Effects of Pre-induction Administration of Allium *Sativum* on Some Biochemical Parameters in Alloxan Induced Diabetic Rats

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Abstract: The aim of the present study was to investigate the effects of pre- and post- administration of garlic extract on serum glucose and some biochemical parameters in alloxan-induced diabetic rats to show the preventive and ameliorating effects in alloxan induced-diabetic rats. Rats were divided into 4 groups; normal control rats, diabetic control rats, diabetic rats post-treated with garlic extract and rats pre-treated with garlic extract before induction. Garlic extract was administered orally for 2 weeks to post-treated rats and 3 weeks to pre-treated rats and they were compared with the normal and diabetic groups, respectively. Serum glucose was reduced significantly in both post-treated and pre-treated groups. The post-treatment with garlic extract reduced serum cholesterol, but pre-treatment with garlic extract produced significant change compare to the diabetic control. The serum creatinine and urea levels were significantly reduced in post-treated group and pre-treated group compare to the diabetic control group. It is concluded that the consumption of garlic produced a significant hypoglycaemic and hypolipidemic effects in diabetic rats and also, garlic is capable of protecting the liver and the kidney functions in alloxan-induced diabetic rats as shown in the activities of serum enzymes and other biochemical parameters examined.

Keywords: Alloxan, diabetes, garlic, pre-induction, pre-treatment, serum enzymes

INTRODUCTION

Garlic is a food commonly used worldwide and its medical properties have been well recognized since the ancient times. Garlic is known for its antibacterial, hypolipidemic, hypoglycaemic, antifungal and as an antioxidant against free radicals. Hippocrates (470-358 BC) has recommended its use in treating infections (Rivlin, 2001). It is cultivated in Nigeria and used as meat tenderizer and spice in many delicacies (Morakinyo *et al.*, 2008). Raw garlic homogenate has been the major preparation of garlic subjected to intensive scientific study, because it is the most common method of garlic consumption. Raw garlic homogenate is essentially the same as the aqueous garlic extract which has been used in various scientific studies. When garlic is chopped or crushed, allinase, enzyme, present in garlic is activated and acts on alliin (present in whole garlic) to produce Allicin (allyl 2-propene thiosulphinate or Diallyl thiosulphinate) the principal bioactive compound present in aqueous extract or raw garlic homogenate (Fenwick and Hanley, 1985; Augusti and Mathew, 1975). Other important sulphur-containing compounds present in garlic homogenate are allyl methyl thiosulphonate, 1-propenyl allyl thiosulphonate and -L-glutamyl-S-alkyl-L-cysteine (Block, 1985).

Animal studies and some early investigational studies in humans, have suggested possible cardiovascular benefits of garlic (Sovova and Sova, 2004). It has been demonstrated that garlic supplementation reduce accumulation of cholesterol on the vascular walls of animals (Sovova and Sova, 2004). Another study had similar results, with garlic supplementation significantly reducing aortic plaque deposits of cholesterol-fed rabbits (Durak *et al.*, 2002). Also, Studies have shown that supplementation with garlic extract inhibit vascular calcification in human patients with high blood cholesterol (Durak *et al.*, 2004). Likewise, information on the hematological effects of *Allium sativum* have been widely documented such as reduced platelet aggregation

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(Rahman, 2007); hypolipidemia (Kojuri *et al*., 2007) and blood sugar levels (Chang and Johnson,1980).

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 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 activities against some disease conditions in diabetic animal model and Nigeria is blessed with many of these medicinal plants which have been used for the treatment of various diseases. The aim of this study was to investigate the efficacy of aqueous extract of raw garlic in preventing elevation of serum glucose levels; and on some vital biochemical parameters in the alloxan-induced diabetic rats.

MATERIALS AND METHODS

This research was carried out in the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria, between early July to August ending, 2011.

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 280 g bred in the animal house of College of Medicine, Bingham University, Karu, Nasarawa state, Nigeria, were used for the study. They were fed ad labitum with water and feed (Guinea feed). They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage for 21 days in the animal house of College of Medicine, Bingham University Karu, Nasarawa state, All animals were maintained under the standard laboratory condition for temperature ($26 \pm 20^{\circ}C$) and light (12 h 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 sec bursts for a total of 10 min. The homogenized mixture was filtered 3 times through cheesecloth, the filtrate was centrifuged at 2000 RCF for

10 min 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 -20ºC until use in the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria.

Induction of diabetes mellitus: Two grams of crystalline powdered alloxan monohydrate (sigma) was dissolved in 50 mL of normal saline to yield a concentration of 40 mg/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 h.

Experimental design: The study was carried out for six weeks in the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria, between July and August, 2011: The rats were allowed to acclimatize under standard photoperiodic condition in a clean rat cage for 21 days in the animal house of College of Medicine, Bingham University Karu, Nasarawa state (All animals were maintained under the standard laboratory condition for temperature ($26 \pm 20^{\circ}C$) and light (12 h day length) and were allowed free access to feed and water) and three weeks for the treatment.

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 30 min. The serum was separated and analyzed for glucose concentration. The animals were then randomly divided into four groups (4):

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

Group 2: The control diabetic group, was administered daily with 1 mL saline through stomach intubation for 7 days before a single intraperitoreal injection of alloxan (150 mg/kg bwt of rat) was administered and subsequently 1 mL 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 1 mL garlic extract (500 mg/kg body weight) (Arti *et al*., 2010; Gorinstein *et al*., 2006) through stomach intubation for seven days before a single intraperitoreal injection of alloxan (150 mg/kg bwt) was administered and subsequently daily with 1 mL garlic extract (500 mg/kg body weight) (Arti *et al*., 2010; Gorinstein *et al*., 2006) was administered daily through stomach intubation for the treatment period.

Group 4: Was administered daily with 1 mL saline through direct stomach intubation for 7 days before a single intraperitoreal injection of alloxan (150 mg/kg bwt) was administered and subsequently 1 mL garlic extract (500 mg/kg bwt) (Arti *et al.*, 2010; Gorinstein *et al.*, 2006) 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 *et al.*, 2007).

Assays: The blood glucose in a protein free serum was determined as described by Sood (1999). Urea, creatinine and total bilirubin concentrations were determined by the methods of Patton and Crouch (1977), Henry *et al.* (1974) and Pearlman and Lee (1974), respectively. Total cholesterol was measured by the procedure described by Allain *et al.* (1974). Serum Aspartate amino Transferase (AST) and Alanine amino Transferase (ALT) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel (1957). Protein content was determined by the method of Lowry *et al.* (1951). All the assays were carried out at the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria.

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 t-test. Statistical analysis was performed using SPSS (Version 17). A level of $p<0.05$ was considered to be significant results.

RESULTS AND DISCUSSION

From this study it was observed that the rats that were pre-admistered with garlic before induction with alloxan showed stable blood glucose level after 72 h 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 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 organosulphur 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 the present study, injection of alloxan caused an increase of serum lipid Profile, the marked hyperlipidemia that characterizes the diabetic state may therefore be as a result of the uninhibited actions of lipolytic hormones on the fat depots due to the absence of insulin (Goodman and Gilman, 1985). The post-treatment of the induced-diabetic rats with Allium sativum extract caused reduction in the

Table 1: Effect of allium sativum extract on serum urea,creatinine,bilirubin and cholesterol levels in diabetic rats

Groups	Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Bilirubin (mg/dL)	Cholesterol (mg/dL)
1	The normal control	26+2.98[b]	0.85+0.19[a]	1+0.38[b]	71.5+3.11[b]
2	Control diabetic group	78+6.68[a]	1.08+0.23[a]	2+0.14[a]	108.75+3.78[a]
3	Pre-and post alloxan-induced garlic-treated group	28+1.77[b]	0.56+0.30[b]	0.75+0.24[c]	81.5+2.65[b]
4	Post alloxan-induced garlic-treated group	37+4.58[c]	0.61+0.31[b]	0.92+0.42[b]	87.67+2.52[b]

Values given represent the Mean±SD of 4 observations; Values with different superscript in the same column are significantly different at p<0.05

Table 2: Effect of allium sativum extract on serum glucose levels in diabetic rats

Groups	Treatment	Day 0 (before induction)	Day 3 (72 h after induction)	Day 7	Day 14
1	The normal control	73.3±5.0[a]	68.8±6.8[c]	63.0±3.7[a]	73.3±5.4[a]
2	Control diabetic group	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	71.0±2.4[a]	67.0±1.7.2[a]	70±1.6[a]	68.0±2.2[a]
4	Post alloxan-induced garlic-treated group	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 3: Effect of allium sativum extract on serum ALT, AST and total protein levels in diabetic rats

Groups	Treatment	ALT	AST	Total protein (mg/dL)
1	The normal control	45+7.85[a]	42+4.50[a]	6.50+1.53[a]
2	Control diabetic group	55+7.67[b]	52+6.50[b]	8+56[a]
3	Pre-and post alloxan-induced garlic-treated group	40+5.56[a]	39+1.73[a]	2+1.44[b]
4	Post alloxan-induced garlic-treated group	50+4.61[b]	40+3.00[a]	5+1.59[a]

Values given represent the Mean±SD of 4 observations; Values with different superscript in the same column are significantly different at p<0.05

levels of serum cholesterol, On the other hand, the pre-treatment with Allium sativum extract followed by induction of diabetes stablises the plasma levels of cholesterol (Table 1). In line with the present data, other workers have reported that administration of fresh garlic extracts was shown to improved lipid profile including reduction of serum cholesterol levels (Knipschild and Ter-Riet, 1989). With respect to the cholesterol lowering property of garlic, it has been suggested that some constituents of garlic may act as inhibitors for some enzymes such as hydroxy methyl glutaryl CoA reductase, which participates in cholesterol synthesis (Gebhardt and Beck, 1996). The serum cholesterol levels of the diabetic rats were significantly higher than the normal levels. After treatment, the serum cholesterol levels of garlic-treated diabetic rats were significantly lower in comparison with the control Diabetic rats. It was concluded that the hypocholesterolaemic effect of garlic could have possibly resulted from the inhibition of cellular cholesterol biosynthesis after the consumption of the extract (Fuhrman *et al.*, 2000). Furthermore, Neess *et al.* (1996) reported that the reduction of cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, which in turn leads to enhanced removal of LDL from plasma, resulting in reduced serum cholesterol concentration.

The effect of garlic extract on the kidney functions was assessed by the determination of the levels of serum creatinine and urea and the study revealed that post-administration of garlic extract to the diabetic rats reduced and normalized the levels of serum creatinine and urea. On the other hand, the pre-treatment with garlic before the induction of diabetes inhibited the higher increase of plasma creatinine and urea resulting from the induced-hyperglycemia by alloxan. Thus the results showed significant ($p<0.05$) increase in the level of serum urea and creatinine in the diabetic groups compare to the control level. These results indicated that diabetes could lead to renal dysfunction. While, pre- treatment of alloxan induced diabetic rats with garlic extract, significantly reduced urea ($p<0.05$) in serum compared to the mean value of diabetic group (Table 1). Similarly, the elevation of creatinine level caused by diabetes was declined after administration of garlic ($p<0.05$), compared with the diabetic group (Table 2). These results are in agreement with other previous studies on garlic (El-Demerdash *et al.*, 2005); onion (Babu and Srinivasan, 1999) and root extract of panax ginseng (Badr El-Din, 1997). In addition, Ajith *et al.* (2007) reported that the presence of polyphenols and flavonoids in plant extract might be responsible for the antioxidant/nephroprotective activities and the reduction of serum urea and creatinine levels.

From the data obtained, it can be concluded that pre-treatment with garlic extract produced a significant improvent of the impaired kidney functions in alloxan induced-diabetic rats.

The decrease in total protein concentrations in the serum of diabetic rats may be ascribed to:

- A decreased amino acid uptake (Garber, 1980)
- A greatly decreased concentration of a variety of essential amino acids (Brosnan and Man, 1984)
- An increased conversion rate of glycogenic aminoacid to CO_2 and H_2O (Mortimore and Manton, 1970)

Administration of garlic maintained the protein level near normal.

The increase in the activities of serum AST and ALT (Table 3) indicated that diabetes may induce hepatic dysfunction. In support of this finding, Larcan *et al.* (1979) had observed that liver was necrotized in diabetic patients. Therefore, the increment of the activities of AS T and ALT, in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al.*, 1993), which gives an indication on the hepatotoxic effect of alloxan. On the other hand, treatment of the diabetic rats with garlic caused reduction in the activity of these enzymes in the serum (Table 3) compared to the mean values of diabetic group. These results are in agreement with those obtained by Ohaeri (2001) and EL-Demerdash *et al.* (2005) in rats. A possible explanation for the differential effects of garlic on the activities of AST and ALT in serum is that these treatments may inhibit the liver damage induced by alloxan. These data agree with those reported by El-Shater *et al.* (1997) and Augusti *et al.*(2001), who found that the enzyme activities (AST, ALT and ALP) in rat serum decreased significantly when they were fed a diet containing 5% *Allium sativum*

Furthermore, the improvement of the liver damage by oral administration of garlic could be confirmed by studying their effects on the level of serum bilirubin. The results in Table 1 showed that the experimentally induced diabetes increased ($p<0.05$) the level of plasma bilirubin compare to the control. Also, garlic intake produced significant ($p<0.05$) decrease in plasma bilirubin of alloxan-diabetic rats compared to the diabetic rats. Rana *et al.* (1996) reported that the increase in serum bilirubin (hyper-bilirubenimia) may resulted from the decrease in liver uptake, conjugation or increase bilirubin production from haemolysis, which has been shown to result in decrease in total erythrocyte counts (El-Demerdash *et al.*, 2005). Also, the elevation in serum bilirubin indicates liver damage as confirmed by the changes in the activities of liver enzymes (AST and ALT) (Table 3).

As shown in Table 4, Induction of diabetes, prevented increase in body weight in diabetic rats compared to the weight gain found in the control rats.

Table 4: Percentage change in the body weight of the treated rats

Group		Treatment	Day 0	Day 14 % change in body weight
1	Normal control	265.28±5.25	266.36±5.17	0.41
2	Diabetic control 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

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, (Table 4),as some studies have shown that the active compounds of garlic such as S-allyl cysteine and organosulphur 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.

CONCLUSION

It is concluded that the consumption of garlic produced a significant hypoglycaemic and hyperlipidemia effects in diabetic rats. In addition, garlic is capable of protecting the liver and the kidney functions in alloxan-induced diabetic rats as shown in the activities of serum enzymes and other biochemical parameters examined.

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