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ORIGINAL ARTICLE

Effects of *Mesembrrybryanthemum forsskalei* Hochst seeds in lowering glucose/lipid profile in streptozotocin-induced diabetic rats

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Abstract The aim of present investigation was to study the effects of *Mesembryanthemum forsskalei* Hochst (Samh) seeds in streptozotocin-induced diabetic rats. Thirty rats were administrated with streptozotocin to induce diabetes and 6 rats were used as untreated diabetic control. Diabetic rats were fed with 5 and 15% Samh seed alone and in combination with fatty diet i.e. 2% Cholesterol for 6 weeks. Effects of Samh seed on blood glucose levels, lipid profiles and enzyme activities of diabetic rats were examined. In addition, total cholesterol (TC), triglyceride (TG) and high density lipoprotein-cholesterol (HDL-C) were determined. Diabetic rats treated with 15% Samh seed diet were significantly decreased the level of TC (40%), TG (46%) and HDL-C (31%) respectively. Whereas, there was no significant effects observed in the glucose level in 15% Samh seeds treated rats for 6 weeks. A decrease in enzymes levels, AST (58.2%), LDH (1.6%), ALT (24.3%) and ALP (5.38%) in 5% Samh seeds diet treated rats were observed and were found near to untreated control. Findings from present study demonstrated that non fatty Samh seeds diet could have hypoglycemic and antihyperlipidemic effects in diabetic rats and could be useful model for the treatment of diabetic patients.

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Introduction

Diabetes, a chronic disease is predominant public health concern worldwide including Saudi Arabia. According to World Health Organization (WHO 2004) report, more than 150 million people throughout the world suffered from diabetes. The total number of people with diabetes is projected to rise from 171 million in 2,000 to 366 million in 2030 (Wild et al. 2004). Diabetes is associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism, which are the major causes of morbidity and mortality of diabetes Taskinen (2002). With increasing rates of childhood and adult obesity, diabetes is likely to become even more prevalent over the coming decade (NDFS 1998). One essential task for researcher/scientists involved in the area concern, is the search for the prevention and treatment of the diabetes. American Diabetes Association issued a Position Statement in 2001 on "Unproven Therapies" that encouraged health care providers to ask their patients about alternative therapies and practices evaluate each therapy's effectiveness, be cognizant of any potential harm to patients, and acknowledge circumstances in which new and innovative diagnostic or therapeutic measures might be provided to patients (ADA 2002). Evidences from the literature showed that has focused on herbs or other dietary supplements. Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world reported by Yaniv et al. (1987) and Covington (2001). Many modern pharmaceuticals used in conventional medicine today also have natural plant origins

(Oubre et al. 1997; Saravanan and Pari 2005; Gao et al. 2009; Kumar and Loganathan 2010). Several plants and herbs have been used since ancient times in Saudi Arabia. *Mesembrrybryanthemum forsskalei* Hochst (Samh) seeds flour are being used as a replacement for wheat for cookies, and high nutrition potential of Samh seeds is reported by Al-Jassir et al. (1995). The composition of Samh seeds calculated as 22.25% protein, 5.7% moisture, 5.6% fat, 4.0% ash, 9.7% crude fiber, and the remainder being total carbohydrates. Linoleic and oleic acids were the principle unsaturated fatty acids. Palmitic acid was the main saturated fatty acid. Amino acid analysis of the Samh seeds showed the presence of seventeen amino acids including eight essential amino acids. The present study was undertaken to investigate the potential of Samh seed powder as a dietary supplements in diabetic rat model to normalize the hyperglycemic/hypercholesteremic effect. Streptozotocin was induced in order to study the effect of *Mesembryanthemum forsskalei* Hochst seeds.

Material and methods

Plant material Mesembryanthemum forsskalei Hochst (Samh) seeds were gifted from Al-Jouf Research Center for Pastures and Animal Wealth Development. The food used in this study was prepared according to American institute of Nutrition (AIN) (Reeves et al. 1993). Plant seeds were grinded and mixed with standard pellet. The calculated food supplemented with 5 and 15% of Samh seeds for the balanced fed and with 15% of Samh seeds for high cholesterol content fed (2%). The fed were kept at cool place at 5^0 C during the study.

Experimental animals and streptozotocin-induced diabetes Two months old wistar albino rats (200±5 g) were purchased from animal house (Faculty of Medicine, King Saud University). All the animals were maintained in a temperature-controlled room at 22±2 °C and humidity 50± 5% on a commercial pellet diet and water ad libitum with a 12/12-h light/dark cycle and cared for in accordance to the policy laid down by Animal Care Committee of King Saud University. The animal experimentation was approved by the Ethical Committee of the University. Diabetes was induced in rats by a single intraperitoneal injection of 65 mg/kg streptozotocin (Sigma Chemical Co, USA) freshly dissolved in 0.05 mol/L sodium citrate buffer (pH 4.5). Control six rats received an injection of citrate buffer. After 48 h of treatment, thirty rats with diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycemia (with a blood glucose level of 300 mg/dL or greater). The schedules and procedures were performed in the Experimental Animal Handling Facility in Faculty of Medicine, King Saud University.

Experimental design Experiment was conducted on 36 adult male rats (30 diabetic surviving rats, 6 control rats) for 6 weeks. The rats were divided into six groups of six rats each. Group 1 (CN): healthy normal rats fed with natural diet. Group 2 (CD): diabetic control rats fed with natural diet. Group 3 (SP1): diabetic rats fed with natural diet supplemented with 5% of Samh seeds. Group (4) SP2: diabetic rats fed with natural diet supplemented with 15% of Samh seeds. Group 5 (HL1): diabetic rats fed with high fat diet (2% cholesterol). Group 6 (HL2): diabetic rats fed with high fat diet (2% cholesterol) supplemented with 15% of Samh seeds. The rats were weight at the start and at the end of study. The introduced food and the remnant of food were weight to calculate the eaten food daily and the validity of feeding. The blood Urea Nitrogen (BUN), Creatinine, total protein, albumin and globulin were determined by colorimetric methods according to Tobacco et al. (1979), Rock et al. (1983, 1998), total billirubin Reitman and Frankel (1957).

Biochemical analysis The blood glucose level was determined at the start of administration of Samh seeds as baseline. Effect of Samh seeds on blood glucose levels of diabetic rats was examined during and at the end of the study. Blood was collected from the tail of fasting (10 h) rats. The tail was embedded in 45 °C water bath and about one millimeter of its end was cut and a drop of blood was used for the blood glucose test with the help of glucometer GX (Ames, USA), further sampling did not need re-cutting of the tail. To measure lipid profile; all the rats were anaesthetized by pentobarbitone sodium (60 mgkg−1) and opened at the abdomen. Blood was withdrawn from the abdominal aorta and centrifuged at 3,000 rpm for 10 min to obtain the plasma. Blood glucose was determined following the methods of Teuscher and Richterich (1971). TC (total cholesterol) was measured following the method of Rattiff and Hall (1973) and TAG (triglyceride) according to Trinder (1969), HDL-C (High density lipoprotein-cholesterol) and LDL-C (Low density lipoprotein-cholesterol) following the method of Arcol (1989). Activity of plasma enzymes, Aspertase Amino transfrase (AST) and (LDH) was determined by (Boehringer Mannheim, Germany). Alanine aminotransferase (ALP) and Alkaline phosphatase (ALP) activities were determined using UDI kits (United Diagnostics Industries-Dammam, KSA) which are based on the method of Reitman and Frankel (1957).

Statistical analysis Statistical analysis of performed using (SPSS, version 11.0). Statistical analysis was performed using one-way analysis of variance (ANOVA) and post-hoc Tukey's test to compare the inter- and intra-group findings. The values depicting $p<0.05$ were considered as statistically significant.

Table 1 Effect of Samh seed in liver function. All the values are presented as Mean±SE of three independent experiments

Groups	Control Normal (*N*=6) CN	Control Diabetic (*N*=6) CD	5% Samh (*N*=6)-SP-I	15% Samh (*N*=6)-SP-II	HL Diet (*N*=6)-HL-I	15% Samh (*N*=6)-HL-II	ANOVA	
							F. value	Sig.
BUN (mg/dl)	7.8±3.53^{a}	8.5±1.33^{a}	5.6±0.75^{a}	9.1±3.91^{a}	16.7±2.50^{a}	16.6±4.88^{a}	1.19	0.351
Creatinine (mg/dl)	49.0±9.69^{ab}	36.4±4.39^{a}	33.0±6.87^{a}	23.7±10.10^{a}	66.2±29.40^{b}	48.0±28.00^{ab}	3.01	0.038*
Total Protein (g/dl)	6.3±3.84^{a}	6.8±2.38^{a}	6.2±4.30^{a}	4.9±2.17^{b}	6.8±1.43^{a}	5.4±1.62^{b}	4.62	0.001*
Albumin (g/dl)	9.0±1.58^{ac}	10.6±1.51^{a}	9.8±0.96^{a}	6.7±1.21^{bc}	10.2±0.96^{a}	7.0±2.00^{c}	5.01	0.005*
Globulin(g/dl)	5.4±3.64^{ac}	5.7±1.11^{a}	5.3±2.41^{a}	4.2±1.03^{b}	5.8±3.07^{a}	4.7±2.64^{bc}	5.23	0.004*
Total Bilirubin (mmol/L)	2.0±0.70^{a}	1.8±1.64^{a}	2.2±0.57^{a}	2.7±0.05^{a}	3.6±2.00^{a}	3.5±0.71^{a}	2.07	0.119

CN Control normal, *CD* Control diabetic, *SP-I* (5% Samh seed powder), *SP-II* (15% Samh seed powder), *HL-I* High lipid diet (2% cholesterol), *HL-II* (High lipid diet-2% cholesterol with 15% Samh seed powder), *N* number of rats, *HL* Hyperlipidemic, *NS* Non Significant. *p<0.05, abc.

Results and discussion

Hyperglycemia is the main cause of secondary complications of diabetes such as coronary diseases, dyslipidaemia, rubifcient and lipid abnormalities (Lenzen and Panten 1988; Gidado et al. 2005; Sochar et al. 1985). Elevation of plasma level of urea and protein are considered as significant markers of renal dysfunction as mentioned by Carlo et al. (1996). Significantly (p<0.05) decrease in the level of creatinine (9.34 and 34.8%), total protein (7.9 and 28.2%), albumin (7.5 and 36.8%) and globulin (7.6 and 25.8%) in diabetic control animals were observed after the treatment in SP-I and SP-II groups. (Table 1). Changes in these proteins, clearly indicates antioxidant activity of organic compounds present in the Samh flour, which may be playing important role to bring down the level of protein. Similar effects of many plants and their extracts have been also reported in normal and diabetic animals by Augusti and Sheela (1996), Babu and Sriniwasan (1999), Jiang et al. (2005). Our findings also confirm that low intakes of Samh flour significantly reduced blood urea of STZ-induced rats, but insignificant effects were observed in case of SP2 group (Table 1). An increase in the level of blood urea produced in SP2 could be due to the high protein diet (22.5%), naturally present in Samh seeds. Blood urea nitrogen (BUN) is primary metabolite derived from dietary protein. In the gut, protein converted into peptide and amino acids, of which more than 90% are carried out to the liver. Plasma biliburin may also be the result from decrease of liver intake (Table 1). However, there were no significant differences in BUN and total biliburin levels in SP-I, SP-II and HL-II treated animals at the end of study of 6 weeks.

The plasma lipid level is usually raised during diabetes and enhances the risk factor for the coronary heart disease and lowering lipid level indicates the decrease in the risk of vascular diseases. Our analyzed data found an increase level of plasma lipid in STZ induced rat, which could be a result of cleavage of lipid molecules and mobilize of free fatty acids from tissues. STZ regulating oxidative mechanism could be indication on lipid per oxidation as a marker. Significantly reduced in total cholesterol (19 and 40%), triglycerides (40 and 46%) and increased level of HDLc (31%) indicates the effect of Samh flour on body cholesterol metabolism and is strongly influenced by hyperlipidimia and hypercholestermia as reported by

Table 2 Blood lipid profile in diabetic rats. All the values represents as Mean±SE of three independent experiments

Groups	Control Normal (*N*=6) CN	Control Diabetic (*N*=6) CD	5% Samh (*N*=6) SP-I	15% Samh (*N*=6) SP-II	HL Diet (*N*=6) HL-I	15% Samh (*N*=6) HL-II	ANOVA	
							F. value	Sig.
Glucose (mol/L) (the last day)	4.8±1.34^{a}	7.6±1.00^{a}	7.6±1.18^{a}	11.1±9.75^{ab}	29.9±13.05^{b}	33.0±17.05^{b}	2.99	0.039*
Cholesterol (mmol/L)	1.7±0.17^{ac}	2.1±0.36^{ab}	1.6±0. 38^{ab}	1.2±0.32^{a}	2.9±1.48^{ab}	2.6±0.63^{bc}	2. 6	0.063
Triglycerides (mmol/L)	1.2±0.62^{a}	0.76±0.48^{a}	0.69±0.19^{a}	0.45±0.45^{a}	0.90±0.89^{a}	1. 3±0. 89^{a}	1.08	0.402
HDLc (mmol/L)	0.34±0.03^{a}	0.47±0.11^{ab}	0.45±0.14^{ab}	0.36±0.18^{a}	0.64±0.28^{b}	0.45±0.09^{ab}	2.94	0.041*

CN Control normal, *CD* Control diabetic, *SP-I* (5% Samh seed powder), *SP-II* (15% Samh seed powder), *HL-I* High lipid diet (2% cholesterol), *HL-II* (High lipid diet-2% cholesterol with 15% Samh seed powder), *N* number of rats, *HL* Hyperlipidemic, *NS* Non Significant. *p<0.05, abc.

Table 3 Effect of Samh seed diet on AST, ALT, ALP and LDH enzymes in blood. All the values represents as Mean±SE of three independent experiments

Groups	Control Normal (*N*=6) CN	Control Diabetic (*N*=6) CD	5% Samh (*N*=6) SP-I	15% Samh (*N*=6) SP-II	HL Diet (*N*=6) HL-I	15% Samh (*N*=6) HL-II	ANOVA	
							F. value	Sig.
Ast (IU/L)	167.0±10.34^{a}	287.2±19.57^{ab}	135.2±24.30^{a}	120.0±19.90^{b}	237.2±22.13^{ab}	162.7±19.30^{ab}	1.20	0.347
Ldh (IU/L)	1160.8±23.68^{ab}	1942.4±128.21^{a}	1132.5±95.33^{ab}	949.6±329.34^{b}	971.5±162.87^{b}	865.3±356.64^{b}	1.76	0.172
Alt (IU/L)	23.8±4.96^{a}	57.4±13.18^{bc}	25.2±2.22^{a}	48.3±1.15^{b}	28.5±8.42^{ac}	47.0±8.00^{b}	6.43	0.02*
Alp (IU/L)	90.0±8.51^{a}	110.2±6.83^{ab}	106.0±13.90^{b}	104.0±4.28^{c}	239.2±27.90^{d}	228.5±36.00^{d}	50.27	0.000*

CN Control normal, *CD* Control diabetic, *SP-I* (5% Samh seed powder), *SP-II* (15% Samh seed powder), *HL-I* High lipid diet (2% cholesterol) *HL-II* (High lipid diet-2% cholesterol with 15% Samh seed powder), *N* number of rats, *HL* Hyperlipidemic, *NS* Non Significant. *$p<0.05$, abc.

Halberstein (2005), Viana et al. (2004), Ramalingam and Leelavinothan (2005). An increase level of HDLc could potentially contribute, inhibit lipid oxidation and protect cell from cytotoxic effects. Our study clearly shows the beneficial role of organic ingredients of antihyperlepidic character in Samh flour to hypercholesterolemia rats. Lowering effects of cholesterol was also noted when high lipid diet given to the animals (Table 2). It is very well known that the beneficial effects on health of organic compounds due to their anti oxidative and anti lipidperoxidation effects by Husain Ali (2002) and Jiang et al. (2005). In case of sugar there was no significance difference in serum glucose level between each group in treated and untreated animals. This study showed much unexpected result of serum glucose in SP1, SP2 and HL2 groups after the treatment. This inconsistency of the result could be of many reasons, it may be the result of high sugar present in the Samh flour (25%) which would not have enough organic ingredients to support the insulin released from B cells to encounter the developed diabetic mellitus in experimental animals. Thamolwan and Thanapat (2005) reported the same result when crude leaves supplement was studies to diabetic animals, but the result were found different if aqueous extract of same leaves were given to animals. However, it is interesting to note that Samh supplement exerted different effects on blood glucose but showed positive effects on lipid profile. It indicated that recovery of dyslipidamia in diabetic animals treated with Samh flour is independent of glucose lowering activity. Currently there is no particular data or known constituents in Samh, which is responsible for the antihyperglecmic effects. Further action is needed of Samh extracts to know the better way of mechanism on diabetic mellitus animals.

The elevated level of enzymes activities in SP-I and SP-II were restored almost close to normal level after the treatment for 6 weeks i.e. AST (52.9 and 58.2%), LDH (41.6 and 51.1%), ALT (38.5 and 5.6%) and ALP (3.8 and 5.38%) respectively. Similar trends were also recorded in HL-II animals (Table 3). The increase in the activities of plasma enzymes such as AST, ALT. LPD and ALP indicates diabetic induced hepatic dysfunction (Larcan et al. 1979). Elevation of enzymes activities in plasma may be mainly due to the leakage of these enzymes from liver into the blood stream (Ohaeri 2001; Navarro et al. 1993). On the other hand, treatment of diabetic rats with Samh flour causing reduction in hepatotoxic effects in the activities of enzymes when compared with mean values of CD rats. These are comparable with earlier report of Ohaeri 2001. Toxicity induced by STZ could restore the activities of plasma enzymes by treatment of Samh seed diet which may

Table 4 Weight and Food Intake. All the values represents as Mean±SE of three independent experiments

Groups	Control Normal (*N*=6) CN	Control Diabetic (*N*=6) CD	5% Samh (*N*=6) SP-I	15% Samh (*N*=6) SP-II	HL Diet (*N*=6) HL-I	15% Samh (*N*=6) HL-II	ANOVA	
							F. value	Sig.
Weight (g)	237.9±92.00^{d}	183.0±25.92^{ac}	207. 1±47.10^{b}	205.9±58.50^{b}	174.4±33.72^{c}	197.8±.49.98^{ab}	13.32	0.000*
Food intake (g/day)	15.8±3.56^{a}	17.0±2.01^{b}	17.9±3.06^{b}	16.5±3.77^{a}	17.0±3.29^{bc}	16.2±4.13^{ac}	4.28	0.001*
Waste food (g/day)	4.1±3.58^{a}	2.9±2.06^{bc}	2.0±3.06^{b}	3.0±3.06^{a}	2.9±3.36^{bc}	3.7±4.14^{ac}	3.92	0.002*

CN Control normal, *CD* Control diabetic, *SP-I* (5% Samh seed powder), *SP-II* (15% Samh seed powder), *HL-I* High lipid diet (2% cholesterol), *HL-II* (High lipid diet-2% cholesterol with 15% Samh seed powder), *N* number of rats, *HL* Hyperlipidemic, *NS* Non Significant. *$p<0.05$, abc.

inhibit the liver damage, our results are consistent with Gayathri and Kannabiran (2008), El Demerdash et al. (2005). Similar trends were also recorded in HL-II animals (Table 3). The body weight regain were calculated as 13.04 and 12.5% after losing weight (12.1%) in STZ- induced diabetic rats. Some improvement in body weight 174.46 to 197.88 (13.1%) was observed after taking HL-II diet of high lipid for 6 weeks (Table 4). There was no significant effect observed in food intake in treated rats when compared with control groups. Highest amount of waste food was recorded in HL-I group. All the animals of diabetic induced showed a significant reduction in body weight as compared to healthy control. Ramesh et al. (2005) and Gayathri and Kannabiran (2008) have observed the decrease in body weight of diabetic and have rat shown the degradation of structural protein is due to diabetic, which contribute to the body weight.

Conclusion

The present study demonstrated that 5% (SP-I) Samh seed diet could normalize cholesterol, blood glucose, triglycerides and body weight and could be use for diabetic patients. Whereas, 15% Samh seed diet (SP-II) could not used due to high protein and carbohydrate contents. Further studies may be required to explain the exact mechanism involved in the process.

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