Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(10): 1429-1437

ISSN- 0975 1556

Research Article

Germination of *Glycine max* Seeds Potentiates its Antidiabetic Effect in Streptozotocin Induced Diabetic Rats

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Available Online: 15th October, 2016

ABSTRACT

Glycine max was a native legume to East Asia and now it is grown worldwide. It contains phytoestrogens, antioxidants and many bioactive compounds. It is used for stimulation of immune system and as adjuvant therapy in diabetes, liver and kidney disorders and some cancers. Objectives: The present study aims to evaluate the impact of germination on the antidiabetic property of *Glycine max* in diabetic rats. Methods*: Glycine max* seeds were germinated and dried then total polyphenol, flavonoid and the antioxidant power were estimated for non-germinated and germinated *Glycine max*. The feeding experiment was done on 24 albino rats for 5 weeks. Diabetes was induced by Streptozotocin. Nutritional assessment was done. Biochemical parameters were determined including hemoglobin, glucose, urea, creatinine, AST, ALT, lipid profile, G6PDH, total antioxidant activity and lipid peroxidation. Results: Results showed that germination increased total polyphenol, flavonoids and antioxidant power. Biochemical analysis revealed that germinated *Glycine max* was more effective to improve blood glucose, deterioration in body weight, alternation of HDL-C and LDL-C and restored antioxidant parameters to some extent. Conclusion: Consequently, it can be concluded that germination of *Glycine max* potentiates its hypoglycemic effect in diabetic rats and prevents or even reduces the complications of diabetes mellitus.

Keywords: *Glycine max*, diabetic rats, germination, antioxidant activity, blood glucose.

INTRODUCTION

Diabetes mellitus is among the major chronic metabolic diseases which affect huge numbers of people worldwide. It affected around 422 million people in the world in 2014 compared to 108 million in 1980[1]. More than 35.4 million people in Middle East and North Africa (MENA) are living with diabetes which is expected to rise to 72.1 million people by 2040. In Egypt, there were about 7.8 million cases of diabetes in 2015[2]. These increasing numbers of diabetic patients are due to increase in age, obesity, and urbanization of the world's population[3]. Many types of diabetes are known. Type 2 diabetes which represents 90 to 95 % of all diagnosed cases and strikes more frequently older people while type 1 diabetes, usually occurs in children and young adults and represents from 5 to 10 % of cases. The third type is the gestational diabetes occurring in some pregnant women[4]. All types of diabetes share the impaired ability of the body to produce energy from food. After ingesting a meal, the body converts most food into glucose which is the principal source of fuel for cells. In diabetic people, insulin level is reduced due to either insufficient production or impaired cellular response to insulin or both leading to hyperglycemia. Recent studies have revealed that damage due to oxidative stress may result in physiological dysfunction, death of cell, pathologies such as diabetes, aging and cancer[5]. Pham-Huy et al. (2008)[6] added that oxidative stress participates in the development of vascular complications in type 2 diabetes. In case of untreated diabetic, glucose accumulates in the blood rather than entering into the cells, thus no energy is formed. After a while, hyperglycemia leads to complications in different body organs such as kidneys, heart, blood vessels, eyes, nerves, feet, and skin. Prevention of these conditions can be achieved through controlling blood glucose, blood pressure, and cholesterol levels[4]. Although several synthetic anti-diabetic drugs are available to ameliorate the defected insulin secretion, insulin resistance, and hyperglycemia that is characteristic for type 2 diabetes mellitus, some of these drugs may have a lot of side-effects at high doses[7,8]. In recent decades, a major objective of current anti-diabetic research is to produce anti-hyperglycemic agents that are characterized by safety and without side-effects. In this respect, some anti-hyperglycemic agents have been found in plants[9,10]. Research in past few years revealed that a higher intake of plant products is correlated to a lower risk of some chronic diseases. These beneficial effects were attributed to the bioactive compounds with their antioxidant activity. Among these bioactive compounds are the phenolic compounds which are a large group of the secondary metabolites widespread in plant kingdom with their potent antioxidant activity[11]. *Glycine max* (soybean) is a rich source of vegetable protein and polyphenols and it is regarded to as a unique and complete food due to its

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rich nutrient content[12,13]. It is one of legume species that was native to East Asia and now it is widely cultivated around the world. In North America, soybean is called soya bean. It is widely grown for its edible bean that has several benefits. Of any plant, soy contains the greatest concentration of the potent antioxidants; isoflavones which are a class of phytoestrogens[14]. *Glycine max* isoflavones include genistein, daidzein, and glycitein. Moreover, the dried bean contains vegetable protein (40%), complex carbohydrates (35%), fat (18-22%), dietary fibre, oligosaccharides, minerals and other phytochemicales like saponines[12]. Its low glycemic index is attributed to its complex carbohydrates and dietary fiber contents, thus it can be beneficial for diabetic individuals[15]. Also, animal studies have found that isoflavones in *Glycine max* improve glucose tolerance and exert an antidiabetic effect[16]. Ademiluyi et al, (2014)[17] reported that there is an inverse correlation between *Glycine max* consumption and incidences of some degenerative diseases such as diabetes. Nutritional studies conducted in animals and intervention studies in humans have revealed that the intake of soy protein with isoflavones improves glycemic control and lowers insulin resistance[18]. Sprouting or germinating *Glycine max* is used to improve its nutritional value giving rise to more nutritive *Glycine max* sprouts. In general, seed sprouts have usually used in the diet as healthy food and recent studies show that besides being a good source of basic nutrients, they also have substantial phytochemicals that protect against diseases and of health promoting properties. Germination significantly improves the content of all enzymes. Also, it mobilizes complex food forms such as protein and concentrated starch into simpler free amino acids and carbohydrates, respectively[19]. Sprouting induced a considerable increase in the content of saponin and isoflavones of seeds as stated by Zhu et al., (2005)[20]. They also added that germination was used in food products to overcome the disadvantages of *Glycine max* seed. Seed sprouts survive during germination in the natural environment by increasing its defensive response through the formation of phenolic compound. This increases significantly the nutritional value of the resulting seed sprouts. Germination also eliminates anti-nutrients in the seed like enzyme inhibitors resulting in sprouts that are more nutritive for the diet. Sprouts have considerable higher nutritional value on the human body due to their high concentration of essential nutrients and proteins in mobilized forms that can be utilized easily by the body[19]. In this respect, Vernaza et al., (2012)[21] reported that germinated *Glycine max* showed higher antioxidant activity and more soluble protein concentration. Since *Glycine max* seeds was reported to have anti-diabetic effect and also, germination was found to increase the polyphenol content of *Glycine max* and enhance its antioxidant activity as well as increasing its nutritive value, the aim of the present study was to investigate the change in anti-hyperglycemic effect of germinated *Glycine max* compared to its non-germinated form in streptozotocin induced diabetes in rats.

MATERIALS AND METHODS

Materials

Most of the ingredients used for preparation of the diet were obtained from the local market. Ingredients used for formulation of vitamin and salt mixtures were obtained from Fluka (Germany) and BDH (England) Chemical Companies. Skim milk powder was obtained from Irish Dairy Borad, Gratten House, Dublin, Ireland. *Glycine max* was purchased from the Agricultural Research Centre, Giza, Egypt. The obtained variety was Giza 82. Animals used in the biological experiment were obtained from the National Ophthalmology Research Centre, Egypt. The study protocol was approved by Scientific Committee at National Research Centre (NRC, Egypt). Animal experiments were conducted according to the guidelines of animal care and ethics committee of the NRC (Approval no: 15058). Streptozotocin used for induction of diabetes was obtained from Sigma-Aldrich Co., USA. Citrate buffer as well as kits used for determination of blood hemoglobin, serum creatinine, serum urea, serum cholesterol, serum HDL-cholesterol, serum LDL-cholesterol, serum triacylglycerols, serum ALT & AST activities, serum lipid peroxide as malondialdehyde, blood glucose, serum total antioxidant and glucose-6-phosphate dehydrogenase (G6PDH) activity in whole blood were obtained from Biodiagnostic Co., Egypt. Folin–Ciocalteu reagent, gallic acid and quercetin were obtained from Sigma-Aldrich Co., USA.

Methods

Glycine max sample was divided into two portions. The first portion was germinated for two days, then, the resultant sprouts were dried in an air ventilated oven at 60 C^{o}. The obtained dry sprouts, as well as the second portion of the non-germinated *Glycine max*, were then milled in a mechanical grinder (Braun, Germany) into fine powder and used in the feeding experiment. The obtained powder of both soy bean and soy bean sprouts was subjected to extraction procedure and chemical analysis prior to the feeding experiment.

Extraction and Determination of total polyphenol & total flavonoid contents

Samples were extracted according to the procedures of Hayat et al., (2010)[22]. The moisture content of the sample was estimated in order to calculate the concentrations on a dry weight basis. Total phenolic content (TPC) of samples was analyzed by Folin–Ciocalteu assay using gallic acid as standard[23]. The total phenolic content was expressed as gallic acid equivalents (mg of GAE/100g dry weight of sample). Total flavonoid content (TFC) was determined according to the colorimetric method of Kim et al., (2006)[24]. The results were expressed as mg of quercetin equivalents (mg of QE/g dry weight of sample) using the calibration curve of quercetin.

Induction of diabetes

Diabetes was induced in a group of rats with a single injection of freshly prepared streptozotocin (STZ) with a dose of 50 mg/kg body weight dissolved in 0.1 mol/l citrate buffer (pH 4.5) by intraperitoneal route[25]. Four days after STZ administration, diabetes was confirmed by determination of fasting blood glucose concentration. The

animals with blood glucose more than 200 mg/dl were selected as diabetic rats.

Animal experiment

Twenty four white female albino rats (Sprague Dawley strain) with a body weight ranging from 88 to 120 g were used. Animals were divided into 2 groups, the first group comprising 6 rats and was considered as a control group, while the other group comprised the rest of animals which were injected with STZ for induction of diabetes as previously mentioned and then were divided into 3 diabetic groups (groups 2, 3 & 4). A standard control diet was prepared according to Reeves et al., (1993)[26] with some modifications. The 4 groups were given diets as follows;

Group 1: was given the control diet and considered as negative control group.

Group 2: diabetic rats given the control diet and considered as positive control group.

Group 3: diabetic rats that were given the control diet + 20% dried powdered of non-germinated *Glycine max*.

Group 4: diabetic rats given the control diet + 20% dried powdered of *Glycine max* sprouts.

Rats were housed individually in separate cages. The experiment lasted for 5 weeks during which, food and water were allowed ad-libtum to each rat. Body weight was followed twice a week. Food intake of each rat was recorded daily. At the end of the experiment, body weight gain and food intake were recorded. Feed efficiency ratio for each rat was calculated. At the end of the experimental period, rats were fasted overnight and in the early morning each rat in different groups was subjected to slight anesthesia by diethyl ether. Blood was withdrawn from sub-orbital vein and delivered into two tubes; heparinized tube and empty dry tube. Whole blood from the heparnized blood sample was used for immediate determination of blood hemoglobin and the activity of glucose-6-phosphate dehydrogenase (G6PDH) which is stable in whole blood for one week at 2-8 C°. Serum was separated from the samples that were delivered into dry tubes after centrifugation at 3000 rpm for 15 min and delivered into clean tubes, then, stored at –70 C° until analysis of other biochemical parameters in serum. Blood glucose was estimated according to the method described by Trinder (1969)[27]. Blood hemoglobin was determined according to Betke and Savelsberg (1950)[28]. Glucose-6-phosphate dehydrogenase (G6PDH) activity in whole blood was determined according to the method of Beutler et al., (1979)[29]. Serum urea and creatinine each was determined according to Fawcett & Soctt, (1960)[30] and Bartles, et al., (1972)[31], respectively. Serum cholesterol was determined as described by NCEP Expert Panel (1988)[32]. HDL-cholesterol was determined according to Lopes (1977)[33]. LDL-C was determined according to Warnick, et al., (1990)[34]. Serum triglycerides was assessed as described by Fossati & Prencipe (1982)[35]. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were determined according to Reitman & Frankel, (1957)[36]. Serum malondialdehyde was determined according to the method of Ohkawa, (1979)[37]. Serum total antioxidants were determined as described by Koracevic et al, (2001)[38]. The optical density of all of the previously mentioned biochemical parameters were measured by a colorimetric technique using a spectrophotometer (Shimadzu UV-2401 PC, Australia), then the concentration of each parameter was calculated according to the aforementioned methods.

Statistical analysis

Results were analyzed statistically using the computerized program SPSS version "20". The one way ANOVA test was done. Data were represented as mean ± SE. Significance was considered at a level of 0.05.

RESULTS AND DISCUSSION

Glycine max (soybean) is considered as a unique and complete food because of its rich nutrient content. This legume contains complex carbohydrates, vegetable protein, dietary fiber, oligosaccharides, phytochemicals (isoflavones, saponins *etc*), and minerals. Its complex carbohydrates and dietary fiber contents contribute to its low glycemic indexes, which benefit diabetic individuals[15]. Also, Kwon et al., (2010)[18] reported that *Glycine max* seeds have a low-glycemic index and are rich in fiber, making them a good addition to the diet of type 2 diabetic patients. Sprouting significantly improves the nutritional value of the *Glycine max*. Germination is considered as an inexpensive and simple method that have multi-effects in enhancing nutritional value and reducing anti-nutritional factors. These include: (1) increasing the vitamin contents (e.g., riboflavin and ascorbic acid) and calcium bioavailability (2) improving the digestibility of protein; (3) hydrolysis of oligosaccharides which cause flatulence; (4) lowering the trypsin inhibitors levels, phytic acid, lectin and lipoxygenase activity that give the undesirable beany flavor; and (5) increasing the phenolics, isoflavone aglycones and saponin glycosides, therefore increasing the antioxidant activity of the sprouted *Glycine max*[39]. Based on these findings, a number of studies mentioned that the use of soy and soy products can led to a better glycemic control[40,41].

Antioxidant power of the non-germinated and germinated Glycine max

The obtained data from the present study illustrated that there was a marked elevation of both total polyphenols and flavonoids by germination of *Glycine max* (table 1). This can be explained on the basis of the benefits of germination. One of these benefits is to increase the polyphenol content of the sprouts to enhance its defensive response against the surrounding environment[19]. This improved the antioxidant power of the sprouts as the five tests which were carried out in this study for the antioxidant activity revealed (table 2). The recorded value of the antioxidant power in each test was always greater in

Table 1: Total polyphenols & flavonoids of non-germinated & germinated *Glycine max*.

Material	Total polyphenols (mg GAE/100g)	Flavonoids (mg QE/100g)
Non-germinated *G. max*	616.7	339.8
Germinated *G. max*	933.3	548.3

Table 2: Antioxidant activity represented by DPPH, TPTZ, peroxide radical, B- carotene & ABTS for non-germinated & germinated *Glycine max*.

Material	DPPH (% Scavenging activity)	TPTZ (Mole T E/100g FW)	peroxide radical	B-carotene	ABTS (µmol T/ gm FW)
Non-germinated *G. max*	36.52	640.85	0.281	50.21	0.144
Germinated *G. max*	39.60	709.13	0.329	82.49	0.173

Table 3: Food intake, body weight gain and food efficiency ratio (FER) of different groups.

Group	Food intake (g)	B. wt. Change (g)	F E R (g)
Control Negative	599.33 ± 3.40 [b]	57.83 ± 7.52 [a]	0.096 ± 0.012 [a]
Diabetic control	497.5 ± 17.95 [a]	-38.17 ± 9.47 [b]	-0.077 ± 0.020 [b]
Diabetic+non-ger. *G. max*	516.17 ± 27.92 [a]	-3.00 ± 3.20 [c]	-0.027 ± 0.015 [c]
Diabetic+ger. *G. max*	572.33 ± 3.02 [b]	3.83 ± 4.14 [c]	0.008 ± 0.007[c]

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level.
*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.

Table 4: Concentration of blood hemoglobin and serum glucose of different groups.

Group	Hb (g/dl)	Glucose (mg/dl)
Control negative	14.06 ± 0.44 [a]	84.67 ± 3.28 [a]
Diabetic control	13.04 ± 0.58 [a]	298.03 ± 14.97 [b]
Diabetic+non-ger. *G. max*	13.24 ± 0.23 [a]	246.19 ± 11.15[c]
Diabetic+ger *G. max*	13.64 ± 0.26 [a]	222.83 ± 5.90 [c]

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level.
*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.

case of the germinated *Glycine max* than the corresponding value of the non-germinated soybean. Vernaza et al., (2012)[21] reported similar findings, since they concluded from their experiments that germinated *Glycine max* flour showed higher antioxidant capacity and soluble protein concentration. Thus, it is obvious that germination raised the antioxidant power of *Glycine max* as it increases the bioactive compounds responsible for the antioxidant activity which are the total polyphenols; particularly the isoflavones that represent a category of phytoestrogens. It is well known that *Glycine max* contains the greatest concentration of isoflavones among all plants[14] and germination increases the concentration of these isoflavones.

Biological evaluation of non-germinated & germinated Glycine max

It can be noticed that induction of diabetes led to a significant reduction of the food intake in the diabetic control group (table 3) as rats lost their appetite because of the deterioration of their overall condition as they became hyperglycemic and the consequent complications. This decrease of food intake began to be improved in the diabetic group that received the *Glycine max* without germination, although the difference between results was non-significant with the diabetic control group. The improvement in food intake proceeded in the diabetic group that was fed on the germinated *Glycine max* and there was a significant increase in values compared to the diabetic control group. The values became more or less near to the value recoded for the control negative group. This was attributed to the improvement in the overall condition of the rats that received the germinated *Glycine max*. Also, the body weight of the diabetic control group showed reduction compared to the control negative group (table 3). The loss in body weight of rats due to STZ injection was reported before[42,43]. This loss in body weight began to be reduced from a value of -38.17 ± 9.47 g in the control diabetic group to -3.00 ± 3.20 g in the diabetic group that received the *Glycine max* without germination then it became a gain in case of the diabetic group that was fed on the germinated *Glycine max* with a value of 3.83 ± 4.14 g. This means that the introduction of *Glycine max* in the diet of the diabetic rats has led to a better utilization of food with its consequent improvement in body weight, in particular the germinated *Glycine max* that improved the body weight from loss in diabetic control group gradually to a gain in the diabetic group that was fed on germinated *Glycine max*, although did not reach the normal value of the control negative group. Similar results were found by Choi et al., (2010)[44] who mentioned that *Glycine max* reduced weight loss in diabetic rats. The ability of germinated *Glycine max* to restore the body weight to some extent may be related to its ability to reduce hyperglycemia due to increase in isoflavones concentration compared to the non-germinated *Glycine max*[45]. Also, it may be due to controlling muscle wasting which means the reversal of gluconeogenesis[46]. A non-significant reduction in the concentration of hemoglobin was noticed in the diabetic control group (table 4). This finding was reported before by Oyedemi et al., (2011)[47] who stated that the occurrence of anemia during diabetes may be explained as follows; the persistent hyperglycemia concomitant to diabetes results in enzymatic glycosylation of body proteins among which the hemoglobin and RBCs membrane proteins. Oxidation of these proteins along with hyperglycemia in diabetes mellitus led to the formation of

Table 5: Concentration of serum urea and creatinine and activities of serum AST and ALT of different groups.

Group	Urea (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)
Control negative	19.73 ± 2.15[ab]	0.51 ± 0.03 [a]	69.60 ± 11.72 [a]	61.60 ± 8.20 [a]
Diabetic control	23.37 ± 2.20[a]	0.52 ± 0.04 [a]	78.50 ± 6.17 [a]	79.00 ± 11.89 [a]
Diabetic+non-ger *G. max*	15.00 ± 0.45[b]	0.49 ± 0.03 [a]	68.50 ± 9.14 [a]	82.67 ± 5.88 [a]
Diabetic+ger. *G. max*	15.75 ± 0.54 [b]	0.58 ± 0.02 [a]	75.00 ± 5.33 [a]	85.42 ± 2.08 [a]

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level.
*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.

Table 6: Concentration of serum triacylglycerols (TG), total cholesterol (TC), LDL-C and HDL-C of different groups.

Group	TG (mg/dl)	TC (mg/dl)	HDL-C (U/L)	LDL-C (U/L)
Control negative	97.32 ± 10.83 [a]	85.66 ± 4.45 [a]	42.84 ± 3.94 [a]	23.77 ± 1.59 [a]
Diabetic control	90.67 ± 10.37[a]	86.17 ± 6.65 [a]	31.17 ± 1.89 [b]	37.27 ± 1.55 [b]
Diabetic+non-ger *G. max*	97.50 ± 3.68[a]	79.33 ± 3.65 [a]	33.67 ± 0.92 [b]	29.80 ± 1.59 [b]
Diabetic+ger. *G. max*	91.00 ± 3.17 [a]	86.75 ± 5.84 [a]	40.33 ± 1.58 [a]	27.88 ± 2.41 [a]

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level.
*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.

Table 7: Whole blood glucose-6-phosphate dehydrogenase (G6PDH) activity, serum total antioxidant activity and concentration of serum lipid peroxidation product; the malondialdehyde (MDA) of different groups.

Group	G6PDH (U/L)	Total antioxidant activity (U/ml)	MDA (n mol/ml)
Control negative	11.92 ± 0.66[a]	0.52 ± 0.07 [a]	7.37 ± 0.29 [a]
Diabetic control	14.24 ± 0.78[a]	0.84 ± 0.08 [b]	8.60 ± 0.37 [a]
Diabetic+non-ger. *G. max*	13.65 ± 1.39[a]	0.80 ± 0.05[b]	8.35 ± 0.57 [a]
Diabetic+ger. *G. max*	12.53 ± 0.77[a]	0.78 ± 0.03 [b]	8.18 ± 0.39 [a]

*Values are expressed as mean ± SE and the mean difference is significant at the 0.05 level.
*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.

lipid peroxides which in turn led to hemolysis of RBCs[48]. The diabetic rat model obtained by injection of STZ showed increased blood glucose (table 4) that may be attributed to destruction of pancreatic β-cells. STZ was mentioned to kill cells by forming a toxic DNA compound and also contributes to glucotoxicity of the β-cells' mitochondria[49]. Mitochondria are very important for the survival of β-cells and for glucose-induced insulin secretion[50]. Therefore, it results in insulin deficiency and insulin resistance leading finally to impaired glucose transport into the cells and consequently accumulation of glucose in the circulation in a state described as hyperglycemia. As shown from the obtained data in this study, feeding diabetic rats on either non-germinated soybean or the germinated soybean reduced significantly blood glucose levels. Similar findings were reported by Choi et al., (2010)[44] who stated that ingestion of *Glycine max* diet improved the glycemic control in streptozotocin induced diabetic rats. Many mechanisms have been postulated for the mode of action of *Glycine max* as anti-hyperglycemic agent, some of which were proved experimentally. It is possible that *Glycine max* fibers, which contains pectins, galactomannans and arabinogalactans with high viscosity, delay gastric emptying and glucose absorption[51]. Also, *Glycine max* was reported by Adedayo et al. (2013)[52] to have inhibitory effects against amylase and glucosidase which are enzymes on the brush borders of the small intestine that catalyze the conversion of carbohydrates into glucose and facilitate the glucose absorption from intestinal lumen. This in turn may help to reduce postprandial hyperglycemia by inhibiting the enzymatic hydrolysis of carbohydrates, and hence may delay the absorption of glucose. Moreover, the *Glycine max* isoflavones; genistein and daidzein, which are phytoestrogens having estradiol effects, were reported to bind to estrogen receptor α that is a key molecule involved in the metabolism of lipid and glucose. This estrogen receptor is found in pancreatic β-cells that regulates the biosynthesis and secretion of insulin from pancreas, also it helps in survival of β-cells. Thus, *Glycine max* isoflavones stimulate insulin synthesis and secretion[53,18]. Thus, decreasing glucose levels in rats fed on *Glycine max* may be attributed to either one of these mechanisms or to all these mechanisms combined together. Germinated *Glycine max* fed group showed better results for reduction of blood glucose level than did the non-germinated *Glycine max* since germination increases the isoflavone content of *Glycine max* as well as increasing the inhibitory action against amylase and glucosidase enzymes[54] thus decreasing the glucose levels more efficiently than the non-germinated *Glycine max.* A non-significant increase in urea concentration was noticed in the diabetic control group compared to the control negative group (table 5). This increase was restored back to the normal value of the control negative group in case of the two groups that received the *Glycine max* either non-

germinated or germinated reflecting the potentiality of the *Glycine max* to ameliorate the deleterious effects due to diabetes or the toxic effect of streptozotocin itself on the kidney. Choi et al., (2010)[44] concluded from their study that *Glycine max* may prevent the deterioration of kidney due to diabetes mellitus. In this respect, Azadbakht et al., (2003)[55] and Stephenson et al., (2005)[56] mentioned that using *Glycine max* protein instead of animal protein in diabetic kidney diseased human subjects resulted in improving renal function reducing protein loss. On the other hand, creatinine concentration did not show any change. The activities of liver enzymes (AST and ALT) showed a non-significant increase in the diabetic control group compared to the control negative group (table 5). This increase was returned back to near the normal value of the control negative group in case of AST in the group that received non-germinated *Glycine max*, while remains unchanged in case of ALT. Different finding for the ALT, was mentioned by Shim et al., (2007)[57] who reported that the increased activity of ALT in STZ induced diabetes in rats was restored when diabetic rats was administered with *Glycine max* isoflavones extract. This contradictory results may be explained on the basis that *Glycine max* isofavones extract is more potent than *Glycine max* itself to ameliorate the liver dysfunction. So, may be longer duration of ingesting *Glycine max* or much more quantities of it are required to ameliorate the liver dysfunction associated with the induced diabetes. No significant change was noticed in this study for either of the triacylglycerols or the total cholesterol in the diabetic control group (table 6). On the other hand, a significant decrease in the concentration of HDL-C and a significant increase in the LDL-C concentration were observed in the diabetic control group compared to the control negative group. Feeding the diabetic rats on the germinated *Glycine max* rendered the HDL-C and LDL-C concentrations near the normal values. Similar findings were reported for LDL-C by Pipe et al., (2009)[58] who mentioned that LDL-C was reduced in diabetic patients after ingestion of *Glycine max* protein isolate. Also, Anderson et al., (1995)[59] from meta-analysis of studies before 1995, mentioned that 50 g of *Glycine max* protein per day caused a reduction of LDL-C by 12%. Jenkins et al. (2010)[60] has attributed the LDL-C lowering effect of *Glycine max* to being a complex protein with seven globulin fractions. These fractions may be digested into simpler peptides which hinder cholesterol synthesis. They also added that the *Glycine max* isoflavones and saponins may be responsible for the lowering effect of LDL-C of the soy. Moreover, some other studies mentioned that *Glycine max* isoflavones enhance clearance of lipoprotein and increase the biogenesis of HDL-C by regulation of gene expression of certain nuclear receptors resulting in regulating the lipid and glucose metabolism[61]. Babashahi et al. (2015)[62], concluded from their study that fermented soy milk increased the HDL-C and decreased the LDL-C than did the soy milk without fermentation. They explained that, fermentation process led to changes in the structure and function into a mixture of amino acids and biologically active peptides that have more potent beneficial effect. The isoflavones glucosides were converted into aglycones with higher physiological activity. The germination process is partially similar to the fermentation process in producing biologically active peptides and thus render the *Glycine max* sprouts more potent than the non-germinated *Glycine max*. The antioxidant activity as measured by the serum total antioxidant activity, the glucose-6-phosphate dehydrogenase (G6PDH) and malondialdehyde for lipid peroxidation (table 7) recorded increase in the diabetic group, this increase was significant only in case of the total antioxidant activity. Increased G6PDH activity was explained on the basis of its essential role for generating NADPH from the pentose phosphate pathway[63]. Hyperglycemia was reported to liberate reactive oxygen species that increasing the oxidative stress in diabetes as evidenced from the experimental and clinical studies[64], hence, more NADPH molecules are required to normalize or counteract these liberating oxidizing molecules and consequently, this may explain the increased levels of G6PDH in the diabetic control group. In a trail to counteract the increased oxidative stress, the body's own antioxidant enzymes increase to overcome the free radicals produced and this is well represented in the present study by increasing of total antioxidant activity. Also, lipid peroxide products increased as a result of the increased oxidative stress due to diabetes which liberates oxidizing free radicals that in turn lead to the lipid peroxidation of the fatty acids forming the membranes of cellular organelles[65], thus increasing levels of lipid peroxide products in the circulation. Addition of the *Glycine max* to the diet of rats restored the value of these antioxidant parameters, although non-significantly. The group that was fed on germinated *Glycine max* showed better improvement compared to the group that received *Glycine max* without germination. Germination is reported to increase the total isoflavones and the saponins in the *Glycine max* which are potent antioxidants thus improving the general antioxidant state of the body. Therefore, inclusion of germinated *Glycine max* (with its potent antioxidant activity) with the diet of diabetics can counteract the increased oxidative stress due to diabetes. Similar results were reported for the lipid peroxidation by Clerici et al. (2011)[66]. They found that individuals with type 2 diabetes when ingesting pasta enriched with soy-germ recorded a reduction in lipid peroxidation products.

CONCLUSION

It is obvious from the obtained data that feeding germinated *Glycine max* seeds to diabetic rats was able to ameliorate the loss in body weight, decrease blood glucose level, counteract the deterioration in kidney and liver to some extent, improve the decreased HDL-C and the increased LDL-C, improve the antioxidant activity much better than the non-germinated *Glycine max*. May be extra amounts of germinated *Glycine max* seeds have to be used with longer duration to obtain more positive results. Also, further studies are required to confirm these findings on human and to establish the exact quantities to be ingested. Therefore, it can be concluded that ingestion of germinated *Glycine max* improves the glycemic control in diabetic rats

and prevent or even reduce the complications of diabetes mellitus.

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