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A dietary supplement to Ameliorate Hyperglycemia and associated complications in Streptozotocin injected rats.

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Abstract: A dietary supplement was formulated from a number of plants and herbs including guar gum, chicory, cape gooseberry fruit, turmeric, blackberry, ginkgo biloba and sumac. These food items were assumed to improve hyperglycemia and to correct the associated complications. Analytical evaluation of this supplement proved that it posses an antioxidant power evidenced by the total polyphenol content (1679.17 ± 96.91 mg GAE /100g dry weight) and flavonoid content as quercetin (802.33 ± 3.35 mg QE/100g dry weight). It is also rich in some phenolic compounds including gallic acid, protocatchuic acid, chlorogenic acid, caffeic acid, vanillic acid, coumarin, rosemarinic acid, cinnamic acid and chyrsin. Biological evaluation of the supplement proved its ability to reduce hyperglycemia in streptozotocin injected rats (from 393.17 ± 25.23 mg/dl for diabetics to 276.33 ± 42.98 mg/dl for diabetics + formula). This dietary supplement proved to be safe with regard to health. It did not affect liver or kidney functions or hemoglobin synthesis. Even the derangement that occurred in these parameters due to injection of streptozotocin and hyperglycemia was corrected when this supplement was included in the diet of hyperglycemic rats.

In conclusion, it is assumed that this supplement can be given to human suffering from diabetes mellitus along with hypoglycemic drugs after carful studies on humans to standardize the conditions most suitable to its practical use.

Key words: Dietary supplement, hyperglycemia, streptozotocin, diabetes mellitus, guar gum, chicory, cape gooseberry fruit, turmeric, blackberry, ginkgo biloba, sumac, antioxidants and polyphenols.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease. It is not mere a state of hyperglycemia but also complications associating the disease. These complications affect so many organs in the body, such as orthopedic complications (1), neuropathy (2), nephropathy (3), retinopathy **(**4), cardiomyopathy and diabetic feet (5). It is thus necessary that the therapeutic strategies for the disease take into consideration to treat or ameliorate its complications.

Several investigations proved that natural products present in plants either fruits, vegetables or herbs have effective roles to control either hyperglycemia or the associating complications. These natural products include lignanes, flavonoids, polyphenols and terpenoids (6).

There are several plants reported to contain active constituents able to control diabetes and its complications. Guar gum is a soluble dietary fiber formed of polygalactomannan derived from seeds of the legume plant, *Cyamopsis tetragonalobus* (7). Partially hydrolyzed guar gum was found to decrease the postprandial blood glucose by lowering the rate of absorption from the small intestine in the rat and by reducing the diffusion of glucose in the lumen (8).

Chicory (*Cichorium intybus* L.) is a rich source of the dietary fiber inulin (9)**.** Supplementation of inulin (10 g/d) to diabetic women for 2 months improved fasting plasma glucose, insulin, and hemoglobin A1c (HbA1c) levels, and decreased malondialdehyde levels compared with maltodextrin supplementation (10). It was reported that chicory root extract could delay or prevent the early onset of diabetes mellitus and improve bowel movements (11)**.**

Kasali et al., (2013) found that the crude aqueous extracts from the leaves of *Physalis peruviana* L. (cape gooseberry fruit) present hypoglycemic activity in animal model, but at high doses the plant may cause severe intoxication.

It was mentioned that more than 7000 published articles have shed light on the various aspects of curcumin including its antioxidant, hypoglycemic, anti-inflammatory and anti-cancer activities (13).

Berries are known to be rich in bioactive flavonoid compounds with antioxidant activities (14).

Ginkgo biloba extracts have drawn attention for their antioxidant and anti-platelet properties that can protect against atherosclerosis in diabetic patients (15). It was reported to enhance the hepatic metabolic clearance rate of insulin and hypoglycemic agents resulting in reducing elevated blood glucose in non-insulin-dependent diabetes mellitus (16). Also, it was proved to prevent diabetic retinopathy in diabetic rats (17) and improved blood flow rate in retinal capillaries in type 2 diabetic patients with retinopathy (18).

Sumac was among the plants listed by McCune (2013) that is associated with higher antioxidant activity and antidiabetic effect.

The association between diabetes and free radicals reported before in many articles means that compounds with antioxidant properties can be helpful to protect against the degenerative effect of these free radicals which may be among the factors contributing to the incidence of diabetes. Diabetics can counteract the progression of their disease by improving insulin sensitivity, enhancing glucose metabolism, and attempting to mitigate the complications of disease.

The proper and effective treatment of diabetes or any other disease has to take into consideration the control of the disease and alleviation of the associated complications. It seems difficult to verify this using only one item. It is necessary to include several plant sources to make use of the diverse compounds with health benefits they contain. In the present study, a dietary supplement was formulated. The formula constituted from several plants and herbs including guar gum (*Cyanopsis tetragonoloba*), chicory (*Cichorium intybus*), cape goose berry (*Physalis peruviana L.*), turmeric (*Curcuma longa* L.), black berry (*Rubus fruticosus*), ginkgo biloba (*Ginkgo biloba*), and sumac (*Rhus coriaria*). These ingredients were selected according to reported investigations indicating the presence of bioactive compounds able to control the disease and cure or minimize associated complications. These ingredients were mixed in a dry powdered form in certain proportion and used as dietary supplement to control streptozotocin induced diabetes in rats and its associated complications.

Materials and Methods

Materials

Animals used in this experiment were Sprague Dawley male albino rats obtained from the animal house of the National Research Centre. The body weight of the animals ranged from 123-180 gm.

The formula used as a supplement composed of guar gum, chicory, cape gooseberry fruit, turmeric, blackberry, ginkgo biloba and sumac. All these constituents were purchased from the local market.

Streptozotocin (STZ) used for induction of hyperglycemia in rats was obtained from Sigma-Aldrich Co., USA. Citrate buffer (pH 4.5 & 0.1 mol/l) in which STZ was dissolved was obtained from Biodignostic Co., Egypt.

Most of the constituents of the diet formulated and introduced to the rats were purchased from the local market, while, casein was obtained from Scerma Co., France. The salts and vitamins mixtures used were of analytical grade obtained from Fluka (Germany) and BDH (England) Chemical Companies.

Folin–Ciocalteu reagent, gallic acid, quercetin and all standards for HPLC analysis were obtained from Sigma-Aldrich Co., USA.

Diagnostic kits used for the determination of alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were obtained from Salucea Co., Netherlands. Glycosylated hemoglobin (Hb A1C) was measured using a kit from BioSystems S. A., Barcelona, Spain. The kit used for determination of glucose-6-phosphate dehydrogenase (G6PDH) activity was from Biochemical Enterprise (BEN) Co., Millano, Italy. Kits used for determination of blood glucose, blood hemoglobin, plasma urea, plasma creatinine, plasma total protein, plasma albumin were obtained from Biodiagnostic Co., Egypt. Insulin was determined using the rat insulin ELISA kit obtained from Glory Science Co., Ltd., USA.

Methods

Preparation of the formula

The selected ingredients (guar gum, chicory, cape gooseberry fruit, turmeric, blackberry, ginkgo biloba and sumac) were dried in an air ventilated oven at 60 C^o till complete dryness. Then, the dried ingredients were milled into fine powder in a mechanical grinder (Braun, Germany). The powdered ingredients were mixed together in suitable concentrations assumed to exert bioactive role and according to panel testing as follows; guar gum (14%), chicory (14%), cape gooseberry fruit (22%), turmeric (14%), blackberry (15%), Ginkgo biloba (14%) and sumac (7%). This powder was subjected to chemical analysis for determination of polyphenolic compounds prior to the feeding experiment.

Extraction and Determination of total polyphenol & flavonoid contents

The powdered formula was extracted with either of two solvents, once with 80% methanol and the other with 80% ethanol according to the method of Hayat *et al*., (2010). The moisture content of the sample was determined in order to calculate the concentrations on a dry weight basis.

Total polyphenol content was determined by Folin–Ciocalteu assay according to the method of Ramful, et al. (2011). Results are expressed in mg of gallic acid equivalent per 100 g of dry weight.

Total flavonoid content (TFC) was determined using a colorimetric method described previously (22). The results were expressed as mg of quercetin equivalent (mg of QE/100 g of sample).

Determination of individual polyphenols by HPLC

Individual polyphenols content of the formula was determined in the two extracts by HPLC according to Kim, et al., (2006) as follows:

The HPLC analysis was performed using a Gilent Technologies 1100 series liquid chromatograph equipped with an auto-sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm. All samples were filtered through a 0.45 µm Acro disc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

Formulation of the diet

The standard control diet was prepared according to Reeves et al., (1993) as shown in table (1). Then the formula was added to the diet of the selected groups on the expense of starch.

Table (1): Composition of diet of control rats (g/100g) diet

Ingredients	Amount
Casein*	22
Sucrose	10
Cellulose	5
Corn oil	4
Salt mixture (AIN-93)*	3.5
Vitamin mixture (AIN-93)*	1
Choline bitartrate	0.25
L- Cystine	0.18
Corn starch	54.07

* Protein content of casein was estimated as 54.6%.
* Control diet was prepared according to Reeves et al., 1993.
* Salt and vitamin mixtures were prepared according to Reeves et al., 1993

Animal Experiment

The experiment was done on a number of 24 rats. A group of 6 rats was separated from the whole group to serve as control. This was denoted as (group 1) and was fed on the control diet. Another group denoted group (2) was given the control diet with 100 gm formula/1kg diet. The rest of the rats were injected intraperitonealy with streptozotocin dissolved in 0.1 mol/l citrate buffer (PH 4.5) which was freshly prepared before injection in a dose of 60 mg/kg B.wt. for each rat to induce hyperglycemia. The fasting blood sugar of these rats was followed afterwards to insure hyperglycemia. Animals with fasting blood sugar of 200 mg/100 ml blood or above were included in the experiment others were excluded. These animals were subdivided into 2 groups each of 6 rats. These groups were:

Group 3: fed on a standard control diet and served as positive control.

Group 4: fed on the standard control diet containing 100 gm of the formula/kg diet.

Animals were kept in stainless steel cages in a temperature controlled room at 25 °C. Light was adjusted day and night. Food and water were allowed ad libtum to the animals during the whole experiment which lasted for 6 weeks. During the experiment the food consumption was followed every two consecutive days and the body weight was also followed.

At the end of the experiment, all rats were fasted overnight and in the early morning fasting blood samples were collected obtained by open heart puncture and divided into two portions; one over heparin to determine parameters of the whole blood and that of the RBCs immediately. The other was also over heparin but subjected for separation of plasma after centrifugation at 4000 rpm for 15 minutes. All biological samples were chilled in crushed ice mixture till handled or for the next step. Plasma was kept in deep freeze at -20 °C until analysis.

The methods used for the estimation of the chemical parameters were as follows:

Blood glucose was estimated according to the method described by Trinder (1969). Blood hemoglobin was determined according to Betke and Savelsberg (1950). Glycosylated hemoglobin was detected as described by the method of Hoelzel et al., (2004). G6PDH was estimated in whole blood following the procedure of Beutler et al., (1979). Insulin assay was determined according to Lomedico et al., (1980). The activities of both ALT & AST were measured according to the method of Henry et al., (1960). Plasma urea and creatinine each was determined according to Fawcett & Soctt, (1960) and Bartles, et al., (1972), respectively. Plasma total protein was determined according to Cannon et al. (1974). Plasma albumin was assessed according to Doumas et al. (1971).

Results

Analysis of the formula (table 2) showed that the total polyphenol content determined as gallic acid of both ethanolic and methanolic extracts were 891.67 ± 51.83 & 1679.17 ± 96.91 mg gallic acid equivalent (GAE) /100g dry weight, respectively, while the flavonoid content as quercetin equivalent of the formula was 312.33 ± 7.26 & 802.33 ± 3.35 mg QE/100g dry weight for ethanolic and methanolic extracts, respectively. From the obtained results it is obvious that methanol gives better extraction for both total polyphenols and flavonoid contents of the formula.

Table (2): Total polyphenols (as gallic acid equivalent) & flavonoids (as quercetin equivalent) of ethanolic and methanolic extracts for the prepared formula

Test / Extract	Total polyphenols (mg GAE/100g)	Flavonoids (mg QE/100g)
Ethanolic extract	891.67 ± 51.83 [a]	312.33 ± 7.26 [a]
Methanolic extract	1679.17 ± 96.91 [b]	802.33 ± 3.35 [b]

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Table (3): Differential pattern of polyphenol contents of the formula (ethanolic and methanolic extracts) as detected by HPLC expressed in mg/ 100g dry weight

Extract / Polyphenol	Ethanolic extract (mg/100g d. w.)	Methanolic extract (mg/100g d. w.)
Gallic acid	32	93.63
Protocatchuic acid	19.26	208.25
Chlorgenic acid	33	22.5
Caffeic acid	13.76	8.3
Vanillic acid		4.9
Coumarin	15	10.15
Rosmarinic acid	611.1	31.54
Cinnamic acid	54.42	4
Chyrsin	665	25.5

The HPLC analysis of the formula (table 3) showed the presence of: gallic acid, protocatchuic acid, chlorogenic acid, caffeic acid, vanillic acid, coumarin, rosemarinic acid, cinnamic acid and chyrsin.

Table (4): Food intake, change in body weight, feed efficiency ratio (FER) of the control group and the different groups

Test / Group	Food intake (g)	Body Weight change (g)	FER
Control Mean± SE	591.00 ± 5.77[b]	146.17 ±16.73 [b]	0.25 ±0 .03[b]
Control+formula Mean± SE	571.33 ± 5.77[b]	138.67 ± 5.12 [b]	0.24 ± 0.01[b]
Control diabetic Mean± SE	493.17± 8.49 [a]	-26.50± 6.72 [a]	-0.048 ± 0.01[a]
Diabetic+formula Mean± SE	574.17 ± 4.81[b]	-7.50 ± 7.80 [a]	-0.01 ±0 .01[a]

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

As mentioned before the food intake and body weight of animals were followed. As shown in table (4). The food intake of streptozotocin injected rats (493.17 ± 8.49 g) was significantly lower than that of controls (591.0 ± 5.77 g). Addition of the formula to the diet caused no marked change in the amount of diet consumed by the control animals while the consumption of diabetic rats that were given the formula was significantly increased relative to diabetic rats not given the formula. The body weight gain of control rats was not much different when the formula was added to the diet. It can be noticed that diabetic rats lost weight (-26.50± 6.72 g) relative to a gain in control amounting to (146.17 ±16.73 g); however the loss in body weight was less significant when the formula was included with the diet (-7.50 ± 7.80 g). The feed efficiency ratio in case of diabetic rats was markedly affected showing a significant drop. The values obtained for control diabetic was (-0.048 ± 0.01) and for diabetic and the formula the drop was less significant (-0.01 ± 0.01) relative to a value of (0.25 ± 0.03) for controls. Controls given the formula did not show any appreciable effect on the FER.

Table (5): Organ weight percentage of the control group and other groups

Test / Group	Pancreas%	Heart%	Liver%	Spleen%	Kidney%
Control Mean± SE	0.22 ± 0.04^{b}	0.31 ± 0.02^{a}	3.16 ± 0.17^{b}	0.39 ± 0.05^{a}	0.64 ± 0.02^{b}
Control+formula Mean± SE	0.22 ± 0.04^{b}	0.34 ± 0.02^{a}	3.09 ± 0.06^{b}	0.33 ± 0.08^{a}	0.67 ± 0.03^{b}
Control diabetic Mean± SE	0.36 ± 0.03^{a}	0.41 ± 0.04^{a}	5.08 ± 0.41^{a}	0.39 ± 0.03^{a}	1.15 ± 0.04^{a}
Diabetic+formula Mean± SE	0.23 ± 0.02^{b}	0.39 ± 0.04^{a}	4.73 ± 0.33^{a}	0.36 ± 0.06^{a}	1.16 ± 0.07^{a}

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Regarding organ weight % (table 5), no marked difference was noticed between the weight percent of pancreas, heart, liver, spleen or kidney of control rats either given the formula with the diet or not. However, the organ weight percent of diabetic rats except the spleen was higher than normal. Adding the formula to the diet of this diabetic group caused decrease in the weight percent of pancreas and heart.

Table (6): Concentration of plasma glucose & plasma insulin of the control group and other groups.

Test / Group	Glucose mg/dl	Insulin mU/L
Control Mean± SE	76.63 ± 1.86^{c}	2.88 ± 0.27^{a}
Control+formula Mean± SE	78.83 ± 7.01^{c}	2.52 ± 0.31^{a}
Control diabetic Mean± SE	393.17 ± 25.23^{a}	2.26 ± 0.09^{a}
Diabetic+formula Mean± SE	276.33 ± 42.98^{b}	2.76 ± 0.22^{a}

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Injection of streptozotocin caused a marked elevation of the fasting plasma glucose level of rats (table 6). The level of plasma glucose of normal rats was 76.63 ± 1.86 mg/dl and that of diabetic animals was 393.17 ± 25.23 mg/dl. Addition of the formula to the diet of control animals caused non-appreciable change in blood sugar while addition of the formula to the diet of diabetic rats caused a significant decrease in plasma glucose. Plasma insulin of diabetic rats was lower than that of controls, however the decrease in plasma insulin was statistically non significant. The values for plasma insulin of control rats was 2.88 ± 0.27 mU/l and for diabetic it was 2.26 ± 0.09 mU/l. Addition of the formula to the diet of diabetic rats caused a moderate increase in the value of plasma insulin (2.76 ± 0.22 mU/l).

Diabetic rats had a low blood hemoglobin level (table 7). The control value of hemoglobin was 14.55 ± 0.49 g/dl while the diabetic value was 12.45 ± 0.30 g/dl. Addition of the formula to the diet of diabetic animals restored the hemoglobin level to near the normal value (14.89 ± 0.33 g/dl).

Glycosylated hemoglobin (HBA1C) level of control rats was not affected by addition of the formula to their diet (table 7). Injection of streptozotcin caused a slight but significant drop in (HBA1C) level, and addition of the formula caused a marked increase in the level of (HBA1C).

Table (7): Hemoglobin concentration, glycosylated hemoglobin concentration (HbA1c) & activity of whole blood glucose-6-phosphate dehydrogenase of the control group and other groups

Test / Group	Hb g/dl	HbA1c g/dl	G6PDH U/g hemoglobin
Control Mean± SE	14.55 ± 0.49^{b}	4.67 ± 0.12^{c}	14.24 ± 0.72^{bc}
Control+formula Mean± SE	15.49 ± 0.59^{b}	4.84 ± 0. 36^{c}	13.13 ± 0.60^{c}
Control diabetic Mean± SE	12.45 ± 0.30^{a}	3.82 ± 0.26^{a}	23.43 ± 1.21^{a}
Diabetic+formula Mean± SE	14.89 ± 0.33^{b}	6.75 ± 0.25^{b}	17.03 ± 1.17^{b}

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

As shown in table (7), the level of glucose-6-phosphate dehydrogenase (G6PDH) was markedly elevated as a result of injection of streptozotocin. The value obtained for normal rats was 14.24 ± 0.72 U/g hemoglobin and for diabetic it was 23.43 ± 1.21 U/g Hb. Adding the formula to the diet of control animals did not cause an appreciable change in the value, while addition of the formula to the diet of the diabetic group caused a significant decrease in the value (17.03 ± 1.17 U/g Hb).

The parameters of liver function were all deranged due to injection with streptozotocin and the resulting hyperglycemia. As shown in table (8) marked increase in the activities of the AST and ALT enzymes was noted. The values reported for AST and ALT for normal rats were 33.83 ± 2.99 and 23.00 ± 1.73 U/l, respectively and the values for diabetic rats were 72.50 ± 7.94 and 72.00 ± 8.23 U/l, respectively. No appreciable drop occurred in these values when the formula was included in the diet of control rats (27.67 ± 3.20 & 21.67 ± 1.59 U/l for AST & ALT, respectively), while the drop was significant in case of diabetic rats having the formula (56.67 ± 14.62 & 44.17 ± 15.88 U/l for AST & ALT, respectively).

Table (8): Activities of aspartate aminotransferase (AST) & alanine aminotransferase (ALT) of the control group and other groups

Test / Group	AST (U/L)	ALT (U/L)
Control Mean± SE	33.83 ± 2.99^{bc}	23.00 ± 1.73^{b}
Control+formula Mean± SE	27.67 ± 3.20^{c}	21.67 ± 1.59^{b}
Control diabetic Mean± SE	72.50 ± 7.94^{a}	72.00 ± 8.23^{a}
Diabetic+formula Mean± SE	56.67 ± 14.62^{ab}	44.17 ± 15.88^{b}

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

The plasma total protein and the albumin levels, as shown in table (9), were significantly decreased in diabetic rats (7.32 ± 0.92 and 3.87 ± 0.56 g/dl) relative to a control value of (10.49 ± 0.73 and 6.39 ± 0.15 g/dl).

Addition of the formula to the diet of the diabetic group made the level of plasma protein and albumin return to near normal.

Table (9): Concentration of total protein and albumin of the control group and other groups.

Test / Group	Total protein g/dl	Albumin g/dl
Control Mean± SE	10.49 ± 0.73[b]	6.39 ± 0.15[b]
Control+formula Mean± SE	9.40 ± 0.93[ab]	6.31 ± 0.40[b]
Control diabetic Mean± SE	7.32 ± 0.92[a]	3.87± 0.56[a]
Diabetic+formula Mean± SE	8.24 ± 0.44[ab]	6.19 ± 0.45[b]

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

The parameters of the kidney function namely urea and creatinine were significantly higher than normal levels in hyperglycemic streptozotocin injected animals (table 10). The reported value for urea in control rats was 27.12 ± 3.15 mg/dl and for creatinine was 0.51 ± 0.03 mg/dl, respectively. In case of diabetic rats the values were 71.48 ± 4.87 and 0.80 ± 0.06 mg/dl for urea and creatinine, respectively. The increase in plasma creatinine that occurred in diabetic animals was not corrected by addition of the formula to the diet, while a significant decrease in plasma urea was noticed by addition of the formula to the diet of diabetic rats.

Table (10): Concentration of plasma urea & plasma creatinine of the control group and all other groups

Test / Group	Urea (mg/dl)	Creatinine (mg/dl)
Control Mean± SE	27.12 ± 3.15[c]	0.51 ± 0.03[b]
Control+formula Mean± SE	29.58 ± 1.49[c]	0.57 ± 0.03[b]
Control diabetic Mean± SE	71.48 ± 4.87[a]	0.80 ± 0.06[a]
Diabetic+formula Mean± SE	54.12 ± 5.63[b]	0.79 ± 0.08[a]

*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Discussion

In this study, a number of plant sources were selected reported to reduce hyperglycemia and ameliorate the associated complications. They were air dried, ground and then mixed in proportions to insure safety use and effective medical action. Guar gum is one of the constituents of the formula used in this study. It is the most promising soluble type of dietary fiber that contains polygalactomannan derived from seeds of legume plant, *Cyamopsis tetragonalobus*. European health claims ensure the role of glucomannan (konjac mannan) from guar gum in the maintenance of normal blood cholesterol levels, and to the contribution of glucomannan in the reduction of body weight (35). Thus, the reduction in body weight of control rats taking the formula with their diet may be attributed to the role of guar gum. Hydrolyzed guar gum has been also reported to decrease postprandial blood glucose and glucose absorption in the rat small intestine (8).

Chicory, which is also a rich source of the dietary fiber inulin, is another constituent of the used formula. It was reported to delay or prevent the early onset of diabetes mellitus (36) and to improve bowel movements (37).

It is postulated that the two constituents used in composition of the formula namely guar gum and chicory that are main sources of dietary fiber either the polygalactomannan or inulin can be factors contributing to the hypoglycemic action reported in diabetic rats receiving the formula with their diet. This action can be attributed to promotion of satiety by slowing the rates of gastric emptying and nutrient absorption.

Curcumin (diferuloylmethane) which is one of the constituents of this formula is a component of turmeric isolated from the rhizome of *Curcuma longa*. Several health benefits were attributed to curcumin including its antioxidant, hypoglycemic, anti-inflammatory and anti-cancer activities (13, 38). The role of curcumin as a hypoglycemic agent thus adds to the antidiabetic action of the formula.

The other 4 constituents of the formula namely blackberry, Ginkgo biloba, cape gooseberry and sumac are known to be rich in several polyphenolic compounds such as anthocyanins, flavonols, flavones, flavanols, flavanones, and isoflavonoids, stilbenes, tannins, and phenolic acids. Total polyphenolic content assay and total flavonoid content assay (table 2) showed that the formula is rich in both polyphenolic and flavonoid contents. The HPLC analysis of the formula as illustrated in table (3) showed that the formula is rich in some phenolic compounds namely; gallic acid, protocatchuric acid, chlorogenic acid, caffeic acid, vanillic acid, coumarin, rosemarinic acid, cinnamic acid and chyrsin. These phenolic compounds are known for their antioxidant characters which protect the body against degenerative diseases. This may explain the protective action of the formula particularly regarding the pancreas, against the free radicals generation known to occur in diabetes (39, 40, 41).

As expected, the plasma insulin level of streptozotocin injected rats was low relative to normal rats however the drop was not significant. Again, adding the formula to the diet of diabetic animals caused a relative increase in the level of plasma insulin. This may indicate a protective action of the bioactive compounds in the formula to pancreatic tissue which prevent any further destruction to pancreatic cells and also the promoting effect on insulin secretion by pancreatic cells. The polyphenols present in these constituents play the major role against the degenerative action of streptozotocin or hyperglycemia (13).

The hemoglobin concentration of normal rats was not affected by consumption of the formula with the diet which indicates that the constituents of the formula do not exert any adverse effect on hemoglobin formation. Even the drop in hemoglobin concentration that occurred in diabetic rats was corrected when these rats were given the formula with the diet. Most investigations on diabetes concentrate on blood glycosylated hemoglobin rather than hemoglobin being considered as an indicator to the diabetic state. In spite of the high blood sugar of the diabetic rats, the value of the glycosylated hemoglobin was low (3.82 ± 0.26 g/dl) relative to a normal value of (4.67 ± 0.12 g/dl). The concordance between blood glucose concentration and glycosylated hemoglobin in human is strongly confirmed. However, in animals the situation seems different. It has been reported that in humans the level of HbA1c reflects the average plasma glucose level over 3 months (42) a condition that does not exist in animals where the diabetic state was induced in rats and the experiment lasted for 6 weeks only. Perhaps if the experiment would last for longer time enough for glycosylated hemoglobin to form, a correlation between blood glucose and glycosylated hemoglobin would be noticed.

The beneficial action of the formula extended to decrease the elevated activity of G6PDH in streptozotocin injected rats (which is most probably due to lysing of the membrane of red blood cells) returning it near the control value. The beneficial effect of the formula leads to a better supply of energy to the red blood cells and keeping on their intact ability (43).

Liver dysfunction was reported in many patients suffering from diabetes mellitus decades ago (44). In this study, the liver dysfunction of diabetic rats evidenced by the increased activity of both plasma enzymes AST and ALT together with decreased level of plasma total protein and albumin was ameliorated by inclusion of the formula with the diet of these animals. As mentioned before the presence of bioactive compounds with anti-inflammatory characters or antioxidant properties are suggested to be behind the protective effect of these constituents that could protect the liver either from the direct effect of the injected streptozotocin or the action of hyperglycemia. Most probably the bioactive polyphenolic compounds are concerned with this role. They protect the liver cells from degeneration caused by free radicals, thus minimize the leakage of the hepatic enzymes from degenerated liver cells into the blood which is the cause of increased activities of the liver enzymes AST and ALT in blood. This phenomenon of liver cell protection is reflected on the capacity of the liver to synthesize different proteins including albumin. This explains the increase in plasma albumin level of

diabetic rats given the formula. It has been reported that the plasma proteins from type 2 diabetics bind and protect the antioxidants less than the ones in healthy blood do and that diabetes diminishes the benefits of dietary polyphenols (45). This means that the hypoglycemic effect of the formula will diminish the formation of glycation products thus render proteins more able to protect the antioxidants and make them able to efficiently scavenge free radicals.

The kidney function of diabetic rats was also defected indicated by the increased level of both plasma urea and creatinine. The development of diabetic nephropathy may be caused by several factors including hyperglycemia, hypertension, oxidative stress, and inflammation (46). In this case the cause of kidney dysfunction due to streptozotocin injection can be similar to that in case of the liver. In turn may be ameliorated by the bioactive compounds that can act as anti-inflammatory or antioxidant present in the different constituents of the formula. This is indicated by improvement in plasma urea when the formula was added to the diet of diabetic rats, although there was no improvement in plasma creatinine.

In conclusion a dietary supplement could be composed from some plant constituents with specific proportion aiming to control hyperglycemia and associated complications. This supplement did not show any health complications evidenced by the normal growth, blood hemoglobin, liver and kidney functions of the rats given the supplement with diet. This supplement succeeded to reduce hyperglycemia in rats after injection with streptozotocin. It is assumed that this supplement can be given to human suffering from diabetes mellitus along with hypoglycemic drugs after carful studies on humans to standardize the conditions most suitable to its practical use.

References

1. Gehling, D. J.; Lecka-Czernik, B & Ebraheim, N. A. (2015), "Orthopedic complications in diabetes.", Bone, In Press, Corrected Proof, Available online 23 July 2015.
2. Salvotelli, L., Stoico, V., Perrone, F., Cacciatori, V., Negri, C., Brangani, C., Pichiri, I., Targher, G., Bonora, E. & Zoppini, G., (2015) "Prevalence of neuropathy in type 2 diabetic patients and its association with other diabetes complications: The Verona Diabetic Foot Screening Program.", Journal of Diabetes and its Complications, In Press, Corrected Proof, Available online 2 July.
3. Houldsworth, A., Hodgkinson, A., Shaw, S., Millward, A. & Demaine, A. G., (2015), "Polymorphic differences in the SOD-2 gene may affect the pathogenesis of nephropathy in patients with diabetes and diabetic complications.", Gene; 569 (1): 41-45.
4. Congdon, N. G., Friedman, D. S. & Lietman, T., (2003), "Important causes of visual impairment in the world today.", JAMA; 290: 2057-2060.
5. Hammes, H. P., (2003), "Pathophysiological mechanisms of diabetic angiopathy", J Diabetes Complications; 17(2 Suppl):16-9.
6. Hung, H. Y., Qian, K., Morris-Natschke, S. L., Hsu, C. S. & Lee, K. H., (2012), "Recent discovery of plant-derived anti-diabetic natural products.", Nat. Prod. Rep.; 29: 580-606.
7. Dogan, V., Kayacier, A. & Ic, E., (2007), "Rheological characteristics of some food hydrocolloids processed with gamma irradiation.", Food Hydrocolloids; 21 (3): 392-396.
8. Takahashi, T., Yokawa, T., Ishihara, N., Okubo, T., Chu, D. C., Nishigaki, E., Kawada, Y., Kato, M. & Juneja, L. R., (2009), "Hydrolyzed guar gum decreases postprandial blood glucose and glucose absorption in the rat small intestine.," Nutrition Research; 29 (6): 419-425.
9. Roberfroid, M. B., (2007), "Inulin-type fructans: functional food ingredients.", J. Nutr; 137: 2493S-2502S.
10. Gargari, B. P., Dehghan, P., Aliasgharzadeh, A., Jafar-Abadi, M. A., (2013), "Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with type 2 diabetes.", Diabetes Metab J; 37: 140-148.
11. Nishimura, M., Ohkawara, T, Toshiyuki, K, Kitagawa, K, Nishimura, H. & Nishihira, J., (2015), "Effects of the extract from roasted chicory (Cichorium intybus L.) root containing inulin-type fructans on blood glucose, lipid metabolism, and fecal properties.", Journal of Traditional and Complementary Medicine; 5 (3): 161-167.
12. Kasali, F. M., Kadima, J. N., Mpiana, P. T., Ngbolua, K. N. Tshibangu, D. S. T., (2013), "Assessment of antidiabetic activity and acute toxicity of leaf extracts from Physalis peruviana L. in guinea-pig.", Asian Pacific Journal of Tropical Biomedicine; 3(11): 841-846 .

13. Ghosh, S., Banerjee, S. & Sil, P. C., (2015), "The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: A recent update.", Food and Chemical Toxicology; 83:111-124.
14. Wu, P., Ma, G., Li, N., Deng, Q., Yin, Y., Huang, R., (2015), "Investigation of in vitro and in vivo antioxidant activities of flavonoids rich extract from the berries of Rhodomyrtus tomentosa (Ait.).", Hassk Food Chemistry; 173: 194-20.
15. Lim, S. & Park, K. S., (2014), "The Use of Ginkgobiloba Extract in Cardiovascular Protection in Patients with Diabetes.", Diabetes: Oxidative Stress and Dietary Antioxidants: 165-172.
16. Kudolo, G. B., (2001), " The effect of 3-month ingestion of Ginkgo biloba extract (EGb 761) on pancreatic beta-cell function in response to glucose loading in individuals with non-insulin-dependent diabetes mellitus.", J Clin Pharmacol; 1(6): 600-11.
17. Doly, M., Droy-Lefaix M. T., Bonhomme, B. & Braquet, P., (1988), "Effect of Ginkgo biloba extract on the electrophysiology of the isolated diabetic rat retina", In: Fungeld EW, Ed. Rokan (Ginkgo biloba)-Recent Results in Pharmacology and Clinic, New York: Springer-Verlag; PP. 83-90.
18. Huang, S. Y., Jeng, C., Kao, S. C., Yud, J. J. H. & Liuc, D. Z., (2004), "Improved haemorrheological properties by Ginkgo biloba extract (Egb 761) in type 2 diabetes mellitus complicated with retinopathy.", Clinical Nutrition; 23: 615-621.
19. McCune, L. M., (2013), "Traditional Medicinal Plants of Indigenous Peoples of Canada and Their Antioxidant Activity in Relation to Treatment of Diabetes.", Ch.: 22, In: "Bioactive Food as Dietary Interventions for Diabetes.", Pages 221-234.
20. Hayata, K., Zhanga, X., Chen, H., Xiaa, S., Jia, C. & Zhonga, F., (2010), "Liberation and separation of phenolic compounds from citrus mandarin peels by microwave heating and its effect on antioxidant activity.", Separation and Purification Technology; 73; 371-376.
21. Ramful, D., Tarnus, E., Aruoma, O. I., Bourdon, E., & Bahorun, T., (2011), "Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps.",Food Research International; 44: 2088-2099.
22. Heimler, D., Vignolini, P., Dini, M. G. & Romani, A., (2005), "Rapid tests to assess the antioxidant activity of Phaseolus vulgaris L. dry beans.", Journal of Agricultural Food Chemistry; 53: 3053-3056.
23. Kim, K., Tsao, R., Yang, R., & Cui, S. W., (2006), "Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions.", Food Chemistry; 95: 466-473.
24. Reeves, P. G., Nielsen, F. H. and Fahey, G. C., (1993), "AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A rodent diet.", J. Nutr.; 123: 1939-1951.
25. Trinder, P., (1969), "Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor.", Ann Clin Biochem; 6: 24-25.
26. Betke, K. and Savelsberg, W., (1950), "Stufenphotometrisho Hemoglobin estimmung mittels cyanohemoglobin.", Z. Biochem.; 320:431.
27. Hoelzel, W., Weykamp C., Jeppsson JO., et al., On behalf of the IFCC Working Group on HbA1c Standardization, (2004), " IFCC Reference System for Measurement of Hemoglobin A 1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study.", Clin Chem.; 50(1): 166-174.
28. Beutler, E., Blume, K. G., Kaplan, J. C., Löhr, G. W., Ramot, B. and Valentine, W. N., (1979), "International Committee for Standardization in Haematology: recommended screening test for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency.", Br. J. Haematol.; 43(3): 465-7.
29. Lomedico, P. T. , Rosenthal, N. , Kolodner, R. , Efstratiadis, A. & Gilbert, W., (1980), "The structure of rat preproinsulin genes.", Ann. N. Y. Acad. Sci.; 343: 425-432.
30. Henry, R. J., Chiamori, N., Golub, O. J. & Berkman, S., (1960), "Revised spectrophoto- metric methods for the determination of glutamic-oxalacetic transaminase, glutamic- pyruvic transaminase, and lactic acid dehydrogenase.", Amer. J. clin. Path.; 34: 381-398.
31. Fawcett, J. K. & Soctt, J. E., (1960), "A rapid and precise method for the determination of urea.", J. Clin. Path.; 13: 156-159.
32. Bartles, H.; Bohmer, M. & Heirli, C.,(1972),"Serum creatinine determination without protein precipitation.", Clin. Chem Acta; 37: 193-7.
33. Cannon, D. C., IN Clinical Chemistry – Principles and Techniques, 2nd Ed., Henry, R. J., Canon, D. C. & Winkelman, J. W., (Eds.), Harper & Row, Hagerstown, MD, 1974, pp 411-421.

34. Doumas, B. T., Watson, W. A. & Biggs, H. G., (1971), "Albumin standards and measurement of serum albumin with bromcresol green.", Clin Chim Acta.; 31(1): 87-96.
35. Kuczora, S., (2015), "Authorised EU health claims for glucomannan, guar gum and hydroxypropyl methylcellulose.", IN: Foods, Nutrients and Food Ingredients with Authorised EU Health Claims: Vol. (2), Woodhead Publishing Series in Food Science, Technology and Nutrition; Pages 175-188.
36. Hu, X. Z., Xing, X. H., Zhang, Z. M., Wu, R. Q., Guo, Q., Cui, S. W. et al., (2011), "Antioxidant effects of Artemis sphaerocephala Krasch. gum, on streptozotocin-induced type 2 diabetic rats.", Food Hydrocolloids; 25(2): 207-213.
37. Bosscher, D., Van Loo, J., Franck, A., (2006), "Inulin and oligofructose as functional ingredients to improve bone mineralization.", International Dairy Journal; 16 (9): 1092–1097.
38. Shabana, M. H., Eman, M. Shahy, Mona, M. Taha, Gehan, M. Mahdy, Maha, H. Mahmoud, (2015), "Phytoconstituents from Curcuma longa L. aqueous ethanol extract and its immunomodulatory effect on diabetic infected rats.", Egyptian Pharmaceutical Journal; 14:36-43.
39. Oberley, L. W., (1988), "Free radicals and diabetes.", Free Radic Biol Med.; 5(2):113-24.
40. Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., Ric de Vos, C. H., et al., (2008), "Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes.", Journal of Agricultural and Food Chemistry; 56: 696–704.
41. Abu-Reidah, I. M., Ali-Shtayeh, M. S., Rana M. Jamous, Arráez-Román, D. & Segura-Carretero, A., (2015), "HPLC–DAD–ESI-MS/MS screening of bioactive components from Rhus coriaria L. (Sumac) fruits.", Food Chemistry; 166: 179-191.
42. Hsu, P., Ai, M., Kanda, E., Yu, N. C., Chen, H. L., Chen, H. W., Cheng, M. H., Kohzuma, T., Schaefer, E. J. & Yoshida, M. (2015), "A comparison of glycated albumin and glycosylated hemoglobin for the screening of diabetes mellitus in Taiwan.", Atherosclerosis; 242 (1): 327-333.
43. American Diabetes Association (ADA), (2012), " Diagnosis and classification of diabetes mellitus.", Diabetes Care; 33 (1): S62–S69.
44. Leevy, C. M., Ryan, C. M. & Fineberg, J. C., (1950), "Diabetes mellitus and liver dysfunction: Etiologic and therapeutic considerations.", The American Journal of Medicine; 8 (3): 290-299.
45. Cao, H., Xie, Y. & Chen, X., (2015), "Type 2 diabetes diminishes the benefits of dietary antioxidants: Evidence from the different free radical scavenging potentia.", Food Chemistry; 186: 106-112.
46. Onozato, M. L., Tojo, A., Goto, A., Fujita, T. & Wilcox, C. S., (2002), "Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: Effects of ACEI and ARB.", Kidney International; 61: 186-194.

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