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Effect of Piperine in Obesity induced insulin resistance and type-II diabetes mellitus in rats

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Abstract

Objective: The present study was undertaken to explore the effect of piperine in obesity induced insulin resistance and type 2 diabetes mellitus. Materials and methods: Male Sprague dawley rats were fed High fat diet (HFD) for first 8 weeks to develop obesity which induced insulin resistance and diabetes mellitus. Later on piperine (400 mg/kg) and sibutramine (5 mg/kg) were administered for 3 weeks along with the continuation of HFD to two separate groups which served as test and standard respectively. Result: Body weight ,serum triglyceride and glucose levels were measured at the end of 4th, 8th (before treatment) and 11th (after treatment) week while insulin tolerance test and fat mass were measured at the end of 11th week in normal, HFD-control, test and standard groups . Conclusion: Piperine significantly reduced not only body weight, fat mass, triglyceride and glucose levels but also improved sensitivity of exogenous insulin. The above results suggest that piperine has significant anti-obesity, antidiabetic activity and also improved insulin sensitivity.

Keywords: obesity, diabetes, insulin, piperine, high-fat diet, sibutramine.

1. Introduction

Obesity results from a greater consumption of energy than used by the body which leads to increased fat accumulation (adiposity) and fat cell enlargement (hypertrophy). Obesity is one of the major health challenges faced by the developed world [1]. Being obese or overweight decreases life expectancy between 3 and 13 years [2], and significantly increases the risk of its associated conditions [3] such as type 2 diabetes mellitus, insulin resistance, different types of cancers, dyslipidemia, steatosis hepatis, coronary heart disease, termed as the metabolic syndrome, represent major challenges for basic science and clinical research [4].

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The consumption of a high caloric (fat) diet is one of the main causes of increased adiposity. Rats fed with a high fat diet (HFD) developed obesity which leads to insulin resistance and hyperglycemia. It has been demonstrated that the size and weight of adipose tissues are increased in rats kept on HFD, and that the hypertrophy of adipocytes leads to changes in the release of adipocytokines such as leptin and adiponectin regulating insulin sensitivity [5].

Piper nigrum/longum is a highly reputed plant in ayurvedic system of medicine Phytochemical review reveals the presence of piperine (1 piperoyl piperidine), which is the active constituent of this plant. Its use is indicated in bronchitis, chronic cold, cough, congestion, hemorrhoids, hepatitis, arthritis, chronic dyspepsia, anorexia, chronic asthma, burning heart, colic, rheumatoid/osteo arthritis, juvenile asthma, etc [6]. The objective of the present investigation was to evaluate the beneficial effect of piperine in decreasing serum glucose and improving exogenous insulin sensitivity in obese animals.

2. Materials and Methods

2.1 Materials

Piperine was purchased from Sigma Aldrich Co., St Louis, USA and Sibutramine was a generous gift from Intas Pharmaceuticals Ltd, Ahmedabad. All other chemicals used were of analytical grade.

2.2 Animals

Male Sprague-Dawley rats weighing 400-450 gm were used for present study. They were housed in clean polypropylene cages (three rats/cage) and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5%) with 12:12 h light and dark cycle. All the rats were fed normal Pellet Diet (NPD) (commercial rat pellets from Pranav Agro Industries Ltd., Baroda, India) and water *ad libitum* before the dietary manipulation. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

2.3 Experimental Protocol

Male Sprague-Dawely rats weighing 400-450 gm were used for the present investigation. Rats were divided into 4 groups of six animals each.

Group I - control group.

Group II - High fat diet (HFD) control group.

Group III - HFD + Piperine (suspended in 0.5% CMC, p.o), for last three weeks.

Group IV - HFD + Sibutramine (solution in deionized water, p.o.), for last three weeks.

Group I was fed normal Pellet Diet (NPD) (commercial rat pellets from Pranav Agro Industries Ltd., Baroda, India) for 11 weeks while Group II, III and IV were fed high–fat diet for 11 weeks. At the end of the 8th week, group III and IV were treated with piperine (40 mg/kg) [7] and sibutramine (5 mg/kg) respectively for three weeks along with the HFD. The composition of HFD [8] is given in table 1. The following parameters were measured: physical parameters like body weight [5], biochemical parameters and Insulin tolerance test (ITT) [5]. At the end of the study, five rats from each group were sacrificed and the fat mass was collected and immediately weighed [5].

2.4 Collection of blood samples

At the end of the 4th week, 8th week and 11th week, blood was collected under inhalation anesthesia by retro-orbital puncture from overnight fasted animals. Blood was allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 4,000 - 5,000 rpm for 15 minutes and analyzed for serum triglyceride (GPO-PAP) and glucose (GOD-POD) levels using commercially available diagnostic kits (Span Diagnostics Ltd., Surat, India).

2.5 Insulin tolerance test (ITT)

To check the endogenous insulin sensitivity, ITT was carried out. At the end of the 11th week, ITT was performed between 0900 h and 1200 h on animals fasted by 10th before the test. Human regular insulin (1 U/kg body weight) was administered intraperitoneally, and blood samples were taken by the retro-orbital puncture method before and 15, 30, 60 and 120 min after insulin administration. Serum glucose levels were measured thereafter as method described above.

2.6. Fat-pad analysis

At the end of the 10th week, rats were decapitated between 09:00 and 12:00 h. They were free to access food and water. After sacrificing by decapitation, the epididymial white adipose tissue and interscapular brown adipose tissue (BAT) were dissected out. The collected fat was weighed immediately and compared with the other groups.

2.7 Statistical analysis

All the values were expressed as mean ± SEM, n=6 in each group. The statistical analysis for determining significant difference was performed using student's paired *t*-test and Tucky (one way ANOVA test) test. Value of p less than 5% ($p<0.05$) was considered statistically significant.

3. Results

3.1. Effect of piperine on body weight

Body weight was measured every week till eleven weeks. Body weight of all HFD groups

Table 1. Composition of high fat diet

Ingredients	(g/kg)
Powdered NPD	300
Lard	275
Casein	200
Cholesterol	10
Vitamin and mineral mix	60
dl-methionine	03
Sodium chloride	02
Sucrose	150

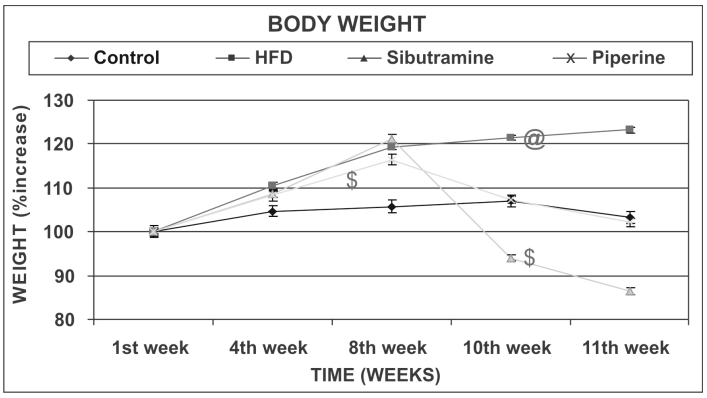

Fig 1. Effect of piperine on body weight in HFD fed animals. Each line represents the mean ± SEM of six observations, n=6. @Data differs significantly ($p<0.05$) when compared against the control group; $Data differs significantly ($p<0.05$) when compared against the HFD-control group.

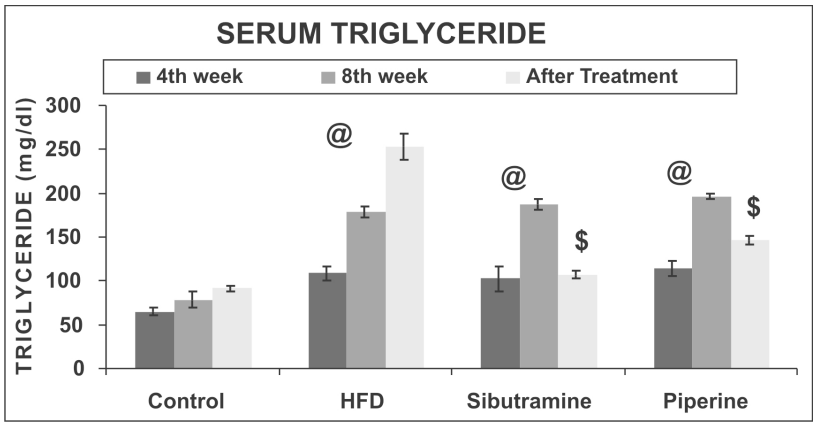

Fig. 2. Effect of piperine on serum triglyceride in HFD treated animals. Each bar represents the mean ± SEM, n=6. @, significant difference from the control group, at $p<0.05$. $, significant difference from the HFD group, at $p<0.05$.

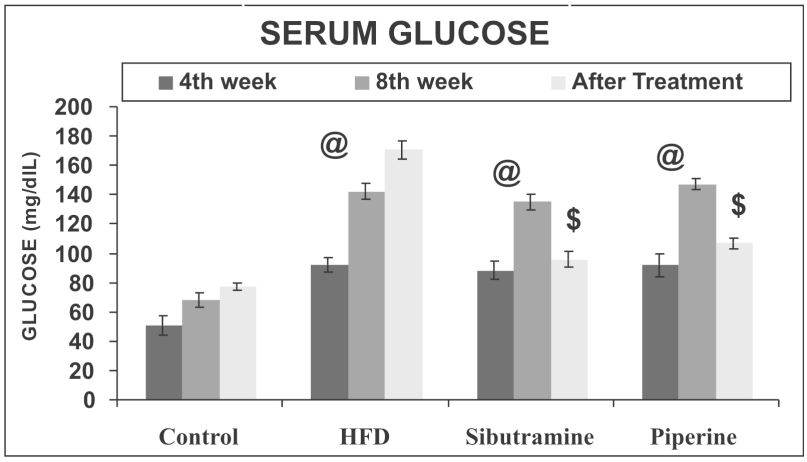

Fig. 3. Effect of piperine on serum glucose in HFD treated animals. Each bar represents the mean ± SEM, n=6. @, significant difference from the control group, at $p<0.05$. $, significant difference from the HFD group, at $p<0.05$.

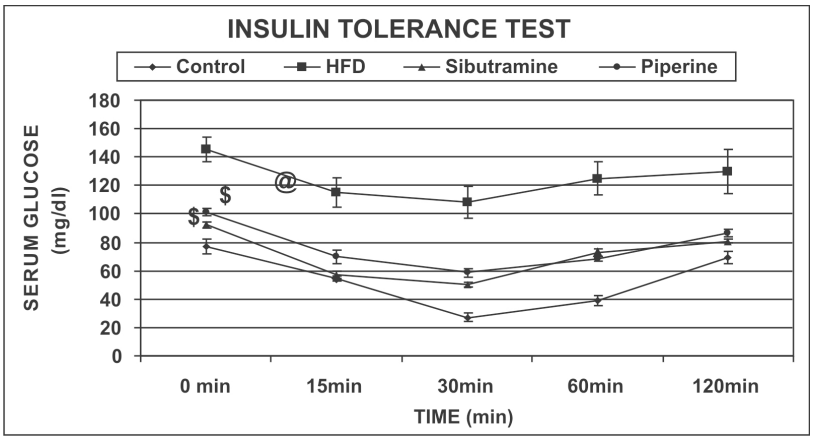

Fig. 4. Effect of piperine on serum glucose levels in ITT in HFD treated animals. Each line represents the mean ± SEM, n=6. @, significant difference from the control group, at $p<0.05$. $, significant difference from the HFD group, at $p<0.05$

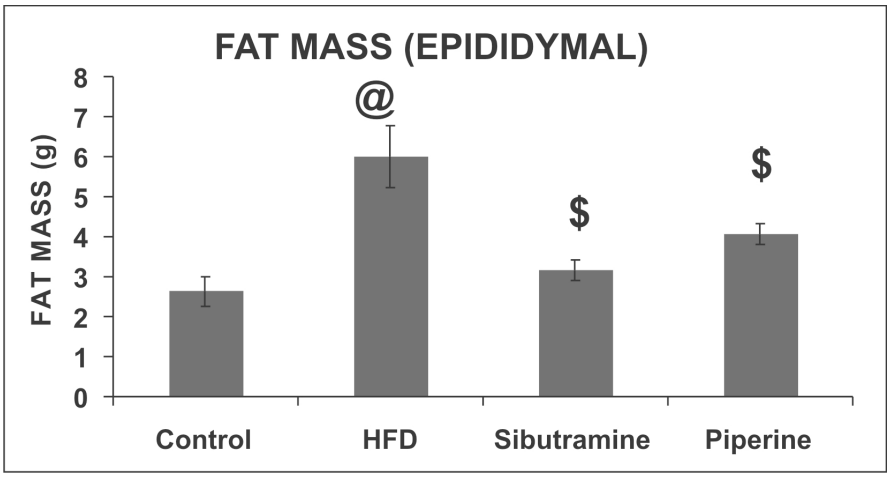

Fig. 5. Effect of piperine on epdidymal fat mass in HFD treated animals. Each bar represents the mean ± SEM, n=6. @, significant difference from the control group, at p<0.05. $, significant difference from the HFD group, at p<0.05.

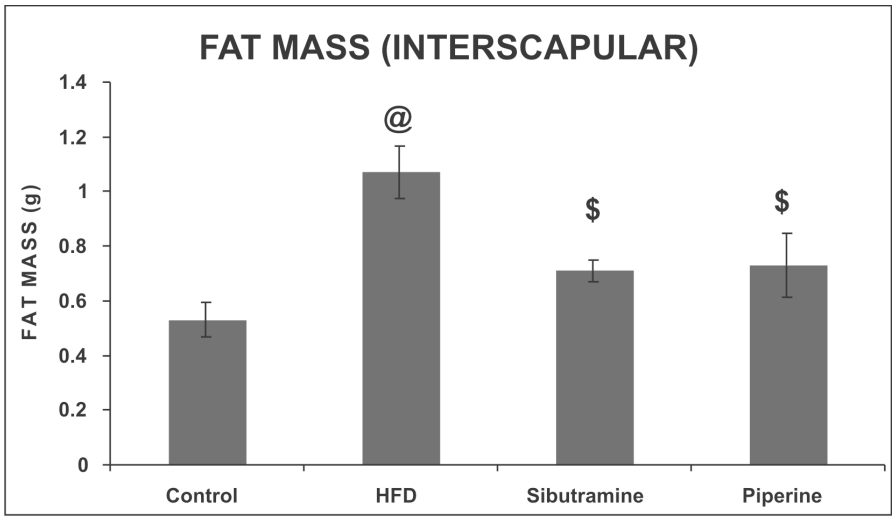

Fig. 6. Effect of piperine on interscapular fat mass in HFD treated animals. Each bar represents the mean ± SEM, n=6. @, significant difference from the control group, at p<0.05. $, significant difference from the HFD group, at p<0.05.

(group II, III, IV) were significantly increased compared to control group (group I) for first 8 weeks while there was further significant increase in the body weight in HFD control group (group II) as it was kept on HFD for 3 more weeks compared to the control group. Piperine treated group showed significant reduction in body weight by 12-15% as compared to the HFD control group (p<0.05), while sibutramine treated group (group IV) exhibited 35-40% weight reduction (Figure1).

3.2. Effect of piperine on biochemical parameters

3.2.1. Effect of piperine on serum triglyceride level

Serum triglyceride level was significantly increased in all the HFD groups for first 8 weeks

compared to the control group. On treatment with piperine for three weeks, the level was significantly decreased compared to the HFD control group ($p<0.05$) (Figure 2). Very similar results were observed with sibutramine treated group.

3.2.2. Effect of piperine on serum glucose level

For the first 8 weeks, there was a significant increase in serum glucose levels in all the HFD groups compared to the control group while after 8 weeks there was further increase in serum glucose level in only HFD control group compared to control group. On treatment with piperine for 3 weeks along with HFD, showed significant reduction in serum glucose levels as compared to the HFD control group (Figure 3).Very similar results were observed with sibutramine treated group.

3.3. Insulin tolerance test

Insulin tolerance test was carried out to check the insulin sensitivity in animals kept on HFD. Insulin sensitivity was decreased in HFD group and thereby showed elevated levels of glucose as compared to the control group. On treatment with piperine for last 3 weeks, there was significant reduction in serum glucose levels as compared to the HFD control group (Figure 4). Similar results were obtained with sibutramine treated group.

3.4. Fat pad analysis

As the animals were kept on HFD for 11 weeks, there was an accumulation of visceral, subcutaneous and interscapular fat. There was a significant reduction in the epididymal (Visceral WAT) and interscapular (BAT) fat mass in piperine treated group compared to HFD control group (Figure 5, 6). This showed the protective effect of piperine in increased adipose tissue.

4. Discussion

This study was initiated with the objective of developing a drug that would simultaneously minimize the outcomes of obesity [9] and its related metabolic disorders. High fat diet is one of the main causes leading to increased fat mass accumulation-obesity which in turn leads to insulin resistance and diabetic condition [10-11]. Thus, High fat diet (HFD) model was used to produce obesity and diabetic condition similar to obesity in humans.

Increase in body weight and fat deposition are the important indicators for gradual progress of obesity. As the animals were fed with HFD, there was an increase in the adiposity which in turn increased fat cell mass. Thus there was an overall increase in body weight. The increased body weight found in HFD rats might be due to the consumption of a diet rich in energy in the form of saturated fats (lard) and its deposition in various body fat pads [10] and decreased energy expenditure as compared to NPD-fed animals [12]. But on treatment with piperine there was a significant decrease in body weight and fat mass which proves its antiobese action.

The hypertriglyceridemia observed in these fat-fed rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic very low density lipoprotein (VLDL) and decreased TG uptake in peripheral tissues [10].

The risk of diabetes increases by increase in each kg in weight [13]. Over 90% of diabetics are overweight or obese. Current theories indicate that the hyperglycemia or impaired glucose tolerance or type 2 diabetes develops when pancreatic insulin output can no longer satisfy the demands imposed by increased insulin resistance [14]. A reduction in sensitivity to insulin can occur through an inherited defect, or it can be acquired as a consequence of obesity.

As body fat increases, the rate of lipolysis rises, leading to increased free fatty acids (FFA) mobilization and consequently to increased FFA oxidation in muscle and liver. In turn, glucose use by muscle declines as FFA is used as an alternate energy source, and hepatic glucose production increases in response to the higher FFA oxidation. These actions result in hyperglycemia and impaired glucose tolerance. Due to the elevated glucose levels, pancreas initially responds by producing more insulin. As long as the pancreas can produce enough insulin to overcome this resistance, blood glucose levels remain normal. Once the pancreas can no longer keep up with producing high levels of insulin, blood glucose levels begin to rise, resulting in type 2 diabetes, thus insulin resistance is a pre-diabetes condition [15]. As per the above results, piperine reduces triglyceride level which decreases the FFA. Due to reduction in FFA levels the glucose utilization by muscle and liver increases which leads to decrease in serum glucose levels and improved insulin sensitivity. As piperine reduces the serum glucose level and improves insulin sensitivity, which proves its role in obesity induced insulin resistance and diabetes condition. The probable mechanism may be the activation of centrally melanocortin-4 receptors [5] or inhibition of the enzyme Diacylglycerol acyltransferase (DGAT) [16]. Moreover piperidine-substituted quinazolinone derivatives were identified as a new class of molecules which causes suppression of food intake and body weight reduction as well as glucose lowering effects [17]. Further work is in progress to identify the possible mechanism of actions for its role in insulin resistance and diabetes.

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