

Effects of Soybean Isoflavone on Lipid Profiles and Antioxidant Enzyme Activity in Streptozotocin Induced Diabetic Rats

¹Mona A. Abd El Latif, ¹Naglaa H. Mohamed, ¹Nahed L. Zaki,
²Mohamed S. Abbas and ²Hassan M. Sobhy

¹Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

²Natural Resources Department,

Institute of African Research and Studies, Cairo University, Giza, Egypt

Abstract: Soy isoflavone-containing diets have been reported to be beneficial in diabetes. This present study investigated the hypoglycemic effects of isoflavones in streptozotocin (STZ)-induced diabetes. Diabetes mellitus was induced by intraperitoneal injection of 50 mg/kg body weight streptozotocin dissolved in 0.2 mole sodium citrate at pH 4.5. Diabetic rats were then randomly divided into 3 groups. The oxidation stress significantly increased serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and very low-density lipoprotein-cholesterol (VLDL-C), while HDL-cholesterol was significantly decreased. Administration of the isoflavones diet improved or returned these values to the normal ones. Oral intake of the experimental diabetic rat groups with isoflavone and diamicon30MR extracts increase the activate of enzymatic antioxidants, Catalase (CAT), Superoxide dismutase (SOD), Total antioxidative capacity (TAC) and Glutathione Peroxidase (GSH-Px) and non-enzymatic antioxidant glutathione reductase (GSH) were reduced.

Key words: Diabetic • Insulin • Rats • Streptozotocin • Soy isoflavones

INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing worldwide and it will be a major health problem in the 21st century. Worldwide prevalence of DM was estimated to be 177 million cases in 2000 and is projected to increase to 366 million by 2030, largely owing to an aging population, increased urbanization and more sedentary lifestyles [1]. The metabolic characteristics of diabetes patients are abnormally high concentrations of glucose in the blood and lipid metabolic abnormality [2]. The serum lipid abnormalities in type 2 DM are characterized by decreased HDL-C level and hypertriglyceridemia, whereas total cholesterol and LDL-C levels are similar to those in non-diabetic patients [3]. Oxidative stress defined as an imbalance between oxidants and antioxidants leads to many biochemical changes and is an important causative factor in several human chronic diseases, such as atherosclerosis, cardiovascular diseases, mutagenesis, cancer, with

several neurodegenerative disorders and the aging process [4]. The important goal of DM treatment is to keep blood glucose, lipid and lipoprotein levels close to normal resulting in a reduction of coronary artery disease, a delay in onset and a major slowing in the progression of complications [5]. Soybean contains complex carbohydrates, protein, dietary fiber, oligosaccharides, phytosterol, saponin, lecithin, isoflavone, phytic acid, trypsin inhibitor and minerals. Complex carbohydrates and dietary fiber contents contribute to low glycemic indexes, which benefit diabetic individuals [6] and reduce the risk of developing diabetes. Also, soybean reduces cholesterol levels [7]. When diabetic patients were fed diets rich in leguminous seeds, in addition to improved blood glucose control, lower serum TG levels were seen than when other carbohydrate sources were used, especially for individuals with raised serum TG levels [8]. It appears from several studies that soy-based diets may provide benefits in conditions associated with impaired glucose

Corresponding Author: Mona A. Abd El Latif, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

tolerance, hyperlipidemia and reduced insulin sensitivity [9-11]. Thus, in the past two decades, investigators have focused on the blood glucose, lipid-lowering effects of soy-based foods [12]. Accordingly, the purpose of this study was to investigate potential glucose-lowering and hypolipidemic effects of soybean in type 2 DM patients comparing to Diamicon 30MR. We also examined the effects of soybean on lipid peroxidation and antioxidant enzymes activity in type 2 DM patients comparing to Diamicon 30MR. Consumption of soy foods/products has been associated with lower incidence of coronary heart diseases, atherosclerosis, type 2 diabetes [13]. The beneficial effects of soybean and by-products have been associated with their effects on insulin [14]. Isoflavones, mainly derived from soybean, are a group of biologically active substances with a chemical structure similar to that of estrogen [15]. Epidemiological studies have associated a diet rich in isoflavones with a lower risk of diabetes and diabetes-related complications such as cardiovascular disease [16]. Evidence suggests that soy isoflavones possess a hypolipidemic effect through their antioxidative, mild estrogenic activity and gene regulation involved in lipogenesis or lipolysis [17]. Recent study has shown that soy consumption lowers elevated plasma glucose concentrations [11]. Nutritional intervention studies performed in animals and humans suggest that ingestion of soy protein associated with isoflavones improves glucose metabolism control and insulin sensitivity [18].

The purpose of this study was to investigate the effect of soybean isoflavone on blood glucose and lipid concentrations and antioxidant enzymes activities in type 2 induced diabetes mellitus (DM) rats.

MATERIALS AND METHODS

Materials: Commercial soybean seeds sample (*Glycin max* L. Clark variety) was obtained from the Soy processing Unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Diamicon 30 MR was purchased from a pharmacy. From Servier Company, French. Streptozotocin: was purchased from Sigma Chemical Co. (St. Louis, Mo). Kits of (Glucose, Total cholesterol, High density lipoprotein cholesterol (HDL), Low density lipoprotein cholesterol (LDL), Triglycerides (TG), Catalase (CAT), Malondialdehyde (MDA), Total Antioxidant (TAC), reduced Glutathione (GSH) and Glutathione peroxidase (GSPX) were obtained from Randox Laboratories Ltd., Diamond Road, Crumlin, Co., Antrim, United Kingdom, BT294QY. The insulin kit was obtained from Biosource Europe S.A., B-1400 Nivelles, Belgium.

Methods:

Extraction of Isoflavones: The soybean seed was extracted using extracted into 80% aqueous methanol (10 ml/g) by stirring 1h at 60°C. The mixture was centrifuged for 10 minutes at 2500 rpm and the supernatant decanted into a round bottom flask. The pellet was resuspended in 80% aqueous methanol (2x5ml) and centrifuged and the supernatant were combined and taken to dryness using rotatory evaporator. The dried extract were then dissolved in 50% aqueous methanol (5 ml) and the lipid were removed by partitioning in hexane(4x20 ml).The aqueous methanol phase was evaporated to dryness using rotatory evaporator and the dried residue dispersed in 10 ml of 80% aqueous methanol, a mixture centrifuged and eppendorf microfuge just prior to analysis by HPLC. Separation of isoflavones was achieved by HPLC on a 30cm x 0.45 cm aqua pore C8 reversed-phase column with mobile phase consisting a gradient of 0- 46.4% acetonitrile in 0.1% (v/v) aqueous trifluoro acetic acid at low rate of 1.5ml/min. The eluting components were detected from their absorbance at 262nm. Concentration of the isoflavones were calculated from the standard curves of area responses for authentic isoflavones standards normalized to the constant amount of fluorescein added to each sample.

Determination of Isoflavones: Isoflavone contents in soybean seeds were determined by HPLC (Hewlett packard 1050, USA) according to Lori *et al.* [19].

Animal Feeding Experiment: Twenty four adult male Albino rats, average weight of 150±5 g., raised in the animal house of the Ophthalmology Research Institute, Giza, Egypt, were used in the present study. The rats were kept under normal healthy laboratory conditions, temperature was adjusted at 25 ± 2 °C and 12 hour light-dark periods. Animals were adapted on free access of water and fed for one week on standard basal diet before the initiation of the experiment.

Composition of the Basal Diet (g/kg): Casein,10%, cellulose, 5%, corn oil,10%, corn starch, 70%, salt mixture, 4% and vitamin mixture,1% according to Kumar *et al.* [20].

Design: After the adaptation period and after an overnight fast, the animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight) streptozotocin dissolved in 0.2 mmole sodium citrate at pH 4.5 according to the method described by Lutz and Pardridge [21]. Blood samples were

collected after 48 hours of injection and glucose levels was determined. Rats with blood glucose levels about 320 mg/dl are considered to be diabetic animals. After feeding on basal diet for one week, rats were divided into two groups. The first group (6 rats) was fed on basal diet for another 8 weeks and was considered as positive control group (control A). The second group (18 rats) was injected intravenously by streptozotocin 50 mg/kg body weight, was dissolved in 0.1 M fresh cold citrate buffer at pH 4.5 to induce hyperglycemia and then the whole group was fed on basal diet for 72 h. where hyperglycemia was developed. To ensure occurrence of diabetes in rats, blood sample was withdrawn after 72h of injection. The diabetic group was divided into three subgroups (6 rats each). The first subgroup was continued to be fed on basal diet and considered as negative control group (control B). Other subgroups were fed on different diets according to the following scheme:

The second subgroup: hyperglycemic rats' received basal diet and isoflavones (20g/kg of basal diet).

The third subgroup: hyperglycemic rats' received basal diet and dimacron30 (60 mg/100g of basal diet)

Biochemical Parameters:

Growth of Rats: The rats were weighed twice weekly, total feed intake of each rat was weighed and feed conversion efficiency, (gain of rat weigh/total feed intake, g) was calculated. At the end of the experimental period, rats were weighed and killed.

Biochemical Assay: At the end of experimental period, blood samples were collected from the animals from the eye plexuses on ice. Each sample was collected into both heparinized tubes to obtain the plasma and into a dry clean centrifuge glass tube without any coagulant to prepare serum. Blood was left for 15 min at room temperature, then the tubes was centrifuged for 15 min at 3000 rpm and the clean supernatant serum was kept frozen at -20°C until the time of analysis. Serum glucose and insulin were determined by Trinder [22] and Temple *et al.* [23]. Total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), VLDL- cholesterol and triglycerides (TG) in serum were determined by using the methods described by Amundson and Zhou [24], Matsuzaki *et al.* [25], Warnick *et al.* [26], Wallach [27] and Cole *et al.* [28]. The lipid peroxidation level (Malondialdehyde, MDA) in serum was determined by the colorimetric method described by Meltzer *et al.* [29]. Catalase in plasma was determined by enzymatic colorimetric according to the method of Aebi [30]. Total Antioxidant in serum was determined by enzymatic

colorimetric method according to Koracevic *et al.* [31]. Total reduced glutathione (GSH) in erythrocytes and glutathione peroxidase activity in blood (GSPX) were measured calorimetrically according to the method of Ellman [32] and Rotruck *et al.* [33].

RESULTS AND DISCUSSION

Isoflavone Concentration: Soybeans contain 0.1 to 5 mg total isoflavones per gram, primarily genistein, daidzein and glycitein the values found in this study were similar to those reported by Velasquez and Bhathena [34]. The results in Table 1 showed that the concentration of total isoflavones in Soybean seed (219.5 mg/100g). The concentrations of aglycones, β -glucosides and malonyl-glucosides, as referred to total isoflavones, for soybean 10.63%, 19.16% and 70.21%, respectively.

Table 2 displays the levels of serum glucose and insulin hormone in normal and experimental animals. The data revealed a significant increased elevation in blood glucose and a significant decline in insulin level in

Table 1: Mean isoflavone concentrations of soybean seed.

Isoflavones	Soybean seed (mg/100g)
Aglycones	
Daidzein	6.71
Genistein	13.48
Glycitein	3.15
β -glucosides	
Daidzin	13.83
Genistin	23.09
Glycitin	5.14
Malonylglucosides	
Malonyldaidzin	52.42
Malonylgenistin	61.1
Malonylglycitin	21.43
Total aglycones	19.15
Total isoflavones	219.5

Means (two duplicates \pm SE) with different superscript letters in the same row are significantly different ($p < 0.05$). Acetylglycosides (acetyldaidzin, acetyl genistin and acetyl glycitin) were not found.

Table 2: Serum glucose level (mg/dl) and insulin level (mmol/U) in rat group, fed on the tested diets.

Groups*	Glucose	Insulin
Control(-)	99.56 ^c \pm 0.82	51.97 ^a \pm 1.61
Control(+)	282.17 ^a \pm 2.06	22.19 ^b \pm 2.38
Isoflavone	107.08 ^b \pm 1.61	50.87 ^a \pm 0.49
Diamicron	108.82 ^b \pm 3.16	45.96 ^a \pm 1.66
LSD _{0.05}	9.44	3.14

Different superscripts (a, b, c....) at the same column are significantly different.

Each value represents the mean \pm S.E.

Table 3: Effect of feeding with different experimental diets on serum TL, TG, TC, HDL-C, LDL-C and VLDL-C (mg/dl) of rats.

Groups*	TL	TG	TC	HDL-C	LDL-C	VLDL-C
Control (-)	329.78c ±0.62	109.61d ±0.82	82.04c ±1.85	55.33a ±0.38	4.47d ±1.55	21.92c ±0.16
Control (+)	405a ±1.28	175.88a ±0.84	225.69a ±1.19	42.92b ±0.73	134.91a ±1.99	35.18a ±0.17
Isoflavone	337.8b ±1.5	119.91c ±0.68	94.98b ±0.33	55.08a ±0.73	15.97b ±0.86	23.98b ±0.13
Diamicon	337.34b ±0.4	123.56b ±1.23	91.86b ±1.00	54.78a ±0.85	12.33c ±1.64	24.71b ±0.25
LSD0.05	2.14	1.85	2.03	1.20	2.48	0.37

Different superscripts (a, b, c....) at the same column are significantly different each value represents the mean ±S.E

The mean value with different superscript alphabets in a column indicate significant differences ($P < 0.05$) using LSD test.

(P, control) diabetic rats compared to (N, control) normal rats. Supplemented administration of isoflavone and diamicon 30MR to diabetic rats significant decreased the level of blood glucose and significant increased the level of insulin compared to (p. control) control diabetic group [34, 35]. The elevation in plasma insulin in the genistein and ISP-treated STZ-diabetic rats could be due to the insulinotropic substances present in the fractions, which induce the intact functional β -cells of the Langerhans islet to produce insulin, or the protection of the functional β -cells from further deterioration so that they remain active and produce insulin, glucokinase activity was decreased in the liver of diabetic rats which may be due to a deficiency of insulin. Genistein and ISP fed diabetic rat showed an elevated activity of glucokinase, which may be associated with reduced blood glucose. Therefore, the ISP appears to be more potent than the genistein [11].

STZ induced diabetes mellitus increases oxidative stress and the presence of oxidized LDL- Cholesterol and other lipoproteins. Oxidation converts LDL-cholesterol to a form that is rapidly taken up and degraded by macrophages and increased degradation of unoxidized LDL-cholesterol. Oxidized lipoproteins play an important role in the development of atherosclerosis [36]. Antioxidants inhibit metabolism of LDL-cholesterol and reduce toxicity of oxidized LDL-cholesterol [37]. The present study observed that there were higher levels of cholesterol of and triglycerides in STZ-diabetic rats. The level of lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. The abnormal high concentration lipids in diabetes are mainly due to increase in adipose tissue lipolysis in absence of insulin and mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. It was also found that there were higher triglycerides amounts in liver and brain.

Similar results were reported by other workers in diabetic rats [38]. The oxidation stress significantly increased serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and very low-density lipoprotein-cholesterol (VLDL-C). While, HDL-cholesterol was significantly decreased as shown in Table 3. Administration of isoflavone and diamicon 30MR improved the tested antioxidants values or returned them to the normal ones. The possible mechanism of the hypolipidemic effects by soy protein intake including the improvement of insulin/glucagon ratio, which involved in lower fatty acid biosynthesis in liver through reducing the gene expression of sterol regulatory element binding protein (SREBP)-1. Moreover, soy protein isoflavones can increase serum cholesterol clearance through stimulating the transcription factor SREBP-2 [39].

GSH plays a central role in antioxidant defense by detoxifying reactive oxygen species, directly or in a glutathione peroxidase catalyzed mechanism. And in the repair of radically caused biological damage [40] and its level reduced in diabetes mellitus [41]. The decrease in GSH levels represents increased utilization due to oxidative stress [42]. The elevated level of GSH protects cellular proteins against oxidation through the glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to STZ [43]. The GSH content increment in the serum of rats treated with, isoflavone and diamicon30MR extractions. isoflavone and diamicon30MR can be directly scavenging the free radicals in diabetic rats, may reduce the utilization of GSH and thereby exhibiting an increase in the GSH content in treated diabetic rats. The GSH content increment in the serum of rats treated with, isoflavone and diamicon30MR extractions may be a responsible factor for inhibition of lipid peroxidation. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species. Table 4 shows the activity levels of enzymatic antioxidants, CAT, SOD and GSH-Px and non-enzymatic antioxidant, GSH, respectively, in normal and experimental rat groups. The activities of enzymatic antioxidants

Table 4: Effect of feeding with different experimental diets on blood S O D, CAT, GSH, GSH-px, TAC (mg/dl) and MDA (mmol/U) of rats.

Groups*	S O D	CAT	G S H	G S H-PX	T A C	M D A
Control(-)	11.25 ^a ±0.42	8.75 ^a ±0.35	8.33 ^a ±0.23	4.43 ^a ±0.44	2.36 ^a ±0.35	3.4 ^b ±0.11
Control(+)	3.56 ^c ±0.13	2.49 ^b ±0.585	3.24 ^c ±0.46	1.63 ^b ±0.15	1.18 ^b ±0.21	8.25 ^a ±0.42
Isoflavone	8.95 ^b ±0.21	8.09 ^a ±0.83	7.11 ^b ±0.15	4.07 ^a ±0.32	3.55 ^a ±0.41	3.96 ^b ±0.42
Diamicon	8.99 ^b ±0.47	8.05 ^a ±0.1	7.09 ^b ±0.26	4.12 ^a ±0.39	2.83 ^a ±0.21	4.05 ^b ±0.18
LSD _{0.05}	0.86	0.91	0.69	0.78	0.91	0.58

Each value represents the mean ±S.E.

The mean value with different superscript alphabets in a column indicate significant differences (P<0.05) using LSD test.

(CAT, SOD, TAC and GSH-Px) and non-enzymatic antioxidant (GSH reduced) were significantly decreased in diabetic rats group (control), when compared with the normal group. Oral intake of the experimental diabetic rat groups with isoflavone and diamicon30MR extracts increased the activates of enzymatic antioxidants, CAT, SOD and GSH-Px and non-enzymatic antioxidant GSH reduced. While, positive control group (diabetic control) was increased to Malondialdehyde (MDA). As shown in Table 4 the TAC and MDA level significantly decrease in Treatment with isoflavone and diamicon 30MR. Chronic treatment of daidzein significantly decreased MDA content and enhanced SOD activity in aortic tissue from diabetic rats, indicating that the improvement in vascular responsiveness from daidzein may be partly due to ameliorating lipid peroxidation and oxidative injury. These results clearly suggested that another cause of the effect of daidzein on improving the endothelial dysfunction is due to its antioxidative [44].

It could be concluded that supplementing the diet with soybean showed a beneficial effect on the improvement of blood glucose control, lipid metabolism and antioxidant enzyme activities in type 2 DM rats.

REFERENCES

1. Wild, S., G. Roglic and A. Green, 2004. Global prevalence of diabetes. *Diabetes Care*, 27: 1047-1053.
2. Coulston, A.M. and C.B. Hollenbeck, 1988. Source and amount of dietary carbohydrate in patients with noninsulin-dependent diabetic mellitus. *Top. Clin. Nutr.*, 3: 17-24.
3. Kjeld, H., C. Marius, S. Mette, B. Birgitte and H. Lars, 2001. Beneficial effects of a soy based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabete. Care*, 24: 228-233.
4. Vijayakumar, M., R. Govindarajan, G.M.M. RAO, Ch. V. RAO, A.R. Shirwaika, S. Mehrotra and P. Pushpangadan, 2006. Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress. *Journal of Ethnopharmacology*, 104:356-361.
5. Venkateswaran, S. and L. Pari, 2002. Antioxidant effect of Phaseolus vulgaris on streptozotocin-induced diabetic rats. *Asia Pacific J. Clin. Nutr.*, 11: 206-209.
6. Jenkins, D.J.A., T.M.S. Wolever and R.H. Taylor, 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am. J. Clin. Nutr.*, 34: 362-366.
7. Slameron, J., J.E. Manson, M.J. Stampfer, G. Colditz, A.L. Wing and W.C. Willett, 1997. Dietary fiber glycemic load and risk of non-insulin dependent diabetes mellitus in women. *JAMA*, 277: 472-477.
8. Simpson, H.C.R., R.W. Simpson and S. Lousley, 1981. A high carbohydrate leguminous fiber diet improves all aspects of diabetic control. *Lancet*, 1: 1-5.
9. Hermansen, K., M. Sondergaard, L. Hoie, M. Carstensen and B. Brock, 2001. Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care*, 24: 228-233.
10. Kang, M.J., J.I. Kim, S.Y. Yoon, J.C. Kim and I.J. Cha, 2006. Pinitol from soybeans reduces postprandial blood glucose in patients with type 2 diabetes mellitus. *J. Med. Food*, 9: 182-186.
11. Lee, J.S., 2006. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities and lipid profile in streptozotocin induced diabetic rats. *Life Sci.*, 79: 1578-1584.
12. Tonstard, S., K. Smerud and L. Hoie, 2002. A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins and plasma total homocysteine in hypercholesterolemic subjects. *Am. J. Clin. Nutr.*, 76: 78-84.
13. Beavers, M. K., S.S. Jonnalagadda and M.J. Messina, 2009. Soy consumption, adhesion molecules and pro-inflammatory cytokines: a brief review of the literature. *Nutr. Rev.*, 67(4): 213-21.
14. Latorraca, M.Q., L.F. Stoppiglia, M.H.G. Gomes, M.S.F. Martins, M.A. Reis, R.V. Veloso and V.C. Arantes, 2011. Flour and bread and their fortification in health and disease prevention. pp: 495-506.

15. Omoni, A.O. and R.E. Aluko, 2005. Soybean foods and their benefits: potential mechanisms of action. *Nutr. Rev.*, 63: 272-283
16. Bhatena, J.S. and T.M. Velasquez, 2002. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am. J. Clin. Nutr.*, 76: 1191-120
17. Xiao, C.W., J. Mei and C.M. Wood, 2008. Effect of soy proteins and isoflavones on lipid metabolism and involved gene expression. *Front Biosci.*, 13: 2660-73.
18. Liu, S., L.M. Lee and Y. Song, 2006. Vitamin E and risk of type 2 diabetes in the Women's Health Study Randomized Controlled Trial. *Diabetes*, 55: 2856-2862.
19. Lori, C., N.C. Barnes, D.R. Kenneth and S. Barnes, 1993. Genistein, daidzein and their B-glucoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *J. Agric. Food Chem.*, 41: 1961-1967.
20. Kumar, S.G., A.K. Shetty, K. Sambaiah and P.V. Salimath, 2005. Anti-diabetic property of fenugreek seeds mucilage and spent turmeric in streptozotocin-induced diabetic rats. *Nutrition Research*, 25(11): 1021-1028.
21. Lutz, L.A. and W.M. Pardridge, 1993. Insulin therapy normalizes glucose transporter mRNA but no immunoreactive transporter protein in streptozotocin diabetic rats. *Metabolism*, 42: 939-944.
22. Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Am. Clin. Biochem.*, 6: 24-27.
23. Temple, R.C., P.M. Clark and C.N. Hales, 1992. Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. *Diabetic Med.*, 9: 503-512.
24. Amundson, D.M. and M. Zhou, 1999. Fluorometric method for the enzymatic determination of cholesterol. *J. Biochem. Biophys. Meth.*, 38: 43-52
25. Matsuzaki Y, E. Kawaguchi and Y. Norita 1990. Evaluation of Two Kinds of Reagents for Direct Determination of HDL-Cholesterol. *J. Anal. Bio. Sci.*, 19: 419-427
26. Warnick, G.R., R.H. Knopp, V. Fitzpatrick and L. Branson, 1990. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clinical Chemistry*, 36(1): 15-9.
27. Wallach, J., 1992. Interpretation of Diagnostic Tests. A Synopsis of Laboratory Medicine. 5th Ed. A Little Brown Hand- Book, Boston.
28. Cole, T.G., S.G. Klotzsch and J.R. McNamara, 1997. Measurement of Triglyceride Concentration, pp: 115-123
29. Meltzer, H.M., M. Folmer, S. Wang, Q. Lie, A. Maage and H.H. Mundal, 1997. Supplementary selenium influences the response to fatty acid induced oxidative stress in humans. *Biological Trace Element Research*, 60: 51-67.
30. Aebi, H., 1984. Catalase *in vitro*. *Method Enzyme*, 105: 121-126.
31. Koracevic, D., G. Koracevic and V. Djordjevic, *et al.* 2001. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.*, 54: 356-61.
32. Ellman, G.L., 1959. Tissue sulphydril groups. *Archives of Biochem. and Biophys.*, 82: 70-77.
33. Rotruck, J.J., A.L. Pope, H.E. Ganther and A.B. Swanson, 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 179: 588-590.
34. Velasquez, M.T and S.J. Bhatena, 2007. Role of dietary soy protein in obesity. *Int. J. Med. Sci.*, 4: 72-82.
35. Park, S., D.S. Kim, J.H. Kim, J.S. Kim and H.J. Kim, 2012. Glyceollin-containing fermented soybeans improve glucose homeostasis in diabetic mice. *Nutrition*, 28(2): 204-211
36. Jafarnejad, A., S.Z. Bathale, M. Nakhjavani and M.Z. Hassan, 2008. Effect of spermine on lipid profile and HDL functionality in the streptozotocin-induced diabetic rat model. *Life Sciences*, 82: 301-307.
37. Ravi, K., B. Ramchandran and S. Subramanian, (2004a). Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin induced diabetic rats. *Biological and Pharmaceutical Bulletin*, 27: 1212-1217.
38. Tanya, G., K. Rachel, V.M. Fatima and Y. Hong, 2011. Soy protein supports cardiovascular health by down regulating hydroxymethylglutaryl-coenzyme A reductase and sterol regulatory element-binding protein-2 and increasing antioxidant enzyme activity in rats with dextran sodium sulfate-induced mild systemic inflammation *Nutrition Research*, 31(12): 922-928
39. Torres, N., I. Torre-Villalvazo and A.R. Tovar, 2006. Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. *J. Nutr. Biochem.*, 17: 365e73.
40. Ananthan, R., M. Latha, K.M. Ramkumar, L. Pari, C. Baskar and V. Narmatha Bal, 2004. Modulatory effects of *Gymnema montanum* leaf extract on alloxan-induced oxidative stress in wistar rats. *Nutrition*, 20: 280-285.

41. Latha, M. and L. Pari, 2003. Modulatory effect of *Scoparia dulcis* oxidative stress-induced lipid peroxidation in streptozotocin diabetic rats. *Journal of Medicinal Food*, 4: 379-386.
42. Dixit, A.K., D. Bhatnaga, V. Kumar, D. Chawla, K. Fakhruddin and D. Bhatnagar, 2012. Antioxidant potential and radioprotective effect of soy isoflavone against gamma irradiation induced oxidative stress. *J.Functional Foods*, 4(1): 197-206.
43. Latha, M. and L. Pari, 2003. Modulatory effect of *Scoparia dulcis* oxidative stress-induced lipid peroxidation in streptozotocin diabetic rats. *Journal of Medicinal Food*, 4: 379-386.
44. Roghani, M., M.R.V. Mahdavi, M.R. J. Nadoushan, T. Baluchnejadmojarad, G. Naderi, F.R. Dehkordi, M.T. Joghataei and M. Kord, 2013. Chronic Administration of Daidzein, a Soybean Isoflavone, Improves Endothelial Dysfunction and Attenuates Oxidative Stress in Streptozotocin induced Diabetic Rats. *Phytother. Res.*, 27: 112-117.