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ORIGINAL ARTICLE

Garlic Attenuates Abnormal Glucose, Insulin Levels and Homa-IR in High Fructose Fed WNIN Rats

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ABSTRACT
The main aim of this study is to investigate the effect of garlic on long term High fructose induced plasma glucose and insulin alterations and HOMA-IR in WNIN rats. Two months old male Wistar NIN (WNIN) rats were maintained either on AIN-93M diet alone (control group) or high fructose (56%) or high fructose (56%) with freeze-dried garlic powder (3%) contains AIN- 93 diet (HF+G group). High fructose (56%) fed WNIN rats shown elevated plasma glucose, insulin levels and increased HOMA-IR at 3 months and end of the experimental period (10 months). These results indicated that insulin resistance associated pre-diabetes at 3 months, maintained pre-diabetes over a period of 10 months. Garlic significantly reduced the HOMA-IR and marginally reduced GAUC, IAUC, GAUC/IAUC after 3 months feeding, but after 10 months of experimental period, garlic had shown marginal improvement in HOMA-IR, GAUC, IAUC and GAUC/IAUC due to its insulin insulin-sensitizing properties.
Keywords- Pre-diabetes, HOMA-IR, bitter gourd, lipid profile and STZ

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INTRODUCTION

Varying the type of carbohydrate in the diet of diabetics can influence blood glucose control and insulin action [16, 17, 14, 1, 2, 7]. For example, rats consuming a high-sucrose diet (69% of calories) have impaired in vivo insulin action both in the liver and peripheral tissues compared with high-starch fed animals [17]. There are a number of reasons why these impairments might occur. First, different sugars are produced when starch and sucrose are digested. Starch is digested to glucose whereas sucrose enters the portal vasculature as fructose and glucose moieties. It may be the fructose component of sucrose that is deleterious. There is certainly in vitro evidence consistent with this notion [18, 19]. Further, fructose feeding and to a lesser extent glucose feeding were shown to produce elevations in plasma triglyceride [20, 21, 16, 6], insulin [14, 16, 21], and sometimes blood glucose levels [20, 6]. Elevated triglyceride levels were associated in a number of circumstances with impaired insulin action [20, 6, 16]. Second, acute feeding trials in humans showed that the rate of digestion of sucrose and (raw) starch differ [14, 7]. Sucrose is digested rapidly whereas raw starch is hydrolyzed 50% more slowly than sucrose. Differing postprandial glycemic curves (glycemic indices) with corresponding differences in stimulation of insulin secretion may then affect insulin action.

Finally, rats with free access to high-glucose diets were shown to have a reduced rate of brown adipose tissue thermogenesis compared with fructose-fed rats [5]. Because there is a close, perhaps causal, link between insulin action and postpranclial energy expenditure [3], these results may implicate glucose rather than fructose in the etiology of sucrose-induced insulin resistance. Therefore, the aim of the present study was to examine the above outlined possibilities in relation to the deleterious effects of fructose feeding on glucose and insulin action and beneficial effects of ginger on abnormal glucose and insulin levels.

MATERIAL AND METHODS

Materials

Fructose, glucose was procured from SRL company, whereas cellulose, vitamin and mineral mixture were obtained from MP Biomedicals. Other dietary ingredients were obtained from the National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition, Hyderabad. Acrylamide, bis-acrylamide, ammonium per sulphate, from Sigma Aldrich. Other reagents were of analytical grade as described earlier in chapter 2 section 2.3.1.

Preparation of Garlic Powder

Fresh garlic was purchased from the neighbourhood market and freeze-dried. The freeze-dried garlic powder was supplemented with the AIN-93 diet containing 56% fructose and used for this experiment.

Experimental Design and Dietary Regimen

Male WNIN rats (two months old), with an average body weight of 195 ± 4 g were obtained from the National Center for Laboratory Animal Sciences, National Institute of Nutrition, (Hyderabad, India) and maintained on an AIN-93 diet alone (control group, n = 9) or an AIN-93 diet supplemented with 56% fructose (HF group, n = 9) and an AIN-93 diet supplemented with 56% fructose and 3% freeze-dried garlic powder (HF+G group, n = 9). Rats were individually maintained in cages with their respective diets for 10 months (Fig 4.1). All animals were housed under controlled conditions (temperature was kept between 18°C and 22°C) with a 12:12 h light-dark cycle with free access to water and food. Oral glucose tolerance test (OGTT) was performed after three months to confirm development of pre-diabetes. OGTT was again conducted before termination of the experiment.

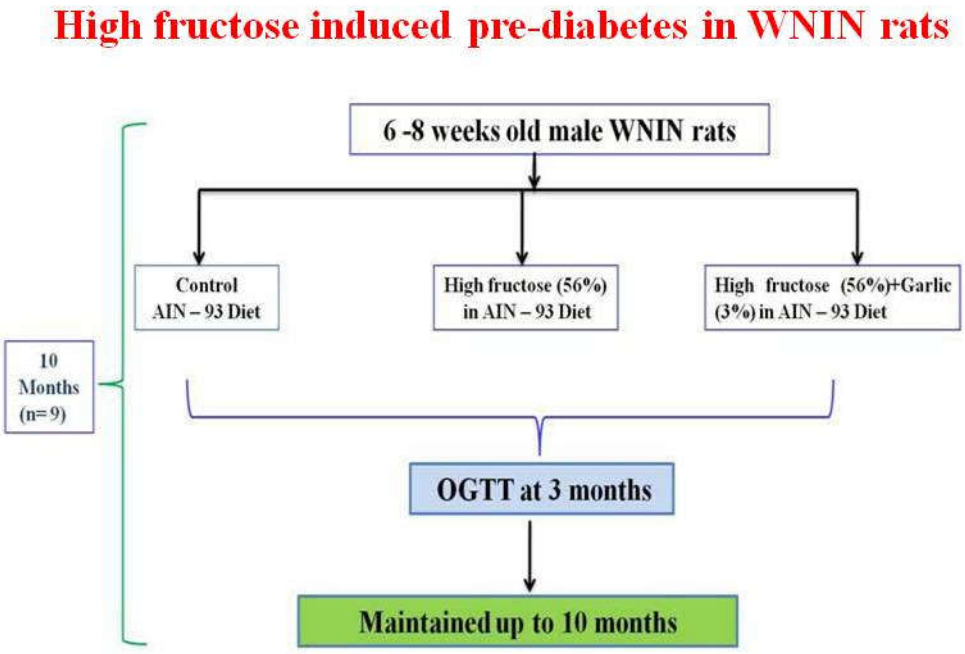

Figure 1: Experiment-3 design. OGTT, oral glucose tolerance test

Fasting and Postprandial Blood Glucose

Fasting and postprandial blood glucose levels in the experimental animals were monitored during the experimental period by using a glucometer (One Touch Horizon).

Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) was conducted at three and ten months after the STZ injection in overnight fasted rats by administering glucose orally as a bolus at a dose of 2.0 g kg^{-1} body weight. Blood samples were collected at 0, 30, 60 and 120-minute intervals to estimate plasma insulin and glucose concentrations to assess IFG, IGT and insulin resistance. Heparin tubes were used for collecting the blood samples and these samples were centrifuged at 4000rpm/15min at 4^0C and plasma was collected. Glucose, insulin, lipids and nephropathy parameters were estimated in these plasma samples as described below. During blood collection, care was taken not to damage the eyeball by passing the capillary from side to side.

Estimation of Plasma Glucose

Plasma glucose was estimated using the glucose oxidase (GOD)-peroxidase (POD) method with a kit obtained from Biosystems (Barcelona, Spain) according to the manufacturer's instructions.

Principle: The GOD-POD method involves oxidation of the GOD enzyme to D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of the POD enzyme oxidizes phenol, which reacts with 4-amino-antipyrene to produce a red-coloured quinonimine dye. The intensity of the colour produced is proportional to the concentration of glucose in the sample.

Method: Working enzyme reagent (1.0 mL) was added to a 0.01 mL of standard/sample, mixed gently and then absorbance was measured at 500 nm against a blank within 30 min.

Sample glucose concentrations were calculated by comparing optical density (OD) of the sample to those of the standard ODs and are represented as mg/dL.

Estimation of Plasma Insulin

Insulin was estimated in plasma samples using a radioimmunoassay (RIA) kit purchased from the Board of Radiation and Isotope Technology (BRIT), Bhabha Atomic Research Centre (BARC) Mumbai, India.

Principle: The radioimmunoassay method is based upon the competition between unlabelled insulin in a standard or sample and radio iodinated (^{125}I) insulin for limited binding sites on a specific antibody. After incubation, bound antibodies and free insulin are separated with a second antibody using a polyethylene glycol (PEG)-aided separation method. The concentration of insulin in the samples is quantified by measuring the radioactivity associated with the bound fraction of sample and standards.

Procedure: Assay buffer (0.3 mL) was added to the individual plasma samples (100 µL). For standards, 100 µL of different insulin concentrations (0, 12.5, 25, 50, 100 and 200 µU/mL) was added to insulin-free serum (100 µL, provided in the kit); assay buffer was then added for a final volume of 0.4 mL. To both samples and standards, 0.1 mL insulin antiserum was added, mixed gently and incubated overnight at 4°C. The next day, 0.1 mL ^{125}I insulin was added to all tubes and incubated for 3 h at room temperature. After incubation, 0.1 mL of second antibody was added, followed by 1.0 mL PEG, and gently mixed. All tubes were kept at room temperature for 20 min followed by centrifugation at 1500 × g for 20 min. The supernatant was carefully decanted without disturbing the pellet. Radioactivity was counted in the precipitate using a gamma counter. Percent binding of radiolabelled insulin antibody was calculated.

Calculations: Percent binding was calculated by setting the binding values measured for the blank samples as 100%. Standard log-logit curves were plotted for percent binding of standards versus concentration of standards. Sample concentrations were calculated from the standard curve and are presented as µU/mL insulin.

Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) Calculation

Insulin resistance was assessed by HOMA-IR as previously described for rats [14] using the following equation: HOMA-IR = [fasting plasma glucose (mg/dL) × fasting plasma insulin (µU/mL)/2,430]. To assess the insulin response of these rats after an OGTT, the area under the curve (AUC) for insulin and glucose was calculated.

Statistical analysis

All statistical analyses had been performed by using SPSS 19.0 and quantitative data had been presented as mean ± standard deviation (SD). One-way ANOVA, followed by Tukey HSD and Student's t-test was used to analyze the differences among means. $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Effect of Garlic on Fasting and Postprandial Blood Glucose

Fasting and postprandial blood glucose levels were measured at 3rd, 6th and 10th month of experimental period. The untreated HF group rats showed marginally increased postprandial blood glucose levels at 3rd and 10th month when compared to control group rats indicating development of glucose dysregulation due to high fructose feeding. However, fasting blood glucose levels were similar among all the group rats. Feeding of garlic to HF group (HF+G) rats showed marginal decrease in postprandial blood glucose levels at 6th and 10th month when compared to HF alone fed group rats indicating its hypoglycemic property (Figure 2). These results were well correlated with recent study on type-2 diabetic subjects [9].

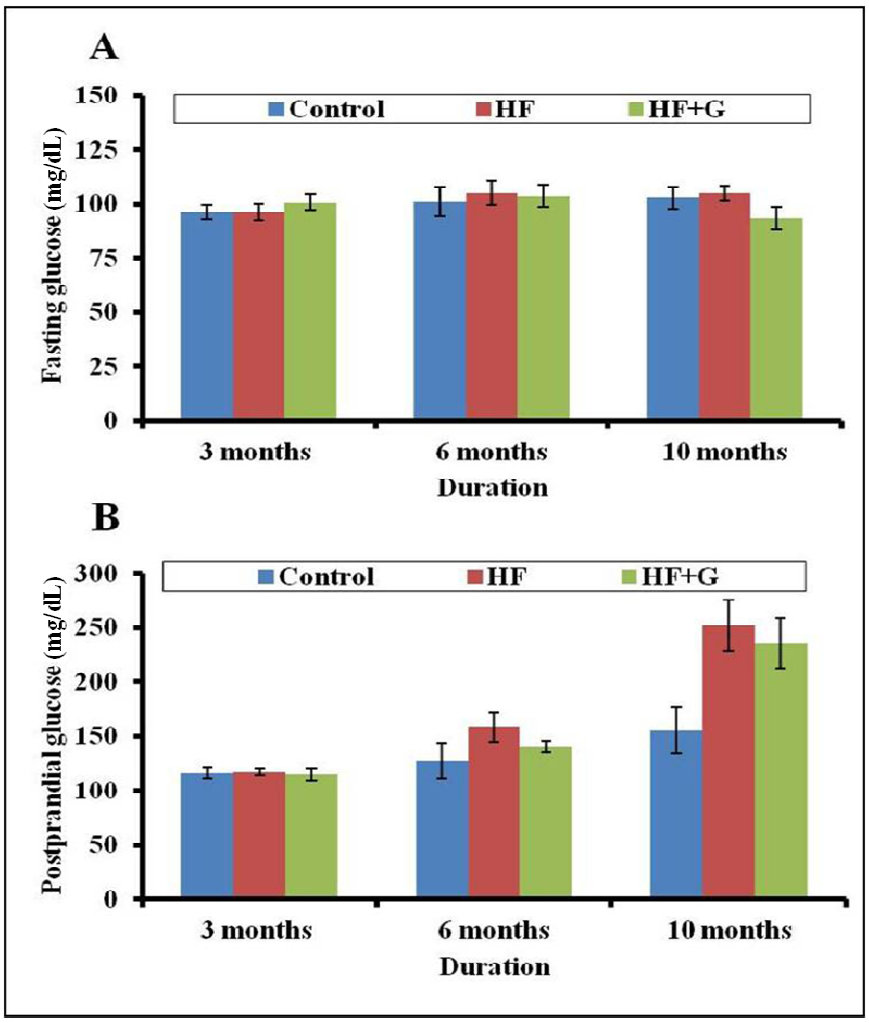

Figure 2: Fasting (A) and postprandial (B) blood glucose levels. Values are mean ± SE, n=9 animals per group.

Effect of Garlic on Plasma Glucose and Insulin Response During OGTT

OGTT results obtained at 3 months (Fig 3) showed a significant higher plasma glucose at 60, 120 min and significant increased plasma insulin at 0 and 120 min in HF group when compared to that of control indicating development of IGT and hyperinsulinemia/pre-diabetes. Feeding of garlic to HF rats animals (HF+G group) has shown marginal reduction in the plasma glucose levels at all-time points except 0 min and also significantly reduced plasma insulin levels at 0 and 120 min compared to HF group rats (at 3 months) indicating its hypoglycemic insulin sensitizing property. Further, these results indicated that feeding of HF to WNIN rats had developed insulin resistance and impaired glucose tolerance by three months. Hence we considered these animals as insulin resistance and impaired glucose tolerance associated pre-diabetic model, which mimics human typical pre-diabetic character. Further these results well correlated with previous studies [10, 11, 12, 13].

Further, OGTT results of HF group rats before termination of the experiment (10 months) showed a significant higher glucose at 120 min and marginally increased insulin (all time points) as compared to control. Garlic fed HF group rats had shown a marginal reduction in the plasma glucose at 120 min and insulin levels at 30 and 60 min as compared to HF group rats (Fig 3).

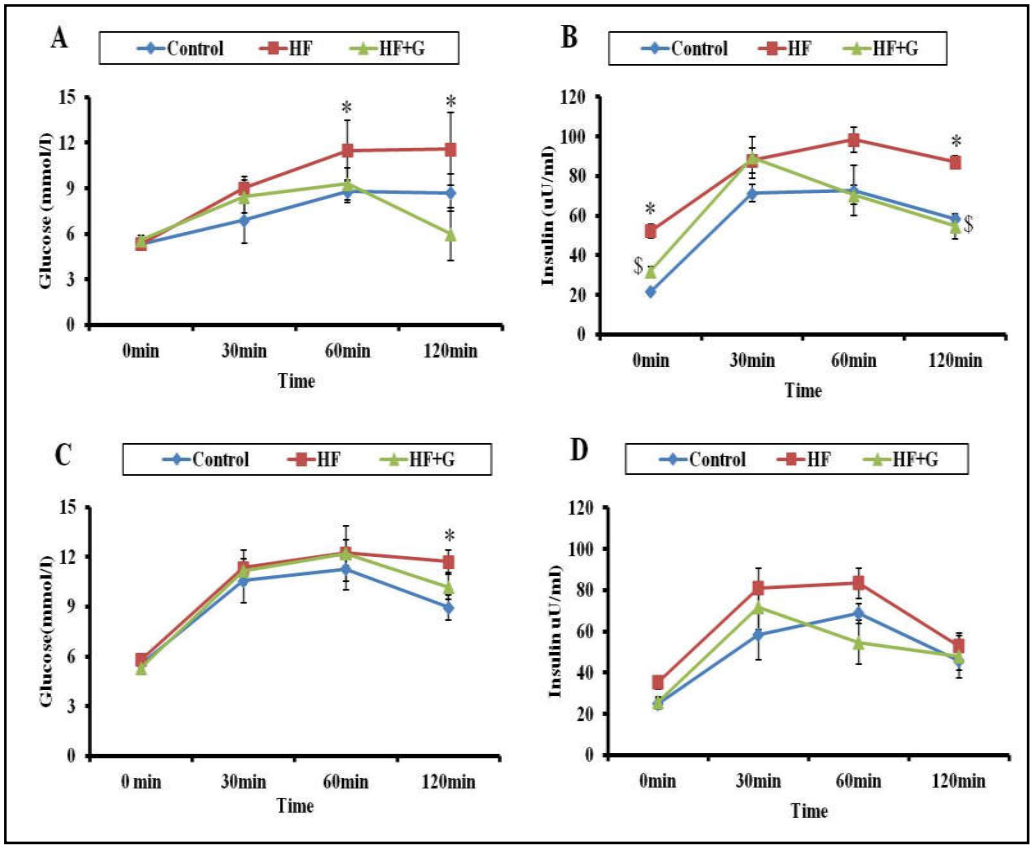

Figure 3: Glucose (A, C) and insulin (B, D) response during oral glucose tolerance test (OGTT) at 3 months (A, B) and 10 months (C, D) after feeding of their respective diets. Values are mean ± SE, n=9 animals per group. *p<0.05 vs. Control; $p<0.05 vs HF. p<0.05 was considered as statistically significant by ANOVA. OGTT, Oral glucose tolerance test.

Table 1: Plasma OGTT parameters of control, HF and HF+G group rats during OGTT at 3rd and 10th month.

Parameters	Groups		
	Control	HF	HF+G
3 months			
GAUC (mmol/h)	16.06±2.29	18.44±3.179	16.52±2.204
IAUC (µmol/h)	127.08±23.46	174.21±20.45*	132.88±15.20$
GAUC/IAUC	0.13±0.025	0.108±0.02	0.127±0.03
HOMA-IR	0.89±0.13	2.09±0.54*	1.32±0.21$
10 months			
GAUC (mmol/h)	15.28±5.59	16.51±3.19	16.25±3.03
IAUC (µmol/h)	95.92±21.82	154.83±26.22*	112.75±18.09
GAUC/IAUC	0.18±0.11	0.11±0.01	0.14± 0.01
HOMA-IR	1.01±0.35	1.59±0.45	1.03±0.38

Values are mean ± SE, n=9 animals per group. *p<0.05 vs Control; $p<0.05 vs HF. p<0.05 was considered as statistically significant by ANOVA. GAUC, glucose area under the curve; IAUC, insulin area under the curve; GAUC/IAUC, ratio of glucose area under the curve/ insulin area under the curve; HOMA-IR, homeostasis model assessment for insulin resistance

In addition to the glucose and insulin response during OGTT at 3 months of experimental period, the results of HOMA-IR, IAUC significantly and GAUC, ratio of GAUC/IAUC marginally increased in HF group rats compared to control rats indicating development of insulin resistance in these animals by three months due to HF feeding and these results were well correlated with previous studies on high fructose rat models [8, 12, 10]. Before termination of experiment (10 months), the IAUC was significantly and HOMA-IR, GAUC, GAUC/IAUC, HbA1c were marginally higher in HF group rats compared to control group rats. Garlic significantly reduced the HOMA-IR and marginally reduced GAUC, IAUC, GAUC/IAUC after 3 months feeding, but after 10 months of experimental period, garlic had shown marginal improvement in HOMA-IR, GAUC, IAUC and GAUC/IAUC due to its insulin insulin-sensitizing properties. Some of these

results were similar with other studies reported earlier on garlic [8, 12, 15]. Together these results indicate that feeding of HF to WNIN rats developed insulin resistance and IGT associated pre-diabetes by three months and maintained these pre-diabetic characteristics till the end of the experimental period of ten months.

CONCLUSION

Feeding HF to WNIN rats led to the development of IR and IGT associated PD. Early intervention of garlic to the HF group (HF+G) delayed the development of IR and IGT, as evidenced by OGTT and HOMA-IR results at 3rd and 10th month.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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