Thomson *et al*., 2007). The results of this study showed that aqueous extract of garlic can prevent diabetes in the people with tendency to develop diabetes as shown in this study. It was observed that the rats that were pre-administered with garlic before induction with alloxan showed stable blood glucose level after 72 hours compare to the diabetic control and no significant difference between this group's blood glucose level and the control. This may be due to the ability of garlic to release bound insulin or increase insulin sensitivity (Thomson *et al*., 2007). The allicin of garlic is responsible for enhancing serum insulin activity due to its free SH group (Mathew and Augusti, 1973). On the other hand, antioxadiative property of S-allyl cystein sulfoxide (allicin) might be another reason of garlic beneficial effect on diabetes (Augusti and Sheela, 1996) as garlic can effectively normalize the oxidative stress in diabetic rats (Anwar and Meki, 2003; Augusti and Sheela, 1996; Banerjee and Maulik, 2002; El-Demerdash *et al*., 2005). Therefore, treatment with garlic extract which contain compounds such as S-allyl cysteine and organosulfor can gradually normalize oxidative stress and causes an increase in serum insulin levels in diabetic rats (Augusti and Sheela, 1996), preventing the effect of alloxan.

In this study, induction of diabetes, prevented increase in body weight in diabetic rats compared to the weight gain found in the control rats. Treatment of rats with aqueous garlic extract compensates for the reduction of body weight, and caused a significant increase in the body weight of treated rats (Baluchnejadmojarad *et al*., 2003; Grover *et al*., 2002). Although in some studies, treatment with garlic extract has been ineffective in increasing the body weight of diabetic rats, this difference in results may be due to the kind of extraction, the dose and how long it is used. In this study, the amount of dose consumed had been effective (500 mg/kg/day) it increased the body weight of the treated diabetic rats and showed significant increase in the body weight of rat pretreated with garlic extract before induction with alloxan, as some studies have shown that the active compounds of garlic such as S-allyl cysteine and organosulphurur can lead to a weight gain in alloxan induced diabetes in rats (Grover *et al*., 2002). The loss in weight of the diabetic rats has also been shown in some studies, in which it was observed that during diabetes mellitus, the blood sugar increases and results in lack of sugar in the cells; forcing, the cells to use amino acids and fatty acids as a source of energy which eventually leads to the reduction of proteins and fats in the body which causes body weight loss.

In conclusion, it was observed from this study that garlic was able to prevent the effect of alloxan thereby preventing the elevation of blood glucose.

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