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Preventive effect of sesame seed cake on hyperglycemia and obesity against high fructose-diet induced Type 2 diabetes in rats

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ABSTRACT

The present study investigates the hypoglycemic and antiobesity effect of sesame seed cake (SSC) on rats fed with high fructose diet (HFD). SSC contained dietary fibre, lignans and phenolic compound. One month of HFD feeding induced significantly the obesity, hyperglycemia, hyperlipidaemia, insulin insensitivity and increased atherogenic index (AI). Treatment of SSC along with feed material decreased the weight gain, normalised the blood glucose (BG) level, reduced the serum cholesterol and improved the glucose tolerance significantly. In the oral glucose tolerance test (OGTT), rats fed with HFD supplemented with 2% and 4% SSC significantly reduced the plasma glucose after 120 min of glucose loading, indicating an improved glucose tolerance. In conclusion, the intake of SSC supplementation can be adopted as a therapeutic strategy for the prevention of obesity induced Type 2 hyperglycemia.

1. Introduction

Diabetes is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have diabetes mellitus and 300 million will subsequently have the disease by 2025. The new millennium has witnessed the emergence of a modern epidemic, the metabolic syndrome, with frightful consequences to the health of humans worldwide. The metabolic syndrome, also referred to as "Diabesity" describes the increasing incidence of diabetes in combination with obesity as a result of changes in human behaviour, available nutrition, and the adoption of more sedentary lifestyles (Wild, Roglic, Green, Sigree, & King, 2004). The potential role for dietary fibre in diabetes was first promoted more than 30 years ago by Trowell on the basis of his experience in East Africa where he noted a virtual absence of what is now known as Type 2 diabetes in association with the consumption of traditional diets, which were extremely high in 'lightly processed' cereal foods (Mann, 2001).

For various reasons, in recent years the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine have been used commonly in India. The consumption of fructose in humans has been increasing for long time, but there is little evidence that fructose could influence carbohydrate and lipid metabolism, which is associated with metabolic abnormalities in humans and animals (Tappy, Le, Tran, & Paquot, 2010). In vitro studies indicated that dietary antioxidants can protect against oxidative damage of body tissues. Sesame seed cake is obtained from the plant *Sesamum indicum* Linn (Fam. Pedaliaceae), which possess significant amounts of antioxidant phytochemicals, phenolic acids and lignans with antioxidant properties (Suja, Jayalekshmy, & Arumughan, 2005). Sesame seed and their by-products contain significant amount of soluble and insoluble fibre (Elleuch, Besbes, Roiseux, Blecker, & Attia, 2007), which are left in the residue after oil extraction. Dietary fibre rich foods play valuable role in lowering of cholesterol, obesity and in management of Type 2 diabetes. The residue left after oil extraction is known as SSC, which is traditionally used as cattle feeding. The valuable dietary fibre and other constituents are left in seed cake in high amounts. Flavonoids from sesame have hypolipidaemic and hypoglycemic activity which profoundly effects hepatic fatty acid oxidation and serum triacylglycerol level altering lipid metastasis in a potentially beneficial manner (Sirato-Yasumoto, Katsuta, Okuyama, Takahashi, & Ide, 2001). Literature survey reveals that the in-vivo study on SSC has not been conducted earlier, so in this project, an attempt has been made to investigate the effect of SSC in HFD induced dislipidaemia, obesity and hyperglycemia on rat.

2. Materials and methods

2.1. Chemicals

Fructose was purchased from Molychem, Mumbai. Metformin was generous gift from Nicholas Piramal Ltd., Mumbai. Glucose,

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casein, dextrose, cholesterol and methionine-DL were purchased from Loba Chem, Mumbai. All other chemical reagents used in the study were of analytical grade.

2.2. Collection and drying

SSC was collected from local oil mill of district Rewa, MP. The collected SSC were shade dried and pulverised with a mechanical pulverizer for size reduction.

2.3. Crude extract

Dried and powdered SSC (20 g) was extracted with 300 ml of 90% ethanol for 16 h in soxhlet extractor. The extract was filtered and concentrated using a vacuum evaporator (Jyoti Scientific, India). The residue was weighed and redissolved in 200 ml ethanol and stored under refrigeration until further analysed qualitatively for the presence of different phytochemicals following the method of Finar (1959), Kokate, Purohit, and Gokhale (2001) and Shellard (1957).

2.4. Purified extract

Twenty-five grams of dried and powdered SSC sample was initially extracted with hexane (three times with a total of 600 ml of hexane) at room temperature. The defatted residue was washed with distilled water (three times with a total of 600 ml of distilled water) to remove soluble sugars and proteins and dried below 70 °C. Fourteen grams of the above purified residue were extracted with 300 ml methanol for 16 h in a soxhlet extractor. The extract was filtered, the solvent removed under vacuum to dryness, weighed and the residue redissolved in 100 ml of methanol to give phenolic and lignans extract and stored in refrigerator until analysed.

2.5. Authentication of isolated lignan by analytical HPLC technique

The HPLC system (Shimadzu, Japan), equipped with CAT-228-39001-38 pump, 228-393000-38 photodiode array detector, LC solution integrated software and a rheodyne injection valve fitted with a 20 μl injection loop was used for the analysis. The baseline resolution of lignan was obtained at 25 ± 2 °C using a Waters μ-Bondapak C_{18} column (250 mm i.d × 4.0 mm) in the reverse phase connected through a guard column of C_{18} (Supelco). An isocratic solvent system consisting of methanol and water in the ratio of 70:30 (v/v) was used. The mobile phase was passed through 0.45 PVDF filter, and degassed before use. The flow rate was kept constant at 1 ml/min and the effluents were monitored at 290 nm. The test solution was prepared by dissolving 220 mg of substance under examination in 100 ml of methanol, filtered and 10 μl were injected (Suja et al., 2005).

2.6. Quantitative determination of sesame seed cake

2.6.1. Dry matter determination

The dry matter was determined by oven drying at 105 °C to constant weight (AOAC Official Methods of the Analytical Chemists, 1990).

2.6.2. Dietary fibre determination

Insoluble and soluble dietary fibres were determined following the method described by Prosky, Asp, Schweizer, De Vries, and Furda (1988). The defatted samples were gelatinized with heat stable alpha amylase (100 °C, pH 6, 15 min) and then enzymatically digested with protease (60 °C, pH 7.5, 30 min) followed by incubation with amyloglucosidase (60 °C, pH 4.5, 30 min) to remove protein and starch. Then, the samples were filtered, washed (with water, 95% ethanol and acetone), dried and weighted to determine the insoluble fibre. Four volumes of 95% ethanol (preheated to 60 °C) were added to the filtrate and to the water washings. Then, the precipitates were filtered and washed with 78% ethanol, 95% ethanol and acetone. After that, the residues (soluble fibre) were dried and weighted. The obtained values were corrected for ash and protein. The total dietary fibre was determined by summing the insoluble dietary fibre and the soluble dietary fibre.

2.6.3. Fat content

This was determined by soxhlet extraction with hexane for 8 h at boiling point of the solvent (68–70 °C). This extraction was carried out to estimate the content of oil (Abaza, Msallem, Daoud, & Zarrouk, 2002).

2.6.4. Total phenolic compound estimation

The total phenolic content was determined by an assay modified from Shetty, Curtis, Levin, Wikowsky, and Ang (1995). One millilitre of homogenised extract was transferred into a test tube and mixed with 1 ml of 90% ethanol and 5 ml of distilled water. To each sample, 0.5 ml of 50% (v/v) Folin Ciocalteu reagent was added and mixed. After 5 min, 1 ml of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 725 nm. The absorbance values were converted to total phenolics and were expressed in micrograms equivalents of gallic acid per gram of the sample. Standard curve was plotted using various concentrations of gallic acid in water.

2.7. Animals and treatment

Five week old laboratory bred Wistar albino rats of either sex weighing between 150–200 g were selected for study and were acclimatised for 1 week before being randomly assigned into experimental groups. The animals were housed in individual cages with free access to water in departmental animal house with a 12:12 h light–dark cycle (8:00 am–8:00 pm), a temperature of 24 ± 1 °C, and a humidity of 55 ± 5%. During the acclimatisation period, each animal were raised on a regular diet *ad libitum*. Animals were randomly divided into 5 groups containing 5 animals in each group. The experimental protocol was conducted in accordance with the internationally accepted principles for laboratory animal use and care as described by CPCSEA guideline after approval of IEAC (IEAC/RCP/2010/10) of Radharaman College of Pharmacy, Bhopal.

The control rat continued to receive either a control diet (CD) or high fructose diet (HFD), and the treatment groups were fed a HFD with either 2 or 4 g/kg of SSC for a period of 1 month. As a positive control, metformin was administered with the HFD at a dose of 50 mg/kg. CD and HFD were self prepared and the nutritional content was similar to those of HFD except carbohydrate content (Table 1). CD, HFD and SSC were given in form of pellets while metformin was given orally to animals. Body weights and BG level

Table 1
Composition of experimental diabetogenic diet.

Control diet	Quantity (g/kg)	High fructose diet	Quantity (g/kg)
Casein	200	Casein	200
Ghee	98	Ghee	98
Corn flour	150	Corn flour	150
Vitamin	3	Vitamin	3
Mineral	15	Mineral	15
Methionine-DL	3	Methionine-DL	3
Cholesterol	5	Cholesterol	5
Dextrose	520	Fructose	520