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Research Article
Amelioration of Hyperglycemia and Associated Health Hazards Using Two Dietary Formulas Composed of Multiple Ingredients

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Abstract

Background and Objective: Diabetes affects considerable percentage of the population all over the world and is associated with multiple health complications. Thus, treatment regimen should be able to alleviate such complications. The present study was designed to investigate the complications that occur due to injection of rats with alloxan. It was evidenced that hyperglycemia was induced in rats associated with a non-significant change in serum insulin concentration, disturbed liver and kidney functions, dyslipidemia and oxidation stress with lowered antioxidant capacity. **Methodology:** Two blends of a mixture from a number of plants assumed to cure or minimize the disease with its complications were formed and used as dietary supplement. The 1st formula is composed of cinnamon (50%), ginseng (20%), ground coffee beans (20%), fenugreek (10%) and the 2nd from jerusalem artichoke 20%, fenugreek (30%), cinnamon (40%), stevia (5%) and pumpkin seeds (5%). **Results:** The HPLC analysis of each of these two formulas showed that they contain several polyphenolic compounds known to possess antioxidant characters. Including these formulas with the diet of the alloxan diabetic rats caused some sort of relief of the hyperglycemia and the associated health complications as evidenced from the analyzed biochemical parameters. **Conclusion:** It was shown that the two formulas composed of the fore mentioned ingredients can be used as dietary supplements able to participate in reduction of hyperglycemia and are also helpful in alleviation of the so many complications associating the disease.

Key words: Diabetes, cinnamon, fenugreek, ginseng, coffee, pumpkin, jerusalem artichoke, stevia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants including vegetables, herbs, spices contain a lot of bioactive compounds that can serve several health functions. These health benefits ascribed to these compounds were attributed to one or more characters such as being antioxidants, chelating agent, detoxifying agent, anti-carcinogenic, anti-inflammatory, anti-allergic, hormonal regulators or replacement and pro-or prebiotics[1]. Diabetes mellitus either type 1 or 2 affects a lot of people all over the world and those who suffer from this disease usually suffer from several health complications such as, the prevalence of diabetic retinopathy[2], various vascular diseases[3], neuropathy, cardiovascular diseases, hypertension and dyslipidemia[4]. It is clear that a successful treatment for the disease is that which put into consideration an effective role that deal with all these disorders. A combination of plants and herbs that contain diverse compounds able to deal with these complications seems to be reasonable. Searching in the literature concerned with this subject revealed that a lot of plant or herbal sources can protect or treat hyperglycemia and most of the associated complications in diabetic patients and this depends on the diversity of bioactive compounds present in these sources. Among these items coffee has been reported to improve glucose level and insulin[5]. Cinnamon contains polyphenols and chromium that can improve insulin sensitivity[6]. Fenugreek is used to treat hyperglycemia in streptozotocin injected animals[7]. Foods containing jerusalem artichoke were shown to decrease the glycemic index to extents depends on the level of artichoke in the food[8]. Each of these sources contains certain bioactive compound that perform specific function, the sum of all lead to relief the health hazards of the disease. The aim of the present study was to form a combination of these plants or herbs in the form of a dietary supplement and to test its effect on hyperglycemia and the associated complications in alloxan injected rats.

MATERIALS AND METHODS

Materials: The materials used in this study were diet ingredients such as corn starch, sucrose corn oil, coffee, fenugreek, cinnamon, ginseng and pumpkin seeds were all purchased from the local market. Jerusalem artichoke and stevia were obtained from Agricultural Research Center, Egypt. Casein was obtained from Sisco Research Laboratories Pvt. Ltd., India. The salts and vitamins used for the preparation of the salt and vitamin mixtures were obtained from Merck, Germany and composed as indicated by (AIN 95) according to Reeves *et al.*[9].

Table 1: Composition of standard normal diet (g/100 g diet)

Ingredients	Amount (g/100 g diet)
Casein	15
Sucrose	10
Corn oil	8
Cellulose	4
Corn starch	58
Salt mixture	4
Vitamin mixture	1

The animals used in the biological experiments (Sprague Dawley rats) were obtained from the animal house of the National Research Centre. The average body weight of these animals was 120-130 g. Kits used for the estimation of the analyzed parameters were obtained from Biodiagnostics and Stanbio Laboratories company.

Methods: The standard normal diet given to the rats during the feeding period was formulated as shown in Table 1. Two formulas were prepared according to the assumed effective dose and the results of panel testing as follows:

- **Formula 1:** Cinnamon (50%), ginseng (20%), ground coffee beans (20%), fenugreek (10%)
- **Formula 2:** Cinnamon (40%), fenugreek (30%), jerusalem artichoke (20%), pumpkin seeds (5%), stevia (5%)

The formula was added to the diet at two levels (10 and 15%). Addition of the formula was at the expense of starch. These Two formulas were extracted with methanol according to the method of Alvarez-Jubete *et al.*[10] and subjected to chemical analyses for determination of polyphenolic compounds. The results are given in Table 2.

Total polyphenol content was determined by Folin-Ciocalteu assay according to the method of Ramful *et al.*[11]. Results were expressed in milligram of tannic acid equivalent per milliliters of extract. Total flavonoid content was determined using a colorimetric method as described by Heimler *et al.*[12]. The results were expressed as milligram of catechin per milliliters extract.

Total antioxidant capacity of the two formulas was determined according to Locatelli *et al.*[13]. Individual polyphenols content of the two formulas were determined in the methanolic extract by HPLC according to Kim *et al.*[14] and the results are given in Table 3.

Design of the animal experiment: Forty two Sprague Dawley male albino rats were used for this experiment. Seven animals were isolated and kept on the standard diet without any injection to serve as normal control (-ve control). The rest of

Table 2: Total polyphenols (as tannic acid equivalent), flavonoids (as catechin equivalent) of methanolic extract and total antioxidant percentage for the prepared formulae

Formula	Tannic acid (Methanol extraction) (mg mL^{-1})	Flavonoids (catechin) (mg mL^{-1})	Antioxidant activity (DPPH assay) (%)
1	1.93	0.15	51
2	1.66	0.13	62

Table 3: Polyphenol contents of the two formulae as detected by HPLC expressed in mg/100 g dry weight

Polyphenol	Formula 1	Formula 2
Syringic acid	122.80	2.14
Rosmarinic acid	88.91	79.44
Coumarin	53.48	69.99
Cinnamic acid	53.72	6.41
Ferulic acid	1.07	2.46
Chrysin	111.58	7.21
Caffeic acid	8.37	-
Vanillic acid	2.27	-
Chlorogenic acid	-	13.49
Sinopec acid	-	5.62

the animals were each injected intraperitoneally with alloxan dissolved in saline (5%), a dose equivalent to (140 mg kg^{-1} b.wt.). The blood glucose level of the injected animals was analyzed before and after injection and those with blood sugar level above 90 mg dL^{-1} were included in the experiment. These animals were divided into 5 groups each of 7 rats. Rats were all housed individually in stainless steel cages and kept in temperature controlled room at 25°C. Food and water were allowed *ad libitum*. A group of 7 rats from the injected animals was kept on the standard diet without the formula (positive control). The other 4 groups (groups 3-6) were each put on a standard diet that contains either formula 1 or 2 with the low or the high concentration (10 or 15%). The body weight and food consumption were followed weekly during the whole period of the experiment which lasted for 6 weeks.

At the end of the experimental period, rats were fasted overnight and in the morning animals were sacrificed by decapitation and the blood from each rat were collected into clean dried centrifuge tubes. They were centrifuged at 3500 rpm in a cooling centrifuge to separate serum which was kept in dry tubes in the freezer at -20°C till analysis. The experimental procedure was carried out according to the institutional ethics community of the NRC Egypt.

Nutritional and biochemical assessment: The nutritional assessment included food consumption, gain in body weight, change in organs weight relative to body weight and the feed efficiency ratio calculated as the result of dividing the gain in body weight by the food consumption.

The biochemical evaluation was done by follow up of the glucose level before injection of alloxan and after injection then at the end of the experiment. Blood sugar level was determined by the procedure described by Trinder[15], insulin assay was determined according to Lomedico *et al.*[16].

Liver function was assessed by determination of serum albumin according to Doumas *et al.*[17], serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to the procedure given by Henry *et al.*[18]. Kidney function was assessed by determination of each of creatinine according to Houot[19] and urea according to Fawcett and Soctt[20]. Lipid peroxidation was measured as malondialdehyde (MDA) following the procedure described by Satoh[21] and Total Antioxidant Capacity (TAC) according to Koracevic *et al.*[22]. Most lipid parameters were determined including total lipids according to Knight *et al.*[23], triglycerides according to Fossati and Prencipe[24] and total cholesterol according to Allain *et al.*[25]. Low density lipoprotein cholesterol (LDL-C) and high density lipoprotein (HDL-C) were also determined by following the procedure described by Levy[26] and Burstein[27], respectively.

RESULTS

Analysis of the two formulas showed that the total polyphenol in the methanolic extract determined as tannic acid were 1.93 and 1.66 TAE mL^{-1} methanol extract, while the flavonoid content as catechin equivalent of the two formulae were 0.15 and 0.13 mg catechin mL^{-1} methanolic extract (Table 2). From the obtained data it is obvious that there was no noticeable difference for both total polyphenols and flavonoid contents of the two formulas.

Antioxidant capacity of the used formulas: The antioxidant capacities of the 2 formulas are presented in Table 2. Table 2 shows that the antioxidant capacity of the formulas are presented by several parameters as tannic acid as catechins and as antioxidant activity (1,1-diphenyl-2-picrylhydrazine, DPPH).

HPLC analysis: The HPLC analysis of the 2 formulas for individual polyphenol contents (Table 3) show the presence of 10 polyphenolic compounds namely syringic, rosmarinic, coumarin, cinnamic, ferulic, chrysin, caffeic, vanillic,

Table 4: Body Weight Gain (BWG), food intake and Feed Efficiency Ratio (FER) for the different experimental groups

	Parameters		
Treatments	BWG (g)	Food intake (g)	FER
G1	33.40±6.27^{bc}	333±13.56^{a}	0.097±0.014^{b}
G2	11.00±2.23^{d}	261±22.50^{b}	0.044±0.009^{c}
G3	50.40±7.83^{a}	339±15.84^{a}	0.147±0.020^{a}
G4	41.80±1.35^{ab}	258±15.62^{b}	0.164±0.012^{a}
G5	12.60±2.24^{d}	231±11.445^{b}	0.053±0.010^{c}
G6	22.80±3.21^{cd}	250±14.832^{b}	0.105±0.005^{b}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

Table 5: Relative liver weight and kidney weight of rats in the different experimental groups

	Parameters	
Treatments	Relative liver weight	Relative kidney weight
G1	3.09±0.120^{ab}	0.83±0.051^{b}
G2	4.03±0.390^{a}	1.10±0.093^{ab}
G3	3.95±0.442^{a}	1.16±0.132^{a}
G4	3.87±0.406^{ab}	1.01±0.089^{ab}
G5	3.54±0.086^{ab}	1.12±0.109^{a}
G6	2.99±0.057^{b}	0.97±0.077^{ab}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

chlorogenic and sinopec. In formula 1 the highest amount was for that of syringic and in formula 2 the highest compound was rosmarinic acid.

Nutritional evaluation: The food intake and gain in body weight during the experiment and the calculated feed efficiency ratio is shown in Table 4.

Table 4 shows that the food intake of normal rats amounted to 333±13.5 g, in case of the alloxan injected rats the food intake was less (261±22.5 g). Rats given the formula No. 1 (the low dose) showed a relative increase in food intake (339±15.84 g), those given the high dose consumed less (258±15.62 g). Rats fed on formula No. 2 consumed less food than the normal rats or those injected with alloxan. Those fed on the low dose consumed even less.

As a result of injection with alloxan, the gain in body weight was significantly decreased. The body weight gain was 11.0±2.23 g relative to a value of 33.4±6.27 g for control non injected rats. There was a marked increase in body weight of rats in groups 3 and 4 given formula 1 either the low or the high dose, even this was higher in case of the low dose. The gain in body weight was 50.4±7.83 g in case of rats receiving formula No. 1 (the low dose) and 41.8±1.35 g in case of the high dose. The gain in body weight of rats given formula No. 2 was limited. It was 12.6±2.24 g in case of the low dose and reached 22.8±3.21 g in case of the high dose.

Table 6: Concentration of glucose and insulin in serum of rats in the different experimental groups

	Parameters	
Treatments	Glucose (mg dL^{-1})	Insulin (mg dL^{-1})
G1	56.94±3.86^{d}	1.02±0.10^{a}
G2	137.08±4.76^{a}	0.97±0.10^{a}
G3	65.95±4.49^{cd}	1.20±0.16^{a}
G4	74.41±6.24^{c}	1.17±0.14^{a}
G5	91.08±5.10^{b}	1.10±0.16^{a}
G6	69.77±3.60^{cd}	1.10±0.11^{a}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

The feed efficiency ratio showed a marked drop due to injection with alloxan, from 0.097±0.014 to 0.044±0.009. When formula 1 was included with diet a remarkable improvement occurred in the FER, it was 0.147±0.020 in case of the low concentration and 0.164±0.012 in case of the high concentration. A limited improvement also occurred when formula 2 was included with the diet. The values reported for the FER were 0.053±0.010 and 0.105±0.005 for the low and high concentrations respectively.

The relative organs weights of the liver and kidney are shown in Table 5. Table 5 shows an increase in organs weight of the rats occurred as a result of injection with alloxan. The relative liver weight was 3.09±0.12 and became 4.03±0.39. In case of the kidney the weight was 0.83±0.051 and became 1.10±0.093. Adding formula 1 or 2 to the diet of animals caused a slight decrease in the relative liver weight; however there was no similar decrease in relative kidney weight except for those given the high level of the 2nd formula.

Blood sugar and insulin levels: The concentration of glucose and insulin in serum of rats after injection with alloxan is shown in Table 6. Table 6 shows that a highly significant increase in blood glucose occurred due to injection with alloxan. The level of blood sugar was 56.94±3.86 mg dL^{-1} and became 137.08±4.76 mg dL^{-1}. The level of insulin was not markedly different. The concentration of insulin before injection was 1.02±0.10 mg dL^{-1} and became 0.97±0.10 mg dL^{-1} after injection. Addition of any of the 2 formulas caused a decrease in blood sugar and a relative increase in insulin concentration. The values reported for blood sugar were 65.95±4.49, 74.41±6.24, 91.08±5.10 and 69.77±3.60 mg dL^{-1} for rats given the low or the high dose from either formula 1 or 2. The values reported for the insulin level were 1.2±0.10, 1.17±0.14, 1.1±0.16 and 1.1±0.11 in the same order.

Total antioxidants capacity and malondialdehyde: The total serum antioxidant capacity and malondialdehyde (MDA)

level of normal rats and those injected with alloxan whether given the control diet or that which contains either formula 1 or 2 is given in Table 7. Table 7 shows that injection with alloxan caused a significant drop in the value of total antioxidant capacity. The value reported for normal rats was 2.22±0.03 mM L^{-1} and that for alloxan injected rats was 1.56±0.19 mM L^{-1}. Addition of formula 1 or 2 the low or high concentration caused an increase in the value of the total antioxidant capacity. The values reported were 2.10±0.12, 2.14±0.14, 2.31±0.04 and 2.40±0.10 mM L^{-1} for rats given either formula 1 (the low and high concentration) or formula 2 (the low and high concentration) respectively. The serum malondialdehyde level was increased due to injection of alloxan. The value reported for normal rats was 4.44±0.19 nmol L^{-1} and became 6.44±0.17 nmol L^{-1}.

Table 7: Concentration of total antioxidant capacity and malondialdehyde of rats in the different experimental groups

	Parameters	
Treatments	Total antioxidant (mM L^{-1})	Malondialdehyde (nmol mL^{-1})
G1	2.22±0.03^{a}	4.44±0.19^{b}
G2	1.56±0.19^{b}	6.44±0.17^{a}
G3	2.10±0.12^{a}	3.59±0.19^{c}
G4	2.14±0.14^{a}	4.00±0.26^{bc}
G5	2.31±0.04^{a}	3.82±0.12^{c}
G6	2.40±0.10^{a}	3.95±0.10^{bc}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

Addition of the formula either 1 or 2 to the diet of hyperglycemic rats appreciably decreased the malondialdehyde level of serum. The values reported were 3.59±0.19, 4.00±0.26, 3.82±0.12 and 3.95±0.10 nmol L^{-1} for rats given formula 1 (low or high concentration)and formula 2 also low or high concentration respectively.

Serum lipid profile: The serum total lipids, total cholesterol, HDL, LDL, VLDL and triglycerides of normal rats and those injected with alloxan either given the formula or not is shown in Table 8. Table 8 shows that a marked increase occurred in the level of most lipid parameters of rats injected with alloxan. The HDL was decreased. The values reported were 253.5±6.6, 95.72±6.17, 24.48±1.91, 46.91±5.93, 24.0±0.90 and 111.67±6.43 mg dL^{-1} for total lipids, cholesterol, HDL, LDL, VLDL and triglycerides respectively. The corresponding values for normal rats were 120.31±8.55, 76.56±3.95, 32.63±5.45, 32.85±4.25, 44.2±29.3 and 75.42±3.89 mg dL^{-1}.

Liver function: The three parameters namely AST, ALT and albumin representing liver function are shown in Table 9. Table 9 shows that a significant increase in the activities of the AST and ALT were noticed after injection with alloxan. The albumin level was low relative to the non-injected rats. The values obtained were 77.0±2.09 and 81.80±1.06 U mL^{-1}

Table 8: Concentration of serum lipid of rats in the different experimental groups

	Parameters					
Treatments	Total lipid (mg dL^{-1})	Triglycerides (mg dL^{-1})	Cholesterol (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
G1	120.31±8.55^{bc}	75.42±3.89^{b}	76.56±3.95^{b}	32.63±5.45^{bc}	32.85±4.25^{b}	44.20±29.34^{a}
G2	253.50±6.60^{a}	111.67±6.43^{a}	95.72±6.17^{a}	24.48±1.91^{c}	46.91±5.93^{a}	24.00±0.90^{a}
G3	100.18±9.408^{c}	52.34±0.50^{c}	84.55±3.90^{ab}	40.47±1.98^{ab}	33.618±2.98^{b}	10.46±0.101^{a}
G4	138.59±16.27^{bc}	61.67±5.39^{c}	89.56±7.03^{ab}	51.57±6.14^{a}	31.194±3.04^{b}	12.33±1.078^{a}
G5	143.04±26.59^{bc}	62.03±1.83^{c}	78.94±4.32^{b}	38.20±3.87^{b}	28.45±2.81^{b}	12.66±0.39^{a}
G6	163.93±13.40^{b}	61.57±3.24^{c}	84.13±3.35^{ab}	42.17±4.20^{ab}	35.538±1.75^{b}	12.45±0.75^{a}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

Table 9: Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin in serum of control rats and other groups

	Parameters		
Treatments	Aspartate aminotransferase (AST) (U mL^{-1})	Alanine aminotransferase (ALT) (U mL^{-1})	Albumin (g dL^{-1})
G1	35.20±1.77^{d}	26.20±1.28^{e}	5.050±0.52^{bc}
G2	77.00±2.09^{a}	81.80±1.06^{a}	4.930±0.20^{bc}
G3	57.40±2.18^{b}	53.00±2.46^{b}	5.296±0.423^{ab}
G4	45.80±2.28^{c}	43.80±1.74^{d}	6.322±0.44a
G5	58.80±2.87^{b}	56.80±1.98^{c}	4.066±0.33^{c}
G6	47.40±1.50^{c}	48.80±1.15^{b}	5.510±0.309^{ab}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

Table 10: Concentration of creatinine, urea, BUN and ratio of BUN/creatinine of rats in the different experimental groups

	Parameters			
Treatments	Creatinine (mg Dl^{-1})	Urea (mg dL^{-1})	BUN (mg dL^{-1})	Ratio of BUN/ creatinine
G1	0.51±0.06^{a}	41.16±2.23^{bc}	19.21±1.04^{bc}	33.41±1.19^{bc}
G2	0.54±0.04^{a}	52.46±2.70^{a}	24.49±1.26^{a}	47.27±5.56^{a}
G3	0.51±0.043^{a}	42.22±4.05^{bc}	19.69±1.88^{bc}	38.88±3.37^{ab}
G4	0.44±0.052^{a}	36.20±2.63^{c}	16.90±1.23^{c}	35.62±2.19^{b}
G5	0.56±0.054^{a}	47.44±2.02^{ab}	22.18±0.93^{ab}	41.11±4.12^{ab}
G6	0.44±0.088^{a}	25.84±1.65^{d}	12.06±0.77^{d}	24.896±3.58^{c}

All values represented as Mean±SE, means with different letters are significantly different ($p<0.05$)

and 4.93±0.20 g dL^{-1} for AST, ALT and albumin levels respectively. The corresponding values for normal rats were 35.20±1.77 U mL^{-1}, 26.20±1.28 U mL^{-1} and 5.05±0.52 g dL^{-1}.

Addition of either formula 1 or 2 caused an improvement in the parameters of the liver function of the alloxan injected rats. The values obtained were more near to the normal values.

Kidney function: The parameters showing the state of kidney function is shown in Table 10. Table 10 shows that the value of creatinine and urea of normal rats were 0.51±0.06 mg dL^{-1} and 41.16±2.23 mg dL^{-1}, respectively. Injection of rats with alloxan caused an increase in these values. They were 0.54±0.04 and 52.46±2.70 mg dL^{-1}. Addition of the dietary supplement either formula 1 or 2 caused relative improvement of these values. They were more near to normal values. The ratio of Blood Urea Nitrogen (BUN)/creatinine was also increased due to injection with alloxan and this increase disappeared when any of the composed formula was given with the diet.

DISCUSSION

Generally it can be stated that injection of rats with alloxan caused a marked increase in blood glucose level and an associated complication represented by disturbed liver and kidney functions together with an abnormal serum lipid pattern. Lenzen[28] stated that alloxan when injected into animals is reduced to dialuric acid with intracellular glutathione. The redox cycling between them generates the reactive oxygen species of the superoxide anion, hydrogen peroxide and then hydroxyl radical and all of this is responsible for the necrotic death of pancreatic cells. The results obtained from this study show a marked increase in the serum malondialdehyde in alloxan injected rats. In the same time, the antioxidant capacity of serum was significantly reduced. This is a typical picture to what has been described by Lenzen[28] that free radicals generated by alloxan injection are among the factors behind hyperglycemia in the injected rats.

In this study disturbed liver function was indicated by increased activities of both AST and ALT enzymes together with a low serum albumin. It has been reported that GGT (gamma glutamyltransferase) is more sensitive to alcohol intake than ALT; however, GGT and ALT could predict T2 DM (type 2 diabetes mellitus) in nonalcoholic liver disorders[29]. These results show that the enzymes ALT and AST can also predict liver injury due to toxic drugs such as alloxan. Such increase of liver enzymes may be due to leakage from the injured liver cells affected by toxicity of the injected alloxan. The decreased albumin concentration in serum indicated the inability of this organ to synthesize albumin.

In the present study it was shown that injection of rats with alloxan caused an increase in the values of both serum urea and creatinine. There is a strong association between increased serum uric acid and the progression of chronic kidney diseases[30]. Plasma urea-to-creatinine (BUN/Cr) ratio was always taken as a good predictor to kidney function denoting the fractional excretion of sodium and urea[31]. It is thus clear that the increased level of both urea and creatinine in serum is an indication to a derangement in kidney function due to alloxan injection.

Elshazly *et al.*[32] reported a significant increase in the mean serum concentrations of total cholesterol and triglycerides and significantly lower mean concentrations of HDL-cholesterol in patients with diabetes when compared with control individuals. Similar pattern was reported in alloxan injected rats. These types of biochemical changes that occur due to alloxan injection show that hyperglycemia and the associated disturbed organ function are to a great extent similar to diabetes in human which mean that findings in experimental animals can be extended to human.

The results reported from this study show that most of the lipid parameters of the alloxan injected rats were disturbed. There was noticed a significant increase in the level of serum total lipids, LDL, cholesterol and triglycerides and a decrease in HDL concentration. Dyslipidemia has been reported in many studies to associate diabetes and perhaps remains for a time after treatment of hyperglycemia[33]. It is an

independent risk factor for atherosclerosis. Patients who suffer from diabetes and dyslipidemia are more subjected to cardiovascular diseases and its consequences[34]. Hepatic lipid homeostasis is regulated by the balance between the import and export of lipids. This imbalance in turn controls the hepatic lipid homeostasis in the body. The diabetic processes leads to increased VLDL secretion or lipid accumulation in hepatocytes, a common finding in subjects with type 2 diabetes. The 2 dietary supplements used in this study are formed from ingredients reported to either protect from diabetes or treat hyperglycemia and associated health complications including dyslipidemia . Cinnamon[35], ginsing[36], ground coffee beans[37] or fenugreek[38] the constituents of formula 1 and jerusalem artichoke[8], stevia[39] of the other formula, all of them have been reported to exert that beneficial effect on diabetes.

Cinnamon and ginger were reported to reduce the burden of diabetes and cognitive impairment with relative safety and at low cost. The mechanism whereby these herbs might induce this action range from energy regulation and mitochondrial function to neurotransmission and protein-folding disorders[37].

Fenugreek has a hypoglycemic effect and was reported to contain saponins, flavonoids, polysaccharides and other active ingredients and act through delayed gastric emptying, reducing glucose and fat absorption, inhibition of glucose transport, stimulation of insulin secretion and sensitivity, diminishing oxidative stress and the modulation of glucagon-like peptide-1[38].

Jerusalem artichoke extract was proved to treat streptozotocin induced hyperglycemia and associated liver damage. It also improved lipid profile that was affected by streptozotocin injection. This effect was attributed to the presence in artichoke of antioxidant compounds such as polyphenols. These compounds can protect against lipid peroxidation and membrane damage caused by reactive oxygen species generated in diabetes. These free radicals play an important role in the production of secondary complications in diabetes mellitus (kidney, eye, blood vessel and nerve damage). The presence of antioxidants also protect the pancreatic β cells from destruction[40].

Glycosides are the compounds in stevia that give the sweetening taste with no caloric value. In addition stevia contain other nutrients such as protein, fibers, carbohydrates, phosphorus, iron, calcium, potassium, sodium, magnesium, rutin (flavonoid), zinc, vitamin C and vitamin A[41]. Animal studies proved that stevioside have antihyperglycemic, insulinotropic and glucagonostatic actions in diabetic rats[42-44]. This herb has been proven in safety and effectiveness for hundreds of years.

This historical illustration show that most of the health benefits reported for the ingredients used for forming the two formulas used in this study were realized. The high antioxidant capacity of the two formulas as reported in results which are assumed to be due to the presence of polyphenols and other antioxidants present in these sources succeeded to protect the pancreatic cells from alloxan toxicity and hence prevent or minimized hyperglycemia. The presence of polyphenols was confirmed from analysis of the methanol extract of these formulas by HPLC. In addition, it is known that flavonoids can inhibit α-amylase activity and trans-chalcone, the intermediary biosynthetic precursor and control glycemic load in streptozotocin injected rats. The effect of polyphenol was found to extend to the amelioration of dyslipidemia associating hyperglycemia and this agrees with our finding in alloxan injected rats. Further explanation to the effect of flavonoids on hyperglycemia and dyslipidemias is the upregulation of hepatic superoxide dismutase activity and the reduction of malondialdehyde together with increase of glucose transporters[45].

The values reported for insulin level in the different treatments did not vary significantly. This indicates that the hypoglycemic effect is not mainly due to insulin insufficiency but due to low sensitivity of insulin as happens in type 2 diabetes. It has been reported that plasma serotonin level and glucose concentration are increased in diabetic patients. Serotonin participate in translocation of insulin receptors in the cell cytoplasm leading to insulin degradation[46,47]. This may explain the insulin resistance caused by alloxan injection in the rats. The inclusion of any of the two formulas used in this study succeeded to lower the high serum glucose inspite of the more or less normal concentration of serum insulin. This means that the ingredients used for formulation of the two formulas were able to prevent the degradation effect of alloxan on insulin thus keep on its sensitivity.

CONCLUSION

Injection of rats with alloxan caused metabolic disturbances in the body represented by hyperglycemia, dyslipidemia, disturbance of liver and kidney functions together with oxidation stress and a drop of the antioxidant capacity. It is assumed that increased malondialdehyde due to alloxan injection is among other factors behind the disturbance of glucose and lipid pattern. This study proved that treatment of alloxan injected rats with formulas that contain ingredients such as coffee, fenugreek, cinnamon, ginseng, jerusalem artichoke, pumpkin and stevia could correct the hyperglycemia due to alloxan

injection and also ameliorate most of the metabolic complications associating it. Antioxidants present in these sources particularly polyphenols are assumed to be the main effective factor.

SIGNIFICANT STATEMENTS

- HPLC analysis of each of these two formulas showed that they contain several polyphenolic compounds known to possess antioxidant characters
- Including these formulas with the diet of the alloxan diabetic rats caused some sort of relief of the hyperglycemia and the associated health complications as evidenced from the analyzed biochemical parameters

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