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ORIGINAL ARTICLE

Effect of samh seeds supplementation (Mesembryanthemum forsskalei Hochst) on liver enzymes and lipid profiles of streptozotocin (STZ)-induced diabetic Wistar rats

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Mesembryanthemum forsskalei Hochst seeds (samh seeds);
Streptozotocin-induced diabetic rats;
Lipid profile;
Lipid peroxidation

Abstract Thirty streptozotocin (STZ)-induced diabetic of Wistar Albino rats were divided into five groups. The rat groups received different food (natural diet or high fat content diet) supplemented with 10% or 15% of samh seeds for 6 weeks. At the end of the study, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phophatase (ALP) and lactate dehydrogenase (LDH) enzymes have been measured in diabetic rats liver. In addition, liver lipid profile (total cholesterol (TC), triglyceride (TAG), lipid peroxide production malondialdehyde (MDA)) and reduced glutathione (GSH) in have been measured in diabetic rats liver, and the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were also determined. The samh seeds diet supplemented with cholesterol significantly increase ($P < 0.05$) the levels of liver peroxide production MDA, TC and TG in diabetic rats comparing to the samh diet not supplemented with the cholesterol. However, the samh seeds significantly decrease ($P < 0.05$) the level of GSH. These data suggest that the samh seeds diet not supplemented with the cholesterol regulated C and TG metabolism and decrease the lipid peroxidation in the diabetic rats.

1. Introduction

Samh plant (*Mesembryanthemum forsskalei* Hochst) is a wild plant grown wildly in spring time on the northern part of Saudi Arabia especially in Al-Jouf region. The samh. Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world (e.g., Native American Indian, Jewish (Yaniv et al., 1987) Chinese (Covington, 2001) East Indian, Mexican). Many modern pharmaceuticals used in conventional medicine today also have natural plant origins. (Pandey et al., 1995; Oubre et al., 1997).

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The seeds of samh (Mesembryanthemum forsskalei) are 'Ostensibly Campylatropous' as they seem externally anatropous but internally they have a curved embryo and both the seed body and the funicle exchange shapes. The embryo occupies a cylindrical seed body and is surrounded by a thin layer of endosperm; the major part of the endosperm occupies an ovoid bulge resembling a seed body (Al-Jassir et al., 1995a).

The chemical composition of samh seed include 22.25% protein, 5.7% moisture, 5.6% fat, 4.0% ash, 9.7% crude fiber, and the remainder being total carbohydrates. Mineral element analysis revealed that potassium, magnesium, sodium and calcium were present as the major elements. Iron, manganese, zinc and copper were found at lower levels. However, lead was not detected in the samh seeds. Gas–liquid chromatographic analysis of the methylester of the fatty acids of the samh seeds oil revealed the presence of 14 fatty acids. 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 17 amino acids including eight essential amino acids. Glutamic acid, arginine, and aspartic acid were the major amino acids. Cystine and proline were present in trace amounts. These results some of which have not been reported elsewhere indicate the high nutritional potential of Saudi samh seeds. The total aerobic bacterial count and total sporeformers of seeds were 19×10^7 and 5×10^4 cfu/g, respectively, thus the enterobacteriaceae, Bacillus cereus and yeast and molds were 5×10^2, 1×10^2 and 7×10^2, respectively. The seeds were Staph free and the samh extract had no antimicrobial effect (Al-Jassir et al., 1995b).

Samh seeds obtained from Al-Jouf area were ground into flour analyzed and used as a replacement for wheat in the ratio of 10%, 20% and 30% for bread and 30%, 60% and 100% for cookies. The samh flour has high protein content and could be used as a replacement for wheat flour up to 30% without adversely affecting the bread specific volume much. Samh flour has improved the cookies appearance specially the colour (chocolate colour) (Mustafa et al., 1995).

Our objective was to investigate the antihyperlipidemic impacts of Mesembryanthemum forsskalei Hochst seeds and dietary supplements for use in diabetes, to propose guidelines that may aid practitioners in advising their patients, and to provide recommendations for future research.

2. Material and methods

2.1. Plant material

Mesembryanthemum forsskalei Hochst seeds (samh seeds) were brought 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 ground and mixed with standard pellets. The calculated food supplemented with 10% and 15% of samh seeds for the balanced fed and with 15% of samh seeds for high cholesterol content fed (2%). The food kept cool at 5 °C during the study.

2.2. Experimental animals and streptozotocin-induced diabetes

Two-month-old rats of Wistar Albino strains, weighing 200 ± 5 g were purchased from animal house (Faculty of Medicine, King Saud University). On arrival, they were housed separately in clear polycarbonated cages and exposed to light for 12 h by two 20-W white fluorescent bulbs (Osram L; Osram Sylvania, Munich, Germany) suspended 30 cm above the cages followed by darkness for the next 12 h and so on. Rats were kept in a temperature-controlled room at 22 ± 2 °C and humidity 50 ± 5%. Animals had free access to water. Diabetes was induced in rats by a single intraperitoneal injection of 65 mg/kg streptozotocin (Sigma Chemical Co., St. Louis, MO) freshly dissolved in 0.05 mol/L sodium citrate buffer (pH 4.5). After 48 h of treatment, 30 rats with diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycemia (i.e. 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.

2.3. Experimental design

A 6-week experiment was conducted on 36 adult male rats (30 diabetic surviving rats and six normal rats). The rats were divided into five groups of six rats each (Table 1). Group 1: diabetic control rats fed with standard diet, group 2: diabetic rats fed standard diet with replace partly by 10% of starch with samh seeds, group 3: diabetic rats fed standard diet with replace partly by 15% of starch with samh seeds, group 4: diabetic rats fed with high fat diet (2% cholesterol) and group 5: diabetic rats fed with high fat diet (2% cholesterol) with replace partly by 15% of starch with samh seeds. The rats were weighted at the start and at the end of study. The introduced food and the remnant of food were weighed to calculate food consumed.

2.4. Preparation of liver homogenate

Liver samples were homogenized in 20-fold volume of 50 mM potassium phosphate buffer (pH 7.5) by using a homogenizer (Ultra-Turrax T 25, IKA; Werke 24,000 r.p.m.j. Germany). Homogenates were centrifuged at 10,000*g* for 30 min at 4 °C and the supernatants were saved at −20 °C until use.

2.5. Liver marker enzymes

Alkaline phophatase (AP) activity in liver homogenates were determined using UDI colorimetric assay kit (United Diagnostics Industry, Dammam, KSA), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using UDI kits (United Diagnostics Industry, Dammam, KSA) which is based on the method of Reitman and Frankel (1957). Lactate dehydrogenase (LDH) activity levels were determined by a commercial assay (Boehringer Mannheim, Germany). Triglycerides (TG) were determined with enzymatic method (UDI kit) using triolein as a standard. Standard procedures (United Diagnostics Industry, Dammam, KSA) were used to determine total cholesterol (TC) with enzymatic colour endpoint method using cholesterol as a standard.

2.6. Oxidative stress markers and antioxidant enzymes

Reduced glutathione (GSH) was determined by the method of Ellman (1959). Liver tissue lipid peroxidation was estimated by

Table 1 The contents of diet produced to rats, the weight in percentage of grams.

Ingredient	Group IV	Group III	Group II	Group I	Control
Cornstarch	46.57	41.91	39.58	44.57	37.58
Casein	14.0	14.0	14.0	14.0	14.0
Dextrinized cornstarch	15.5	15.5	15.5	15.5	15.5
Sucrose	10	10	10	10	10
Soybean oil	4.0	4.0	4.0	4.0	4.0
Fiber	5.0	5.0	5.0	5.0	5.0
Mineral mix	3.5	3.5	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0	1.0	1.0
L-Cystine	0.18	0.18	0.18	0.18	0.18
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Tert-butylhydroquinone	0.0008	0.0008	0.0008	0.0008	0.0008
Samh-seed		4.66	6.99		6.99
Cholesterol				2	2

Table 2 Effect of samh diet on AST, ALT, ALP and LDH enzymes in rat liver. Data represents mean ± SEM of three independent experiments.

Variables	Control	Group I	Group II	Group III	Group IV	*P*
ALT	40.85 ± 1.78	43.80 ± 4.07	41.73 ± 2.29	38.70 ± 3.08	39.57 ± 2.39	0.76
AST	88.20 ± 2.23	104.80 ± 16.70	111.0 ± 16.0	109.7 ± 5.09	97.33 ± 7.10	0.22
ALP	39.88 ± 0.90	43.53 ± 1.77	42.30 ± 2.21	45.00 ± 1.19	43.07 ± 1.68	0.26
LDH	10.99 ± 1.18	9.200 ± 0.92	9.200 ± 0.60	11.13 ± 1.28	10.73 ± 1.88	0.63

the assessment of thiobarbituric reactive species (TBARS) level as described by Ohkawa et al. (1979). The quantity of lipid peroxides is expressed as nmol malondialdehyde (MDA) equivalents/mg protein. SOD was determined according to the method of Misra and Fridovich (1972). Catalase (CAT) was assayed colorimetrically as described by Luck (1971). Glutathione peroxidase (GPX) activity was measured according to the method described by Rotruck et al. (1973). Supernatant was assayed for glutathione content by the method of Ellman (1959) as mentioned before. GPX activity was expressed as μg GSH utilized/min/mg protein.

2.7. Statistical analysis

For statistical analysis of results obtained from three independent experiments, GraphPad Prism (GraphPad Software; Science Inc., San Diego, CA) was used by applying the student *t*-test with a *P*-value for significance set at least at 0.05.

3. Results

Effect of samh diet on AST, ALT, ALP and LDH enzymes in rat liver: As presented in Table 2, our data shows that there was no significant ($P < 0.05$) in AST and ALP levels in rat livers during experimental time period in STZ-induced diabetic rats, when compared to control rats. While, there was no significant difference in ALT and LDH among all groups of the studying. From this study, the administration of samh seeds caused difference in most liver enzymes levels which lead to changes in lipid metabolism.

In lipid profile, samh seeds show decreased in total liver TC levels in groups I and II. There was a significant increase ($P < 0.05$) in TC TG levels in groups III and IV compared with control group. However, there was decrease in the level of TG among group II with no significant differences. This might explain that the high level of TG in samh seeds diet increase the levels of cholesterol and TG compare with the samh diet without cholesterol supplement (Fig. 1).

In addition, the effect of samh seeds on liver peroxide production (MDA) and GSH in diabetic rats liver have shown an increase in MDA in groups III and IV compare with control group ($P < 0.05$). While other groups have shown MDA values closer to normal values. The GSH was significantly decreased in groups III and IV compare with control group ($P < 0.05$) while other two groups (II and III) have shown no significant differences. This might have an effect of samh seeds diet supplemented with cholesterol which influenced in an increase the levels of liver peroxide production (MDA) and GSH in diabetic rats compare with the samh diet without cholesterol supplement (Fig. 2).

The samh seeds have also shown no significant changes in diabetic rats liver antioxidant enzymes among all study group. The levels of SOD, CAT and GPX show values closer to normal values meaning that, the samh seeds diet supplemented with or without cholesterol have no effect on the level of antioxidant enzymes in diabetic rats livers compare with control rats (Fig. 3).

4. Discussion

Most of the literature has focused on herbs or other dietary supplements. This finding parallels results from prevalence surveys that report herbal remedies or other dietary supplements taken by mouth to be consistently among the top CAM therapies used, regardless of the sample surveyed (Egede et al., 2002).

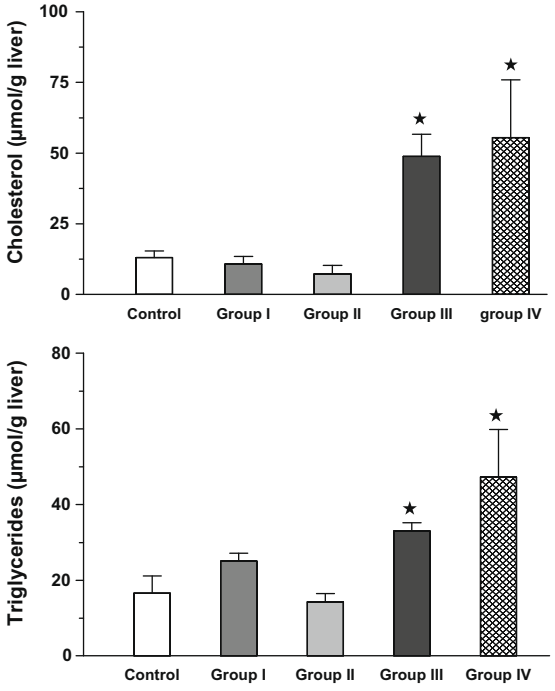

Figure 1 Liver cholesterol and triglycerides. Data represents mean ± SEM of three independent experiments. *Statistically significant at $P < 0.05$ from the control.

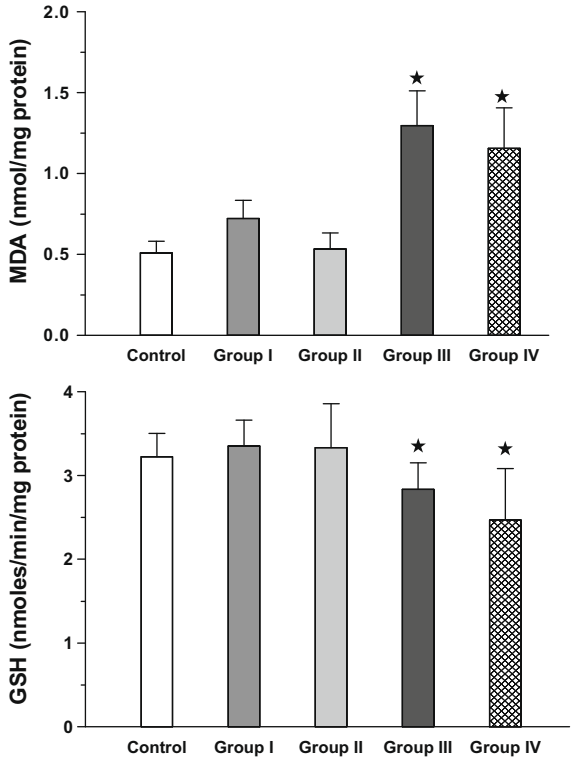

Figure 2 Lipid peroxide production (MDA) and GSH in rats liver. Data represents mean ± SEM of three independent experiments. *Statistically significant at $P < 0.05$ from the control.

This study has investigated the impact effect of samh seeds (Mesembryanthemum forsskalei Hochst) as hypoglycemic and

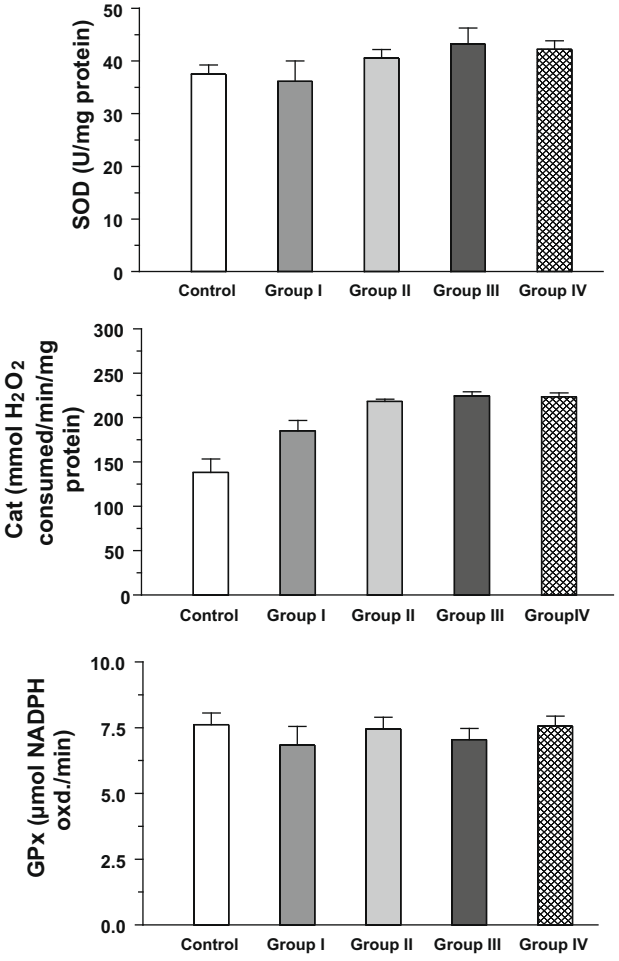

Figure 3 Antioxidant enzymes in control and treated rats liver. Data represents mean ± SEM of three independent experiments.

antihyperlipidemic in healthy and streptozotocin-induced diabetic (STZD) rats. Samh is grown wildly in Al-Jouf (northern Saudi Arabia) desert during the rainy season. Until now, people of Al-Jouf use it like flour for bread, cookies or mix with dates. The samh flour has high protein content and could be used as a replacement for wheat flour up to 30% without adversely affecting the bread specific volume much. Samh flour has improved the cookies appearance specially the colour (chocolate colour) (Mustafa et al., 1995).

In our knowledge, there is very limited number of publication about the samh or the samh seeds. Al-Jassir et al. (1995b) have investigated the chemical composition of samh seed. Mineral element analysis revealed that potassium, magnesium, sodium and calcium were present as the major elements. Iron, manganese, zinc and copper were found at lower levels. However, lead was not detected in the samh seeds. Macromolecules like total protein, carbohydrates and fatty acids have been found in reasonable amount. Al-Jassir et al. (1995b) have also reported that the samh seeds oil presence of 14 fatty acids and linoleic and oleic acids were the principle unsaturated fatty acids, whereas, palmitic acid was the main saturated fatty acid. Amino acid analysis of the samh seeds showed the presence glutamic acid, arginine, and aspartic acid as the major amino acids and cystine and proline as minor ones. Moreover, in food

industry, Elgasim and Al-Wesali (2000) have reported that ground beef patties with samh seed flour increased ash content of the patties significantly.

Diabetes mellitus is one of the most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia diabetes mellitus is one of the most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complications of both clinical and experimental diabetes.

Furthermore, in diabetes, hyperglycemia results in the generation of free radicals due to autoxidation of glucose and glycosylation of proteins. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation. The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenging systems. Hence, in this study, we investigate the effect samh seeds in the rodent animal model of STZD on AST, ALP, TC, TG, MDA, GSH, SOD, CAT and GPX.

Results of this study are consistent with the following conclusions: First, in STZD animals there was no significant in AST and ALP levels in livers. Second, there was decrease in TC in groups I and II, whereas, significant increase in TC in groups III and IV. Also, TG level was increased in studying groups III and IV with significant differences in comparison with control group ($P < 0.05$). Third, the level of MDA has been increased groups III and IV incontrast with control group. Fourth, the GSH was significant decrease ($P < 0.05$) in groups III and IV, while other groups (groups II and III) show no significant differences. Fifth, the levels of SOD, CAT and GPX show values closer to normal values which is mean that the samh seeds diet supplemented with/without cholesterol not affected the level of antioxidant enzymes in diabetic rats livers compared with control rats.

Recently, Sahach et al. (2008) have reported that the STZ up regulates oxidative (lipid peroxidation as a marker) and nitrosative (protein nitrosilation as a marker) stresses as well as ROS (O(2−), H_2O_2, OH) generation in rats. In general, STZ is a commonly employed compound for the induction of diabetes mellitus in experimental rats. It causes DNA strand breaks in pancreatic islets, stimulates nuclear poly (ADP-ribose) synthetase, and thus depletes the intracellular NAD+ and NADP+ levels, which inhibits proinsulin synthesis and induces diabetes. The decrease in body weight in diabetic rats shows that the loss or degradation of structural proteins is due to diabetes, and the structural proteins are known to contribute to the body weight (Ramesh et al., 2005).

Diabetes mellitus has been reported to generate reactive oxygen species (ROS). ROS, such as free hydroxyl radicals ($^{\cdot}OH$) and superoxide ($O_2^{\cdot-}$), can cause lipid peroxidation. The products of lipid peroxidation are capable of interacting with DNA and cause oxidative damage (Zhang and Tan, 2000). In our study, the lipid peroxidation markers such as MDA were significantly increased in the plasma of STZ-diabetic rats as reported earlier.

Oxidative stress occurs when there is an imbalance between free radical reaction and the scavenging capacity of the antioxidative defense mechanism of the organism. The nonenzymatic antioxidants such as GSH, vitamin C, and vitamin E are interrelated by recycling process. Glutathione is the most important non-protein compound-containing thiol group, which acts as a substrate for glutathione transferase and glutathione peroxidase involved in preventing the deleterious effect of oxygen radicals (Zhang and Tan, 2000). In our study, diabetic rats showed a significant decrease in the level of GSH, which may be due to increased utilization. In samh seed fed diabetic rats, a significant improvement in GSH was observed. This could be due to the decreased utilization of GSH. In this context, number of other plants has also been reported to have different effects (Latha and Pari, 2003).

In summary, this paper is the first paper which has investigated the effect of samh seed in diabetes and/or any health related disease. Our data observed that samh seeds decreases GSH level significantly in groups III and IV (diabetic rats fed with natural diet supplemented with 15% of samh seeds and diabetic rats fed with 2% cholesterol, respectively). This might have an effect of samh seeds diet supplemented with cholesterol which influenced in the levels of liver peroxide production (MDA) and GSH in diabetic rats compare with the samh diet without cholesterol supplement. Although the concentration of hepatic cholesterol was significantly high in groups III and IV, the TC level has not been changed on the other group. The seeds were also confirmed to suppress the levels of hepatic triglyceride in groups I and II. These data suggest that the samh seed have some influence in lipid profile and liver antioxidant enzymes of diabetic rats.

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