

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 antigenotoxic, 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 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)

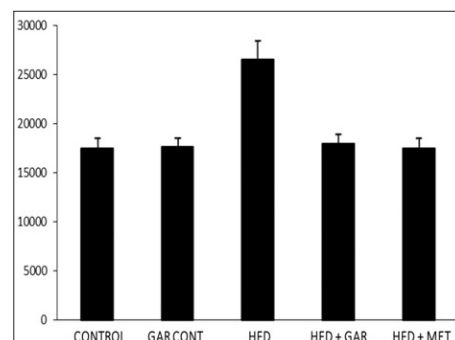
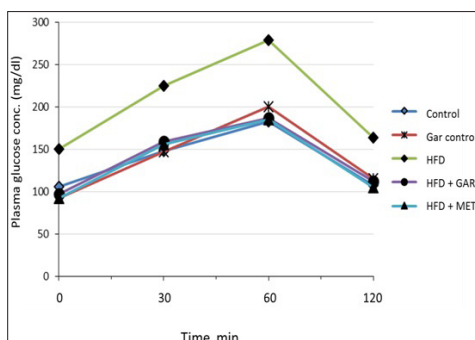
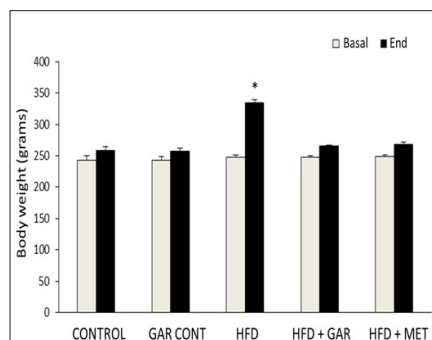
At the end of the experimental period, IPGTT was performed in all groups of rats. The 12 hour fasted animals were challenged with a glucose at a dose of 2 g / kg body weight intraperitoneally. Blood samples were collected at 0 min (before glucose administration), 30, 60 and 120 min after glucose administration [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 (Diaclone, 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 [8,9]. The whole blood reduced glutathione (GSH) was measured using Ellman's reagent (5,5'-Dithio-bis-2-nitrobenzoic acid,) as described by Beutle et al., [10]. Haemoglobin content of blood 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 with (ANOVA) with Tukey post hoc test. All statistical analysis was carried out using SPSS version 19.0.



[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))

Groups	Fasting plasma Glucose (mmol/L)	Plasma insulin (μIU/ml)	HOMA-IR	TNF-α (pg/ml)	Groups	MDA(μM/L)	CAT Katal /ml	GPx(mg/gHb)	GSH mg/g Hb	TAOS (μM/L)
Control	6.0 ± 0.51	31.25 ± 13.86	8.32 ± 3.86	6.52 ± 3.28	Control	2.62 ± 0.41	44.04 ± 16.9	43.3 ± 7.2	6.74 ± 1.4	1066.7 ± 82.41
Control+garlic	5.2 ± 0.41	22.59 ± 9.61	5.16 ± 1.85	5.49 ± 2.50	Control+garlic	2.75 ± 0.24	49.12 ± 19.6	53.15 ± 13.1	9.87 ± 1.4	1149.7 ± 69.97
HFD	8.2 ± 0.80 ^{b,c}	105.90 ± 20.80 ^{a,b,c}	38.7 ± 5.76 ^{a,b,c}	102.73 ± 28.55 ^{a,b,c}	HFD	4.23 ± 0.87 ^{a,b,c}	30.99 ± 4.64 ^{a,b,c}	29.89 ± 5.0 ^{a,b,c}	3.59 ± 0.6 ^{a,b,c}	272.4 ± 22.08 ^{a,b,c}
HFD+garlic	5.4 ± 0.44	60.97 ± 26.32	14.5 ± 6.41	8.60 ± 3.99	HFD+garlic	2.60 ± 0.30	51.18 ± 12.7	50.14 ± 2.2	6.69 ± 3.4	697.8 ± 54.19
HFD+metformin	5.2 ± 0.81	56.38 ± 12.04	12.9 ± 3.27	4.47 ± 0.99	HFD+metformin	2.53 ± 0.39	57.54 ± 16.5	52.13 ± 6.0	7.40 ± 1.2	687.2 ± 51.9
p Value	p<0.05	p<0.05	p<0.05	p<0.05	p Value	p<0.05	p>0.05	p<0.05	p<0.05	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

Dietary 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 insulinemia 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.

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