Attenuation of Oxidative Stress, Inflammation and Insulin Resistance by *Allium Sativum* in Fructose–Fed Male Rats

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**ABSTRACT**

Background: Fructose is widely used as a food ingredient and has potential to increase oxidative stress. Moreover, the beneficial health effects of medicinal plants are frequently attributed to their potent antioxidant effects.

Objectives: The present study was aimed to explore the effects of garlic (*Allium sativum*) extract on insulin resistance, inflammation and oxidative stress in male wistar rats fed with high fructose diet.

Material and Methods: Diabetes was induced in male albino Wistar rats by feeding 60% fructose rich diet. The fasting plasma glucose, insulin, insulin resistance, tumour necrosis factor alpha, total antioxidant status and the whole blood reduced glutathione, erythrocyte antioxidant enzyme activities were measured. Differences between the groups were assessed by using One Way Analysis of Variance with (ANOVA) Tukey post–hoc test.

Results: The diabetic rats showed a significant increase in plasma fasting glucose, insulin, insulin resistance, tumour necrosis factor alpha and malondialdehyde level and decreased levels of total antioxidant status, reduced glutathione, catalase and glutathione peroxidase. Treatment with garlic extract restored all these biochemical changes.

Conclusion: The garlic extract is effective in improving the high fructose induced oxidative stress, inflammation and insulin resistance in male wistar rats.

Key words: High fructose diet, *Allium Sativum*, Insulin resistance, Inflammatory Response, Oxidative stress

**INTRODUCTION**

The metabolic syndrome characterized by insulin resistance, dyslipidemia, and hypertension is associated with increased risk of type 2 diabetes and cardiovascular disease, resulting in reduced quality of life and increased risk of mortality and morbidity. The prevalence of metabolic syndrome has dramatically increased worldwide due to a modern lifestyle and an increase in consumption of refined sugar particularly fructose [1]. It was demonstrated that in rats fed with high-fructose diet, the concentration of free radicals was three times higher than in the control group. Moreover, fructose addition brought about lowering of antioxidant levels. The human consumption of fructose has been increasing over the years. Epidemiological studies revealed direct association between fructose intake and coronary heart diseases [2].

Garlic (*Allium sativum*; Liliaceae) is one of important ingredients of Indian traditional medicine. Garlic has medicinal property and has been used in the treatment of several diseases for centuries. Pharmacologically, garlic has been suggested to be hypolipidemic [3], anticoagulant and anticancer [4]. Several other reports also suggest effectiveness of garlic in exerting antitoxic, anticlastogenic effects by modulating oxidative stress [5].

However, little is known about the potential effects of garlic on body weight regulation or blood glucose lowering in vivo animal models of diabetes. Furthermore, the beneficial effects of garlic on insulin resistance and antioxidant status remain unclear. Hence, the present study was conducted in order to find the therapeutic potential of garlic as oral hypoglycaemic and antioxidant agent. The effects produced by these treatments are compared with the standard drug, metformin.

**MATERIAL AND METHODS**

Chemicals  
Thiobarbituric acid and reduced glutathione (GSH) were procured from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

Preparation of garlic extract  
Fresh garlic (*Allium Sativum L.*) was purchased from local market. The 100g of garlic bulbs suspended in distilled water was boiled for 20 minutes at 100°C. The cooled water was removed by filtration and lyophilized to obtain a powder form. The prepared extract was stored at 4°C.

Animals  
Male albino Wistar rats in the age of 5 months, weighs between 200 to 250 g were housed in polypropylene cages with stainless steel grill. The animals were maintained at standard conditions with a 12-/12-hour light/dark cycle. The animal procedures were approved by the institutional animal ethics committee (Jip/Micro/Jiaec/2012 date 29.3.2012).

Experimental design  
A total of 30 rats were selected for the study and randomized equally (6 rats) to all the study groups. The sample size was determined by convenience subjected to the Institute Animal Ethic Committee approval.

Group 1: control rats, received standard rat chow.

Group 2: control + garlic extract (25mg/kg body weight/day/rat) in aqueous solution orally for 60 days.

Group 3: High Fructose Diet (HFD) rats were given 60% fructose mixed with standard rat chow.

Group 4: HFD + garlic extract (25 mg/kg body weight/day/rat) in aqueous solution orally for 60 days.

Group 5: HFD + metformin (50mg/kg body weight/day/rat) in aqueous solution orally for 60 days [6].

**Body weight gain**  
Changes in body weight of rats in all groups were noted throughout the study.

**Body composition**  
The changes in body composition of the animals were assessed in terms of fat mass (FM) and lean mass (LM) through the dual energy X-ray absorptiometry technique which was used for the assessment of body composition.

**Blood glucose**  
The blood glucose levels were recorded using glucose oxidase method.

**Insulin resistance**  
Insulin resistance index (IRI) was calculated by the formula:

\[
\text{IRI} = \left( \frac{\text{fasting plasma glucose (mg/dL)}}{\text{fasting plasma insulin (mU/mL)}} \right)^2
\]

**Insulin**  
The plasma insulin levels were measured by ELISA.

**HOMA**  
Homeostasis model assessment (HOMA) is an index of insulin resistance and is calculated by the formula:

\[
\text{HOMA} = \frac{\text{fasting plasma glucose (mg/dL)} \times \text{fasting plasma insulin (mU/mL)}}{405}
\]

**HbA1C**  
HbA1C levels were determined using an automated method.

**Triglyceride**  
Triglyceride levels were measured using enzymatic method.

**Cholesterol**  
Cholesterol levels were measured using enzymatic method.

**Urea**  
Urea levels were measured using enzymatic method.

**Creatinine**  
Creatinine levels were measured using enzymatic method.

**Lipid profile**  
Serum total cholesterol and triglyceride elevated concomitantly to insulin resistance.

**Liver injury**  
Liver injury index (LII) was calculated by the formula:

\[
\text{LII} = \left( \frac{\text{ALT}}{\text{AST}} \right) \times 100
\]

**Kidney injury**  
Kidney injury index (KII) was calculated by the formula:

\[
\text{KII} = \frac{\text{serum creatinine (mg/dL)}}{\text{serum urea (mg/dL)}}
\]

**Histopathological analysis**  
Renal and liver sections were examined for histopathological changes.

**Confocal microscopy**  
Renal and liver sections were evaluated for the expression of NF-kappa B and AP-1 using confocal microscopy.

**Biochemistry**  
Blood and tissue samples were collected and used for biochemical analysis. The reduced glutathione levels were determined using enzymatic method.

**Oxidative markers**  
The levels of oxidative stress markers such as thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) were determined using enzymatic method.

**Total antioxidant status**  
The total antioxidant status (TAS) was determined using enzymatic method.

**Erythrocyte antioxidant activity**  
The erythrocyte antioxidant enzyme activities were measured.

**Histopathological analysis**  
Histopathological analysis was performed to evaluate the extent of liver and kidney damage.

**Confocal microscopy**  
Renal and liver sections were evaluated for the expression of NF-kappa B and AP-1 using confocal microscopy.

**Statistical analysis**  
Data were analyzed using one-way ANOVA followed by Tukey’s post-hoc test. The results were considered significant at *p* < 0.05.
the experimental period. The weight of each rat was recorded on day one of the study and at weekly intervals throughout the study.

**Intraperitoneal glucose tolerance test (IPGTT)**

At the end of the experimental period, IPGTT was performed in all groups of rats. The 12 hour fasted animals were challenged with a glucose at a dose of 2 g / kg body weight intraperitoneally. Blood samples were collected at 0 min (before glucose administration), 30, 60 and 120 min after glucose administration [7].

**Biochemical analyses**

At the end of the experiment, the plasma glucose was estimated using the glucose oxidase-peroxidase method in a fully automated clinical chemistry analyzer (Olympus AU-400) and the remaining samples were stored at -80°C for estimation of TNF-alpha (Diacclone, France) and insulin (Crystal chem, inc, USA) by ELISA method according to the manufacturer’s instructions. The Homeostasis Model Insulin Resistance (HOMA-IR) was calculated using the formula (fasting glucose (mmol/L) X fasting insulin (µIU/mL))/22.5. The plasma malondialdehyde were estimated by TBARS method [8]. The whole blood reduced glutathione (GSH) was estimated by cyanmethemoglobin method with Drabkin reagent and catalase assay the method of Aebi. The Total Antioxidant Status (TAS) was measured by FRAP method [11-13].

**STATISTICAL ANALYSIS**

All values were expressed as mean ± S.D. Differences between the groups were assessed using one way analysis of variance (ANOVA) with Tukey post hoc test. All statistical analysis was carried out using SPSS version 19.0.

**RESULTS**

[Table/Fig-1] illustrates the effect of body weight on the control and experimental groups of rats. The HFD induced diabetic rats showed a significant (p < 0.05) increase in the body weight. A significant (p < 0.05) decline in the body weight was observed on oral administration of garlic as well as metformin.

[Table/Fig-2] shows that, the effect of glucose tolerance test in male wistar rats. High fructose feeding showed impaired glucose tolerance and an increase in AUC. The analysis of the IPGTT at the end of the feeding period and the comparison between area under the curve (AUC) between control and experimental groups showed that fructose-fed rats developed glucose intolerance [Table/Fig-3]. Treatment with garlic as well as metformin significantly improved the glucose intolerance caused by high fructose.

[Table/Fig-4] depicts the effect of garlic on the plasma glucose, insulin and TNF-alpha in control and experimental groups of rats. The plasma glucose, insulin, IR and TNF-alpha levels in HFD-induced diabetic group of rats were significantly (p < 0.05) increased, when compared with control group of rats. The oral treatment with garlic as well as metformin significantly (p < 0.05) decreased the glucose, insulin and TNF-alpha when compared with HFD group of rats.

[Table/Fig-5] indicates the effect of garlic in the malondialdehyde level and antioxidants enzymes in the control and experimental groups of rats. In high fructose rats, there was a significant elevation in the level of malondialdehyde and a concomitant decrease in the levels of catalase, GPx, GSH and TAS when compared with control rats. Oral treatment with garlic brought back them to near normal levels as was the case with metformin treatment. There was no statistical significant difference in these parameters between garlic treated and the control rats. This illustrates the complete normalization of oxidant status with garlic treatment.

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**Table/Fig-1**: Illustrates the effect of body weight of the control and experimental groups of rats. (Significantly different with control, bSignificantly different with garlic group, cSignificantly different with HFD+metformin at 5% level of significant (p<0.05)

**Table/Fig-2**: Effect of garlic administration on intraperitoneal glucose tolerance test. (Significantly different with control, bSignificantly different with garlic group, cSignificantly different with HFD+metformin at 5% level of significant (p<0.05)

**Table/Fig-3**: Incremental area under the curve (AUC) of IPGTT. (Area under the curve (AUC) of different groups was calculated using NCCS software. Significantly different with control, bSignificantly different with garlic group, cSignificantly different with HFD+metformin at 5% level of significant (p<0.05))

**Table/Fig-4**: Comparison of the levels of plasma glucose, insulin, HOMA-IR and TNF-α between the groups. (aSignificantly different with control, bSignificantly different with garlic group, cSignificantly different with HFD+metformin at 5% level of significant (p<0.05).

**Table/Fig-5**: Antioxidants effects of garlic in control and experimental groups of rats. (aSignificantly different with control, bSignificantly different with garlic group, cSignificantly different with HFD+metformin at 5% level of significant (p<0.05))
DISCUSSION

Daily fructose is a monosaccharide which can induce metabolic syndrome which is of pathophysiologic importance for the development of diabetes and atherosclerosis. There are many reports in the literature describing an increase in body weight, hyperglycemia, and insulin resistance with the consumption of high fructose diets in both humans and animal models [14-15]. Our results are consistent with earlier studies which found that the consumption of high fructose diets markedly induce hyperglycemia associated with hyperinsulinemia.

The significant increase in AUCs of glucose level after glucose loading during OGTT are seen in rats fed with high fructose diet, indicating that the ability of insulin to stimulate glucose disposal is markedly impaired in tissues by fructose feeding. As per the emerging evidence that prolonged consumption of high fructose diets contributes to excessive formation of Reactive Oxygen Species (ROS), this leads to oxidative stress and insulin resistance [16]. Moreover, an increase in cellular ROS directly triggers the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase and nuclear factor-kappa B that in turn phosphorylate multiple targets including the insulin receptor and the Insulin Receptor Substrate (IRS) [17]. Increased serine phosphorylation of IRS directly decreases its ability to undergo tyrosine phosphorylation and accelerates the degradation of IRS-1, causing impaired glucose uptake in tissues [18]. Our findings on the significantly salutary effect of garlic on glucose tolerance test are particularly promising and requires further elucidation. The blood glucose level regulated by garlic compounds (S-methylcysteine, S-allylcysteine and S-allylmercaptocysteine), may possibly contributed to this effect [19]. Reduction of body weight gain could also be responsible for improving insulin sensitivity in fructose fed rats. High-fructose diet contributes to excessive formation of reactive oxygen species. This leads to oxidative stress and its associated complications like chronic inflammation, characterized by abnormal cytokine production (TNF-α) and the activation of a cascade of inflammatory signaling pathways [20]. TNF-α has been shown to enhance adipocyte lipolysis, which further increases free fatty acids and also elicits its own direct negative effects on the insulin signaling pathway by altering the tyrosine/serine phosphorylation of Insulin Receptor Substrate (IRS). Therefore, it is presumed that the reduction of pro-inflammatory mediators by garlic treatment might have facilitated/improved IRS-phosphorylation including metabolic syndrome. Such improved cellular events be able to reverse the inhibitory effect on insulin signalling pathways, mainly the enzymes of fatty acids, glucose uptake process and depletion of free fatty acids synthesis.

Increasing evidence in both experimental and human studies indicates that oxidative stress plays a major role in the pathogenesis of Type 2 diabetes. Free radicals are generated in diabetes by glucose oxidation. High levels of free radicals and the simultaneous decline of exogenous and endogenous antioxidants can lead to damage of cellular organelles and development of insulin resistance and decreased antioxidant enzymes [21]. In the present study, high fructose feeding increased oxidative stress. The significant restoration of redox status by treatment of garlic indicates its antioxidant property of garlic and may be administered as an adjuvant in the treatment of diabetes.

CONCLUSION

In conclusion, the supplementation of garlic to rats fed a high fructose diet prevents the development of oxidative stress and its associated complications include hyperglycemia and hyperinsulinemia. Further human studies are essential to explore the molecular mechanism of garlic in controlling the insulin resistance and its metabolic complications.

REFERENCES