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Original article

Evaluation of the antihyperglycemic, antilipidemic and antioxidant potential of *Cucurbita ficifolia* in human type 2 diabetes

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Summary. *Background and Aim:* The present study has been undertaken to analyze the antihyperglycemic, antilipidemic and antioxidant potential of *Cucurbita ficifolia* juice in type 2 diabetic subjects. *Methodology:* A total 34 subjects were selected for the study out of which 14 subjects were with type 2 diabetes mellitus, 10 subjects were selected as a normal control group and 10 subjects were selected as a normal treated group. The patients had type 2 diabetes for more than 2 years, their mean age was less than 45 years, and subjects were then asked to start the *Cucurbita ficifolia* juice therapy starting at day 0 and continuing up to 40 day both for diabetic and normal subjects. Fasting blood glucose, glycosylated hemoglobin, lipid profile and anti-oxidant levels were measured two times at 0 days and continuous 40 days *Cucurbita ficifolia* therapy. *Results:* This study compared the measurement of glucose levels, lipid biomarkers, antioxidant and lipid peroxidation in all three groups [normal control, normal treated, and type 2 diabetic patients treated], at 0 days and after therapy of 40 days with *Cucurbita ficifolia* juice. *Conclusion:* The results suggest that the *Cucurbita ficifolia* juice therapy in type 2 diabetes may have the potential to regulate hyperglycemia, hypercholesterolemia and enhance the antioxidants enzymes along with reduction of lipid peroxidation.

Key words: *Cucurbita ficifolia,* type 2 diabetes, antihyperglycemic, antilipidemic, lipid peroxidation, antioxidant enzymes

Introduction

Diabetes Mellitus, characterized by hyperglycemia, is genetically and clinically a heterogeneous group of disorder of glucose intolerance that is associated with the disorder of carbohydrate, and protein metabolism. The number of adults with diabetes in the world is expected to rise from 170 million in the year 2000 to more than 360 million in the year 2025 (1). Of the total worldwide diabetes rate, 90% to 95% are living with type 2 diabetes but it has been evaluated that up to half of these individuals are not aware of their condition (undiagnosed diabetes) (2). The risk factors for diabetes such as obesity, metabolic syndrome, physical inactivity, and smoking are increasing widely in developing countries including India (3, 4).

Type 2 diabetes occurs from the progression of insulin resistance and the affected patients habitually have insulin insufficiency (5). An important part of tissue damage and cell death associated with chronic

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hyperglycemia is mediated by free radicals. Hyperglycemia, the main symptom of diabetes, elevates the oxygen free radicals along with a sharp reduction of antioxidant defenses (6, 7). Oxidative stress occurs in the case of imbalance between free radical production and antioxidant capacity of non-enzymatic and enzymatic substances present in tissue. To prevent the production of reactive oxygen species (ROS) and their deleterious effects, antioxidant system acts as ROS scavengers, such as superoxide dismutase (SOD), catalase and glutathione peroxidase, glutathione (8).

The allopathic drugs such as sulphonylurea, biguanides and α – glycosidase inhibitors are used in the treatment of hyperglycemia in diabetes mellitus in the past decades. Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects (9). Some of the anti-diabetic drugs are exceedingly costly and pose unwanted contraindications as heartburn, vomiting, skin rashes, etc. (10-12). Moreover, it does not reinstate a permanent glucose homeostasis, however when certain patients faced snags alternative treatments were searched for.

Plants have been the major sources in Indian system of medicine and other ancient system in the world (13). There are many antidiabetic plants, which might provide useful sources for the development of drugs, in the treatment of diabetes mellitus. While numerous herbal remedies have been proposed for the treatment of diabetes mellitus only a few have been scientifically established. Herbal remedies such as diamed (14) coagent db (15) and hyponidd (16), diabegon (17) are natural, free from side effects and are even effectual in the treatment of diabetes mellitus (18). Not much literature is available regarding the antihyperglycaemic, antilipidemic and antioxidant potential of *Cucurbita ficifolia.* Previously few reports have suggested that *Cucurbita ficifolia* may use as an herbal medications in the treatment of diabetes mellitus (19, 20). Therefore, the present study was aimed to assess the effect of *Cucurbita ficifolia* juice in human type 2 diabetic subjects for its antihyperglycaemic, antilipidemic and antioxidant potential. The various biochemical parameters like fasting glucose, glycosylated hemoglobin, lipid parameters, antioxidant enzymes and lipid peroxidation were studied.

Material and methods

Cucurbita ficifolia

Cucurbita ficifolia is a perennial climber, family cucurbitaceae cultivated for its edible seeds, fruit, and greens. In India it is also known as "Kumra", while in English it is called as chilacayote, chiverre, fig-leaved gourd, malabar gourd, Malabar squash, pie melon, or shark fin melon. The growth of *Cucurbita ficifolia* is done from the northern Mexico to Argentina and Chile. It's also wide spread in Europe (France and Portugal) and Asia (India)

Selection of subjects and experimental design

A diabetes clinic is organized by School of Studies in Biotechnology, Jiwaji University under the supervision of an Ayurvedic physician. The diabetic patients are provided with regular counseling on the causes, symptoms, complications of diabetes, apart from traditional Ayurvedic medicines. From the diabetes clinic a total 34 subjects were selected for the study out of which 14 subjects were with type 2 diabetes mellitus, 10 subjects were selected as a normal control group and 10 subjects were selected as a normal treated group. The patients had type 2 diabetes for more than 2 years, their mean age was less than 45 years, and weight 45-80 kg. The chosen individuals were not undertaking any antihyperglycemic, antihypertensive or antihyperlipedemic allopathic drugs.

Cucurbita ficifolia juice formulation

The Fresh immature fruits of *Cucurbita ficifolia* measuring ~12 cm were obtained from commercial vendors. The selected fruits were of the variety that is grown in Gwalior region of Madhya Pradesh in India. The fruit was cleaned with potable water and disinfectants, the fruit (the shell and seed were removed) subsequently diced into smaller pieces to be put in an electric domestic extractor, and the juice was then collected. 65 ml of the extract corresponded to 100 g of the starting crude material. The obtained extract was administered orally, 4 ml/kg bodyweight (21) to the selected subjects.

Duration of therapy

After the selected subjects were diagnosed with type 2 diabetes, subjects were then asked to start the *Cu-*

curbita ficifolia juice therapy starting at day 0 and continuing up to 40 day both for diabetic and normal subjects.

Analysis of biochemical parameters (sample collection)

The selected subjects were fasted overnight for 12 hours and their venous blood was withdrawn, the serum was separated and kept at -20°C for analysis of lipid profile, glucose measurements and the remaining blood sample was stored at 4°C for analysis of markers of oxidative stress.

Glucose tolerance test

All the subjects were fasted overnight for 12 hours and their fasting venous blood sample was withdrawn, then the subjects were asked to drink 75 g of glucose dissolved in 250 ml of potable water (22). Their venous blood glucose levels (glucose kit: Crest Biosystems, India Pvt. Ltd) were monitored at different time interval viz. 30 min, 60 min, 90 min and 120 min and tolerance curve was plotted (23).

Analysis of glucose concentration

Fasting and plasma glucose level was determined by glucose oxidase method (24) employing commercial kits manufactured by Crest Biosystems, India Pvt. Ltd. The measurement was followed up after 40 days of therapy.

Glycosylated hemoglobin

The glycosylated hemoglobin was estimated by ion exchange method (25) employing commercial kit (Kamineni Life Sciences, India).

Lipid profile

The lipid profile parameters such as total serum cholesterol (cholesterol oxidase- peroxidase method) (26), serum triglyceride (Glycerol 3 peroxidase method) (27), and serum HDL-cholesterol (Polyethylene Glycol Precipitation method) (28), Low Density Lipoprotein Cholesterol (LDL-C) (Freidewald's Formula) and Very Low Density Cholesterol (VLDL-C) (Freidewald's Formula) were estimated by spectrophotometric assays from fasting serum samples using commercial kits manufactured by Crest Biosystems India Pvt. Ltd.

Measurement of oxidative stress markers

Oxidative stress markers like reduced glutathione (GSH) was estimated in whole blood whereas, malondialdehyde, superoxide dismutase (SOD) were estimated from haemolysate. Haemolysate preparation: The plasma and the buffy coat were removed from whole blood by centrifugation at 2000 rpm for 10 min at 4°C. The red cells were washed three to four times with normal saline and haemolysate was prepared. Malondialdehyde level was prepared by mixing 1.9 ml of chilled distilled water with 0.1 ml of packed cell volume (PCV) suspension. Analysis of GSH by Ellman (29), SOD by winterbourne et al. (30), thiobarbituric acid reactive substances (TBARS) by Ohkawa et al.(31) were used to analyzed there concentration. Malondialdehyde, a decomposition product of lipid hydro peroxides, used as an indicator of oxidative damage to cells and tissues.

Statistical analysis

The study results were expressed as mean ± standard deviation expressed in the figures. The data obtained from the experiments were analyzed using paired t test and one way ANOVA where applicable in the study data by employing sigma stat, statistical software, version 1.0 (Jandal Corporation, USA). For computing data, software application programs like Microsoft Excel, Sigma Direct were used. The values were tested for significance at $P<0.001$, $P<0.05$.

Results

This study evaluate the effect of *Cucurbita ficifolia* on human type 2 diabetes patients.

Effect of Cucurbita ficifolia on fasting glucose levels

After 40 days of *Cucurbita ficifolia* juice therapy there was 17.62% ($P<0.001$) decline in fasting blood glucose levels in case of diabetic treated subjects, while there was a mild increase in normal treated group with no significant difference. The blood glucose levels of normal untreated control group remained fairly constant during the course of the study (Figure 1).

Effect of Cucurbita ficifolia on glucose tolerance

All participants in the study were tested for glucose tolerance test (oral administration of 75 g of glucose in 250 ml of water) at baseline and after a therapy of *Cucurbita ficifolia* juice for 40 days.

The blood glucose concentration after therapy showed a decline of 10% (P<0.01), 8.3% (P<0.01), 10.2% (P<0.001) and 17% (P<0.01) after 30 min, 60 min, 90 min and 120 minutes respectively in diabetic treated subjects. While in normal untreated and normal treated groups, there was a slight elevation observed after 30 min however there was reduction at every time interval viz. 60 min. (P<0.01), 90 min and 120 min (Figure 2).

Effect of Cucurbita ficifolia on glycosylated hemoglobin

After 40 days of *Cucurbita ficifolia* juice therapy there was a decline by 22.5% and 5% for diabetic and normal subjects respectively. Increased glycation of protein has been found to be a consequence of diabetic complications. A number of proteins, including hemoglobin, are glycated to a greater degree in diabetes (32). The increase in glycosylated hemoglobin is directly proportional to the fasting blood glucose level (33) (Figura 3).

Effect of Cucurbita ficifolia on lipid profile

After 40 days of *Cucurbita ficifolia* juice therapy to diabetics, there was no considerable effect on the lipid profile of the studied subjects. However, the total serum cholesterol levels was decreased in diabetic treated to 3.5%. The serum HDL-Cholesterol levels were slightly elevated to 2.1% in normal treated group while in diabetic untreated there was a decline

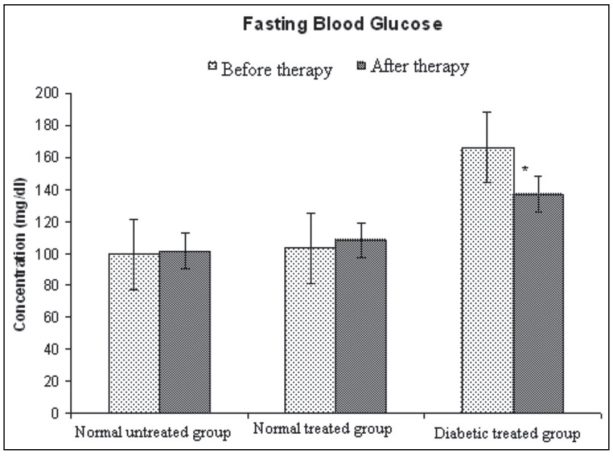

Figure 1. The effect of 40 days of *Cucurbita ficifolia* therapy on fasting blood glucose levels. Participants categorized into three groups (normal untreated control, normal treated and diabetic treated).
Values expressed as Mean ± SE, *Significant Change P<0.01, **Significant Change P<0.001

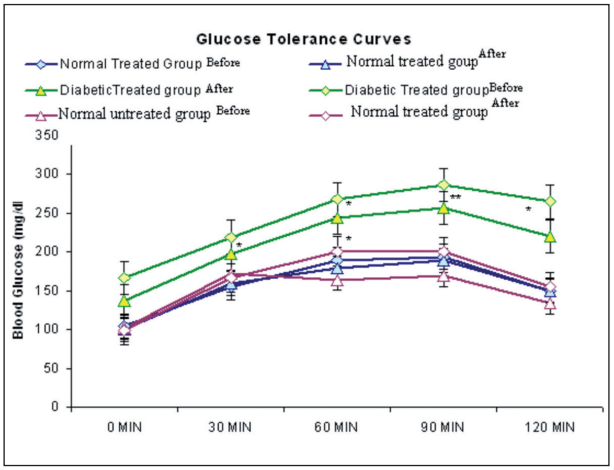

Figure 2. Effect of 40 days of *Cucurbita ficifolia* therapy on glucose tolerance. Blood glucose levels of 12-hour fasting subjects used the procedure of glucose tolerance test (GTT) once before therapy and at end of therapy.
Values expressed as Mean ± SE, * Significant Change P<0.01, **Significant Change P<0.001

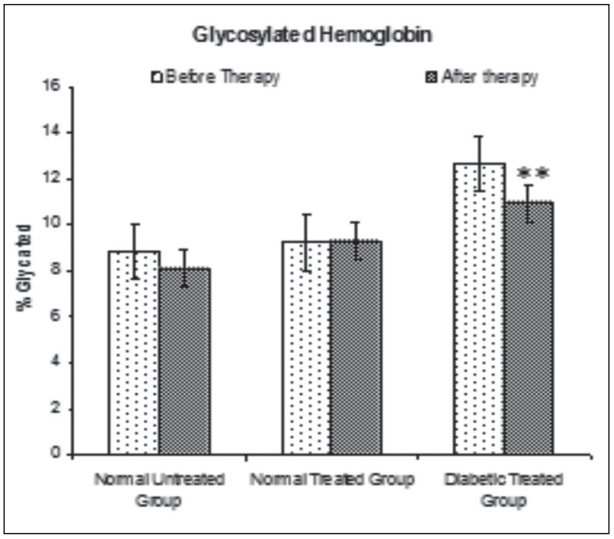

Figure 3. The effect of *Cucurbita ficifolia* on glycosylated hemoglobin before and after therapy of 40 days in normal untreated group, normal treated group and diabetic treated group.
Values expressed as Mean ± SE,* Significant Change P<0.01, **Significant Change P<0.001

of 4.7%. VLDL-Cholesterol levels were found to have decreased to 11.6% in normal treated group, while in diabetic treated VLDL-Cholesterol levels increased to 9.3%. LDL-Cholesterol levels increased to 7.6% and 0.37 % in diabetic treated and normal subjects respectively (Figure 4).

Effect of Cucurbita ficifolia on antioxidant levels and lipid peroxidation

After 40 days of *Cucurbita ficifolia* juice therapy there was an increase of 17.3% and 4.5% for SOD activity and GSH levels, while the blood malondialdehyde level was decreased by 9.3% (P<0.05) in the case of diabetic treated subjects. In normal treated group there was an increase of 3.1% and 17.9% for SOD activity and GSH levels, while the malondialdehyde level was increased by 2.9% (Figure 5 a, b).

Discussion

On a total 14 diabetic subjects were selected for *Cucurbita ficifolia* juice therapy, subjects showed significant improvement in glucose and antioxidant levels. Diabetic subjects showed significant increase in tolerance to glucose evident after *Cucurbita ficifolia* therapy (Figure 2) (P<0.01), fasting blood glucose level (Figure 1) (P<0.001), and glycosylated hemoglobin (figure 3)

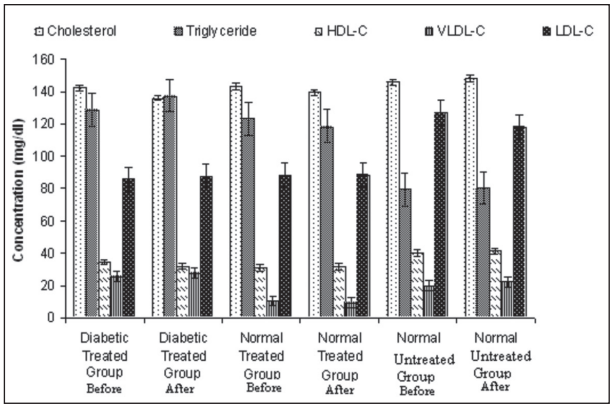

Figure 4. Effect of 40 days of *Cucurbita ficifolia* therapy on fasting lipid profile. (normal untreated group, normal treated group and diabetic treated group) was analysed from venous blood samples once before therapy and at end of therapy.
Values expressed as Mean ± SE,* Significant Change P<0.01, **Significant Change P<0.001

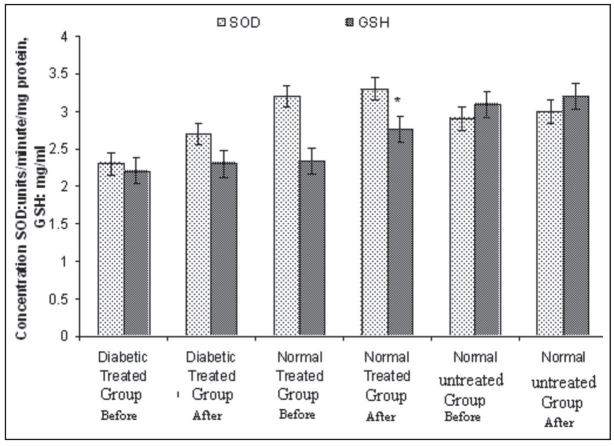

Figure 5a. Effect of 40 days of *Cucurbita ficifolia* therapy on antioxidant levels (normal untreated group, normal treated group and diabetic treated group) were analyzed from venous blood samples once before therapy and at end of therapy.
Values expressed as Mean ± SE,* Significant Change P<0.01, **Significant Change P<0.001

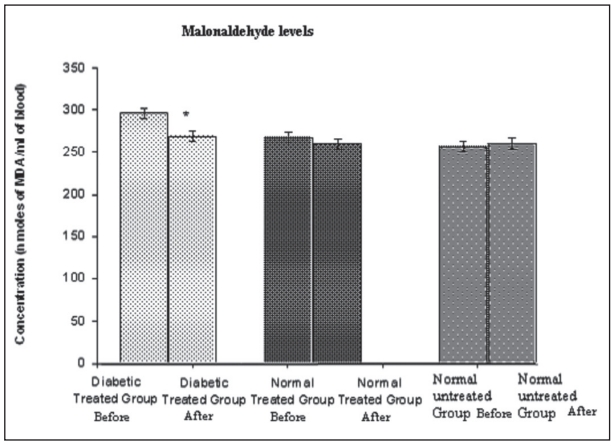

Figure 5b. Effect of 40 days of *Cucurbita ficifolia* therapy on lipid peroxidation (normal untreated group, normal treated group and diabetic treated group) were analyzed from venous blood samples once before therapy and at end of therapy.
Values expressed as Mean ± SE,* Significant Change P<0.01, **Significant Change P<0.001

(P<0.001). This effect is probably due to the reticence of the crucial gluconeogenic enzyme glucose-6-phosphatase (34, 35). In a recent previous study on alloxan-induced diabetic mice, Jessica et al. evaluated the hypoglycemic effect of aqueous extract of *Cucurbita ficifolia* was due to moderately liver glycogen accumulation (36). One human study have been carried out previ-

ously on *Cucurbita ficifolia* regarding its anti-diabetic potential, an oral administration of *Cucurbita ficifolia* decline the blood glucose levels, from 12.07±1.69 mM (217.2±30.4 mg/dl) to 9.42±1.96 mM (169.6±35.3 mg/dl) after 3 hours and following 5 hours a reduction to 8.37±1.74 mM (150.8±31.3 mg/dl) was observed (21). Not much work is available on human subjects; however a few animal studies potentiates the anti-diabetic potential of *Cucurbita ficifolia* fruit extract on diabetic rats that exhibited a noteworthy diminution in the blood glucose, glycosylated hemoglobin levels and showed an improvement in the plasma insulin and total hemoglobin levels (37, 38).

The effect of *Cucurbita ficifolia* has been seen to be under the glucose tolerance curve, in comparison with the control which was 26.4% lesser *Cucurbita ficifolia*, or tolbutamide-treated (14.3%) normal rabbits with weekly oral glucose tolerance test was done (39). *Cucurbita ficifolia* has hypoglycemic activity, similar to tolbutamide (stimulates the secretion of insulin by the pancreas), and it is thought that *Cucurbita ficifolia* could increase either the insulin secretion from the existing pancreatic β-cells or its release in a different form. A compound -D-chiro-inositol was isolated and identified in *Cucurbita ficifolia* and functions as an insulin mediator (40). In another trial the polysaccharide granules of the plant and its polysaccharide liquid has been administered to type 2 diabetes patients, which resulted in a consequential decrease in the post-prandial and fasting glucose levels (41). The current study shows that *Cucurbita ficifolia* fruit extract showed a decrease in glycosylated hemoglobin levels of 22.5% in diabetic subjects which is in conjunction with the study carried out by in streptozotocin induced diabetes in rats (42).

An inevitable but harmful consequence of diabetes is the occurrence of oxidative stress which made the radical scavenging system inefficient to remove all free radicals formed due to cell damage and tissue degeneration (6,7). The ever-reducing scenario prevalent in every living body depends on the efficiency of the enzymes which stably input metabolic energy into the system. However, this balance gets disrupted when the normal redox state is overturned thus releasing the toxic peroxides and free radicals which pose harm to the underlying tissues and also to their components as protein, lipid and deoxyribose nucleic acid (DNA) (8). In the current study after 40 days of therapy there was an increase in SOD activity and GSH levels, while the blood malondialdehyde level was decreased ($P<0.05$) in diabetic treated subjects (Figure 5a, b). Previous reports have shown that serum and liver activities of SOD, glutathione peroxidase (GSH-Px) in mice administered with pumpkin extracts were appreciably elevated ($P<0.01$), and MDA levels in mice given Cucurbita extracts were radically lesser than the Placebo group ($P<0.01$) (43, 44).

Conclusion

From our study it can be effectively concluded that post 40 days of therapy with *Cucurbita ficifolia* juice a reduction in the blood fasting blood glucose level, glycosylated hemoglobin and oxidative stress in diabetic subjects was evident. Moreover the therapy with *Cucurbita ficifolia* in type 2 diabetes reduced the lipid peroxidation. Thus, the study may affirms the anti-hyperglycemic and antioxidant property of *Cucurbita ficifolia* in type 2 diabetes and hence it may advisable to patients with hyperglycemia.

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