

Beneficial Effects of Substitution of Vegetable Soy Protein instead of Animal Protein Casein in Diets for Diabetic Male Rats

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Abstract: The effect of substitution of vegetable Soy protein (SP) for dietary casein protein (CP) on body weight, serum liver enzymes, blood lipids, glucose, insulin, kidney function and activities of antioxidant enzymes in renal tissue of alloxan-diabetic rats were evaluated. Fifty four male Sprague Dawley rats were randomized into 6 equal groups. Group (1) was fed on basal diet (Negative control) containing 20% CP, while the other 5 groups were rendered diabetic by intraperitoneal injection of alloxan (120 mg/kg/day) for 5 days. The diabetic rats were assigned to one of five diets as follows: group (2): 20% CP (Positive control); group (3): 5 % SP + 15 % CP; group (4): 10 % SP + 10 % CP; group (5) 15% SP + 5% CP and group (6): 20 % SP for 6 weeks. The rats were weighed at the beginning and at the end of the feeding period and weight gain was also calculated. Blood samples were collected for separating the serum for biochemical analyses. Kidneys were dissected out and renal homogenates were prepared to assay activities of tissue antioxidant enzymes. The results showed that feeding diabetic rats on diets containing 20 % SP or 15% SP + 5% CP or 10% SP+10% CP significantly increased body weight gain and decreased the elevated serum levels of liver enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, total cholesterol, triglycerides, low density lipoprotein and blood glucose level while there were an increased in insulin level in diabetic rats fed on the previous diets. Blood urea and creatinine levels were normalized and activities of liver superoxide dismutase, glutathione peroxidase, and catalase antioxidant enzymes were increased. In conclusion, substitution of Soy protein for dietary casein or more Soy protein and less casein increases body weight gain and produces antidiabetic, hypolipidemic, hepatoprotective, nephroprotective, and antioxidant effects in diabetic rats. Therefore, consumption of Soy protein instead of casein may be beneficial for diabetic patients as it prevents body weight loss, normalizes blood glucose and lipids, and reduces the adverse effects of diabetes on the liver and kidneys in diabetic rats. This study recommends that diets containing vegetable Soy protein instead of animal protein casein or more Soy protein and less casein may be beneficial for diabetic patient.

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1. Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia due to insulin deficiency, or insulin resistance, or both. Hyperglycemia occurs when the cells become unable to utilize glucose and/or the liver and skeletal muscles cannot store glycogen (Luis-Rodríguez *et al.*, 2012). In renal damage or failure, the kidney threshold for glucose decreases and so glycosuria occur. Diabetes mellitus deteriorates the kidney function and causes diabetic nephropathy that characterized by the presence of proteinuria and hyperlipidemia (Siddiqui *et al.*, 2013). Diabetic nephropathy is one of the most frequent and serious complications of diabetes mellitus. The increased extracellular and intracellular glucose concentrations result in oxidative stress due to the increased production of reactive oxygen species (ROS) and the sharp decrease in antioxidant body defenses (Lucchesi *et al.*, 2013). Oxidative stress plays a key role in the onset and development of diabetes

complications, notably diabetic nephropathy (Wang *et al.*, 2011). Because of the synthetic chemical drugs prescribed for treating diabetes have many adverse side effects; therefore there is a great need to search for alternative safe natural agents to be used for diabetics.

Soybean (USA) or soya bean (UK) (Glycine max) is a species of legumes native in East Asia and grown for its edible beans which have nutritional and medicinal uses. Soybeans have a good quality and cheap protein (Soy protein) which has been consumed for many years in most Oriental countries. Dry Soybeans contain by weight 40% protein. Soy protein has been used for its functional properties as an ingredient in a variety of foods such as salad dressings, soups and vegetarian foods. Soy protein intake regulates expression of the hepatic transcription factor (sterol regulatory element binding protein, SREBP-1). The reduction of this factor reduces the expression of several lipogenic enzymes and decreases hepatic triglycerides, LDL and VLDL

cholesterol in diabetics (Torres *et al.*, 2006). The reduction of serum cholesterol and triglycerides by Soy protein intake produces beneficial effects on the kidney by preventing the inflammatory response and by increasing the renal flow via releasing endothelial nitric oxide synthase, so facilitating the synthesis of nitric oxide (Lopez *et al.*, 2007). Consumption of high-isoflavones Soy protein not only lowers glucose level, but also reduces the incidence of cataracts in diabetic rats. The beneficial effects of Soy isoflavones were attributed to increased insulin secretion, better glycemic control, and antioxidant protection (Lee, 2006 and Lu *et al.*, 2008). Previous studies showed that a soybean diet could prevent the progression of diabetes mellitus, and increased urinary albumin excretion and serum total cholesterol diabetic rats (Lee, 2006 and Choi *et al.*, 2010) and in diabetic patients with nephropathy (Azadbakht and Esmailzadeh, 2009; Yang, *et al.*, 2011 and Urita *et al.*, 2012).

The present study aimed to evaluate the effects of substitution of vegetable Soy protein by an animal protein casein on body weight, serum levels of liver enzymes, blood lipids and glucose, and insulin hormone as well as on the kidney function and the activity of renal tissue antioxidant enzymes in alloxan-diabetic rats.

2. Material and Methods

Material

Soy protein and casein

Soy protein concentrate was purchased from Soy Products Factory, Jeddah, Saudi Arabia, in the form of a yellowish powder. Casein was manufactured by Loba Chemie Company., India, (Batch No.V33505) as a yellowish white fine powder and imported by Sigma Company., Saudi Arabia.

Alloxan and biochemical kits

Alloxan was obtained from Sigma. Company., Saudi Arabia in the form of white powder packed in brown bottles each containing 25 gm alloxan monohydrate. Glucose enzymatic kits, the other biochemical kits and insulin hormone were purchased from Sigma Company., Saudi Arabia.

Rats

Fifty four adult male Sprague-Dawley rats weighing 200-210 g body weight and 8-9 weeks old were used in this study. Animals were obtained from the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were housed in a well ventilated animal room under standard conditions of 24 °C temperature, 50-52% relative humidity and 12 hr light/12 hr dark cycles. Basal and experimental diets and water were provided *ad libitum*. Rats were acclimatized to the laboratory environment for 7 days before the start of the experiment.

Preparation of basal diet

Basal diet was consisted of 20 % protein (casein), 10 % sucrose, 5 % corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fiber (cellulose). The remainder was corn starch up to 100 %, according to Reeves *et al.*, (1993).

Design of the experiment

The experiment was performed on fifty four mature Sprague Dawley rats randomly distributed into 6 groups, of 9 rats each. Group (1) was fed on basal diet and kept as a negative normal control, while the other 5 groups were rendered diabetic by intraperitoneal injection of alloxan (120 mg/kg/day) for 5 days according to Ashok *et al.*, (2007). Rats with fasting plasma glucose \geq 140 mg/dl were considered to be diabetic (Zhang *et al.*, 2008). The diabetic rats were fed on the following experimental diets:

Group (2): fed basal diet containing 20 % casein (CP) (Positive diabetic control)

Group (3): fed basal diet containing 5 % Soy protein (SP) + 15 % CP

Group (4): fed basal diet containing 10 % SP + 10 % CP

Group (5): fed basal diet containing 15 % SP + 5% CP

Group (6): fed basal diet containing 20 % SP

The initial and final body weights of rats were recorded and body gains were calculated. Rats were then euthanized by prolonged exposure to ether anesthetic and blood samples were withdrawn by cardiac puncture. Blood was left to clot and centrifuged at 4000 rpm for 15 minutes for separating the serum which kept frozen until biochemical analyses. Kidneys were dissected out and renal tissue homogenates were prepared and used to assay the activity of antioxidant enzymes.

Biochemical analyses

Serum aspartate aminotransferase and alanine aminotransferase (Bergmeyer *et al.*, 1978) and alkaline phosphatase (Roy, (1970) liver enzymes; total cholesterol (Allain *et al.*, 1974); triglycerides (Buccolo and David, 1973) and high density lipoprotein cholesterol (Lopes-virella *et al.*, 1977) were chemically determined using specific kits and measured using spectrophotometer (Model T80, UV/visible, double beam, UK). Low density lipoprotein cholesterol was calculated using the formula: LDL-c = TC – (TG/5) – HDL-c according to Friedewald *et al.*, (1972). Atherogenic index (AI) (LDL-c/ HDL-c) was then calculated (Nwagha *et al.*, 2010). Blood glucose was determined using glucose enzymatic kits (BioMerieux, France) according to Siest *et al.* (1981). Insulin was estimated using radioimmunoassay (RIA) method according to Yallow and Bauman (1983). The kidney function was

assessed by chemical estimation of blood urea nitrogen (Patton and Crouch, 1977); uric acid (Fossati *et al.*, 1980) and creatinine (Husdan and Rapoport, 1968) concentrations in the serum.

Assessment of antioxidant enzymes

One gram of kidney tissue was washed with ice-cooled 0.9% NaCl solution and homogenized in 100 ml of ice-cooled 1.5% solution of potassium chloride and 50 mMol potassium phosphate buffer solutions (pH 7.4) to yield 1% homogenate (W/V). Homogenization was performed using Sonicator, 4710 Ultrasonic Homogenizer. Kidney homogenates were centrifuged at 4000 rpm for 10 minutes at 4°C and the supernatants were used to assay the activity of antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) according to Paglia and Valentaine (1979), Spitz and Oberley (1989), and Sinha (1972) respectively.

Statistical analysis

Data were presented as mean \pm SE. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (Snedecor and Cochran, 1986) using SPSS computer program (version 15). Differences between experimental groups were considered significant at $P < 0.05$ level.

3. Results

Rats that have been administered alloxan (120 mg/kg, i.p.) for 5 days became diabetic at a frequency of 77%. Diabetes was associated with loss of body weight and reduced weight gain when compared to the normal (Negative) control group fed on basal diet.

Feeding diabetic rats on diets containing 20% Soy protein (SP) or 15% SP + 5 % casein (CP) or 10% SP + 10% CP for 6 weeks significantly ($P < 0.05$) increased the body weight gain as compared to the positive control group (Table 1).

Diabetes caused significant increases in serum levels of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in rats. Diets containing 20% SP or 15% SP + 5% CP or 10% SP +10% CP decreased the elevated serum levels of AST, ALT and ALP in diabetic rats when compared with the positive control group as depicted in Table (2).

Data in Table (3) showed that diabetic rats had significant high serum levels of total cholesterol (TC) and triglycerides (TG) when compared to the negative control group. Feeding the diabetic rats on diets containing 20% SP or 15 % SP + 5% CP or 10 % SP +10% or 5 % SP +15% CP for 6 weeks significantly ($P < 0.05$) decreased the elevated levels of serum TC and TG when compared to the positive control group.

The results revealed that diabetes induced significant decrease in high density lipoprotein (HDL-c) cholesterol and increases in low (LDL-c) and very low density lipoprotein (VLDL-c) and atherogenic index (AI) in rats fed on basal diet. Feeding diabetic rats on diets containing 20% Soy protein (SP) or 15% SP +5% casein (CP) or 10% SP +10% CP or 5 % SP +15% CP for 6 weeks significantly ($P < 0.05$) increased serum HDL-c and decreased LDL-c, VLDL-c and AI when compared with the positive control group as depicted in Table (4).

Table 1. Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on body weight (B.wt) and body weight gain (BWG) in diabetic rats.

Parameters Groups	Initial B.wt (gm) at Week 0	Final B.wt (gm) at Week 6	BWG (%)
Group (1) -Ve control	210.0 \pm 4.0 ^a	280.00 \pm 4.12 ^a	33.33 \pm 1.30 ^a
Group (2) + Ve control 20% CP	200.0 \pm 3.5 ^a	225.50 \pm 5.52 ^c	12.75 \pm 1.50 ^c
Group (3) 5% SP+ 15% CP	210.0 \pm 5.5 ^a	235.50 \pm 7.45 ^c	12.14 \pm 1.64 ^c
Group (4) 10% SP+ 10% CP	205.0 \pm 2.6 ^a	255.50 \pm 6.37 ^b	24.63 \pm 1.82 ^b
Group (5) 15% SP+ 5% CP	205.0 \pm 4.5 ^a	262.50 \pm 8.30 ^b	27.07 \pm 1.15 ^b
Group (6) 20% SP	205.5 \pm 5.5 ^a	269.00 \pm 7.15 ^b	31.14 \pm 1.72 ^a

Means \pm SE with different letters superscripts (a, b, c) in the same column are significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group.

Table (2). Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on serum levels of aspartate aminotransferase (AST), alanine amino-transferase (ATL) and alkaline phosphatase (ALP) in diabetic rats.

Parameters Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Group (1) - Ve control	45.0 ± 3.11 ^d	35.0 ± 2.12 ^d	87.9 ± 3.15 ^d
Group (2) +Ve control 20% CP	85.0 ± 5.12 ^a	65.0 ± 3.41 ^a	105.0 ± 8.17 ^a
Group (3) 5% SP+ 15% CP	75.0 ± 4.68 ^a	59.0 ± 4.33 ^a	100.0 ± 4.11 ^a
Group (4) 10% SP+ 10% CP	63.0 ± 4.28 ^b	44.0 ± 3.11 ^b	95.0 ± 4.24 ^b
Group (5) 15% SP+ 5% CP	55.0 ± 4.88 ^c	40.0 ± 3.12 ^b	90.0 ± 6.20 ^b
Group (6) 20% SP	51.0 ± 3.77 ^c	37.0 ± 2.44 ^c	86.0 ± 5.14 ^c

Means ± SE with different letters superscripts (a, b, c, d) in the same column are Significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group

Table (3): Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on serum total cholesterol (TC) and triglycerides (TG) in diabetic rats.

Parameters Groups	TC (mg/dL)	TG (mg/dL)
Group (1) - Ve control	99.50 ± 3.1 ^d	75.00 ± 3.4 ^d
Group (2) +Ve control 20% CP	135.70 ± 6.1 ^a	105.00 ± 5.2 ^a
Group (3) 5% SP+ 15% CP	122.80 ± 4.8 ^b	100.00 ± 6.1 ^b
Group (4) 10% SP+ 10% CP	120.60 ± 3.7 ^b	95.50 ± 3.5 ^b
Group (5) 15% SP+ 5% CP	118.50 ± 3.6 ^c	90.50 ± 4.3 ^c
Group (6) 20% SP	116.70 ± 3.6 ^c	81.50 ± 3.6 ^c

Means ± SE with different letter superscripts (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group

Table 4. Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on serum levels of high (HDL-c), low (LDL-c) and very low density lipoprotein (VLDL-c) cholesterol and atherogenic index (AI) in diabetic rats.

Parameters Groups	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)	AI LDL-c / HDL-c
Group (1) - Ve control	67.00 ± 3.5 ^d	17.50 ± 0.2 ^d	15.00 ± 0.3 ^d	0.261
Group (2) +Ve control 20% CP	82.40 ± 5.1 ^a	32.30 ± 0.5 ^a	21.00 ± 0.6 ^a	0.391
Group (3) 5% SP + 15% CP	74.30 ± 3.8 ^b	28.50 ± 0.1 ^b	19.00 ± 0.4 ^b	0.383
Group (4) 10% SP + 10% CP	75.50 ± 4.7 ^b	26.00 ± 0.4 ^b	19.10 ± 0.3 ^b	0.344
Group (5) 15% SP + 5% CP	76.00 ± 3.6 ^b	24.40 ± 0.3 ^b	18.10 ± 0.2 ^b	0.321
Group (6) 20% SP	78.00 ± 2.5 ^c	22.40 ± 0.1 ^c	16.30 ± 0.3 ^d	0.287

Means ± SE with different letters superscripts (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group.

Diabetes induced by alloxan significantly ($P < 0.05$) increased blood glucose and decreased insulin level in rats fed on basal diet containing 20% casein

(CP) when compared to the negative control group. Feeding diabetic rats on diets containing 20% Soy protein (SP) or 15% SP + 5% CP or 10% SP + 10%

CP or 5 % SP +15% CP for 6 weeks significantly ($P < 0.05$) lowered blood glucose and elevated insulin serum level when compared with the positive control group as depicted in Table (5). Percentages of the decrease in blood glucose level were 2.28, 11.25, 21.80 and 42.70% in rats fed on diets containing 20% SP, 15% SP +5% CP, 10% SP +10% and 20% SP, respectively.

Results in Table (6) showed that diabetes significantly ($P < 0.05$) increased blood urea nitrogen

(BUN) and creatinine (Cr) serum concentrations in rats fed on basal diet containing 20% casein (CP) when compared to the negative control group. Feeding diabetic rats on diets containing 20% Soy protein (SP) or 15% SP + 5% CP or 10% SP +10% CP or 5 % SP +15% CP for 6 weeks significantly ($P < 0.05$) decreased the elevated BUN and Cr levels when compared with the positive control group as recorded in Table (6). No significant changes in serum uric acid levels were observed.

Table 5. Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on blood glucose (BG) and insulin levels in diabetic rats.

Parameters Groups	BG (mg/dL)	Insulin (ng/ml)
Group (1) - Ve control	116.50 ± 2.9 ^d	2.95 ± 0.15 ^a
Group (2) +Ve control 20% CP	210.50 ± 4.8 ^a	0.89 ± 0.13 ^d
Group (3) 5% SP + 15% CP	190.70 ± 3.4 ^b	1.92 ± 0.24 ^c
Group (4) 10% SP + 10% CP	176.80 ± 2.3 ^c	2.63 ± 0.12 ^b
Group (5) 15% SP + 5% CP	164.60 ± 2.6 ^c	2.72 ± 0.16 ^b
Group (6) 20% SP	120.60 ± 3.2 ^d	2.87 ± 0.14 ^a

Means ± SE with different letters superscripts (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group.

Table 6. Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr) levels in diabetic rats.

Parameters Groups	BUN (mg/dL)	UA (mg/dL)	Cr (mg/dL)
Group (1) - Ve control	36.3 ± 1.4 ^d	1.50 ± 0.01 ^a	0.75 ± 0.01 ^d
Group (2) +Ve control 20% CP	62.0 ± 2.4 ^a	1.49 ± 0.06 ^a	1.84 ± 0.04 ^a
Group (3) 5% SP + 15% CP	54.1 ± 2.6 ^b	1.48 ± 0.02 ^a	1.63 ± 0.02 ^b
Group (4) 10% SP + 10% CP	52.8 ± 2.3 ^b	1.49 ± 0.04 ^a	1.62 ± 0.02 ^b
Group (5) 15% SP + 5% CP	46.5 ± 2.1 ^c	1.50 ± 0.01 ^a	1.40 ± 0.03 ^c
Group (6) 20% SP	39.6 ± 1.8 ^d	1.48 ± 0.01 ^a	0.70 ± 0.01 ^d

Means ± SE with different letters superscripts (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group.

Rats inflicted with experimental diabetes had a significant ($P < 0.05$) decreases in renal tissue levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes when compared with the control negative group. Feeding diabetic rats on diets containing 20%

Soy protein (SP) or 15% SP + 5% casein (CP) or 10% SP +10% CP or 5 % SP +15% CP for 6 weeks normalized the elevated renal tissue levels of SOD, GPx and CAT enzymes when compared to the positive control group as shown in Table (7).

Table 7. Effect of diets containing Soy protein (SP) or casein (CP) or both (SP+CP) on activities of tissue superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes in diabetic rats.

Parameters Groups	SOD (U/mg protein)	GPx (nmol/min/mg protein)	CAT (nmol/min/mg protein)
Group (1) - Ve control	59.70 ± 2.24 ^a	0.69 ± 0.01 ^a	0.195 ± 0.01 ^a
Group (2) +Ve control 20% CP	39.50 ± 2.88 ^d	0.28 ± 0.04 ^d	0.148 ± 0.02 ^d
Group (3) 5% SP + 15% CP	45.74 ± 3.46 ^c	0.35 ± 0.03 ^b	0.155 ± 0.01 ^b
Group (4) 10% SP + 10% CP	48.95 ± 2.58 ^c	0.45 ± 0.01 ^b	0.168 ± 0.01 ^b
Group (5) 15% SP + 5% CP	55.25 ± 2.73 ^b	0.55 ± 0.01 ^c	0.185 ± 0.02 ^c
Group (6) 20% SP	56.25 ± 2.73 ^b	0.62 ± 0.01 ^c	0.190 ± 0.02 ^c

Means ± SE with different letters superscripts (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test. n= 9 rats/group. Unit of GPx = nmol of GSH utilized/min/mg protein. Unit of CAT = nmol of H₂O₂ utilized/min/mg protein.

4. Discussion

The aim of this study was to evaluate the effect of substitution of vegetable Soy protein (SP) instead of animal protein casein (CP) on body weight, serum levels of liver enzymes, blood lipids, glucose, and insulin as well as on the kidney function and activity of renal tissue antioxidant enzymes in alloxan-diabetic rats.

The medicinal plants, vegetables and legumes which possess antidiabetic and antihyperlipidemic activities for treating diabetes and obesity have gained much attention, especially those with little toxicity properties. The biological value of the plant materials depends on their bioactive constituents such as saponins, anthocyanins, flavonoids, polyphenols, diterpenes, triterpenes and other phytochemicals (Veermuthu *et al.*, 2006 and Patel *et al.*, 2012).

Soybean (Family, Fabaceae or *Leguminosae*) has a good quality and cheap protein named Soy protein. Consumption of high-isoflavones soy protein lowers blood glucose level delays progression and complications of diabetes mellitus (Lu *et al.*, 2008; Choi *et al.*, 2010 and Urita *et al.*, 2012).

Results of the present study showed that experimental diabetes induced by alloxan was characterized by decreased weight gain; elevated serum liver enzymes; hyperlipidemia and hyperglycemia; decreased insulin levels; high blood urea and creatinine serum levels and decreased activities of renal tissue antioxidant enzymes in rats. These findings were similar to those previously reported by Adewoye *et al.*, (2009); Al-Hariri *et al.*, (2011); Kaushik, *et al.*, (2013) and Samarghadian *et al.*, (2013). The previous authors concluded that diabetes induced by alloxan or streptozotocin (STZ) in rats causes body weight loss, elevates serum liver

enzymes (AST, ALT and ALP) and produces hyperlipidemia, hyperglycemia, and hyperinsulinemia. Diabetes in rats also deteriorates kidney function as caused diabetic nephropathy due to oxidative stress caused by alloxan (Kocic *et al.*, 2007 and Siddiqui *et al.*, 2013).

The present results revealed that diabetic rats fed on experimental diets containing 20% Soy protein instead of casein or more Soy protein and less casein gained more body weight that those fed on basal diet (20% casein). This result was partially agreed with that reported by Kim *et al.*, (2012) who found that Soy protein isolate caused improvement in body weight gain and reduction in adipose tissue weight by affecting the activities of hepatic lipogenic enzymes in rats fed on high-fat diet.

Feeding diabetic rats on diets containing 20% Soy protein instead of casein or more Soy protein and less casein produced hepatoprotective effect. This effect was evident from significant decreases in the elevated serum levels of liver enzymes (AST, ALT and GGT) in diabetic rats. The decrease in serum liver enzyme levels was found to be dependent upon the percent of Soy protein added to diets. These findings agree with those reported by Tovar *et al.* (2005) who concluded that feeding Soy protein compared to casein reduces effects of diabetes on the liver of diabetic rats as it reduces high liver enzymes (AST and ALT) and decreases hepatic lipotoxicity in hyperinsulinemic obese rats. The hepatoprotective effect of Soy protein was in accordance with that reported by that demonstrated by Torres *et al.*, (2006) and Zhou *et al.*, (2014). The later authors reported that Soy protein isolate intake restored β -catenin signaling and alleviated hepatic fat accumulation and liver damage in the obese rats fed high-fat diet.

In this study, feeding soy protein to diabetic rats for 6 weeks produced hypolipidemic effect. The hypolipidemic activity of Soy protein could be attributed to increased insulin secretion, a better glycemic control, and antioxidant protection (Torres *et al.*, 2006; Lee, 2006; Lu *et al.*, 2008 and Ma *et al.*, 2012). The previous authors concluded that dietary Soy protein intake may modulate serum lipid levels through increasing insulin serum level.

The hypoglycemic and hyperinsulinemic effects of Soy protein when fed to diabetic rats in this study were in agreement with those previously reported by Lee, (2006) and Choi *et al.*, (2010) in diabetic rats and by Azadbakht and Esmailzadeh, (2009); Yang, *et al.*, (2011) and Urita *et al.*, (2012) in diabetic patients with nephropathy.

In the study the results showed that feeding diabetic rats on diets containing Soy protein instead of casein passively altered kidney function (increased serum urea and creatinine concentration) and decreased the activity of antioxidant enzymes (SOD, GPx and CAT) in renal tissues. These findings could be explained by hyperglycemia (resulted by alloxan) which deteriorates kidney function and causes renal oxidative stress. It is known that oxidative stress plays a key role in the onset and development of diabetes complications, notably diabetic nephropathy (Wang *et al.*, 2011; Matsuda and Shimomura, (2013) and Siddiqui *et al.*, 2013). The nephroprotective effect of Soy protein was previously reported by Anderson *et al.*, (1998) and Chen *et al.*, (2006). The nephroprotective effect of Soy protein intake could be due to preventing the inflammation of kidney and increasing the renal flow via facilitating synthesis of nitric oxide (Lopez *et al.*, 2007). The antioxidant activity of Soy protein intake was evident in this study from the increased activities of renal tissue antioxidant enzymes (SOD, GPx and CAT) in diabetic rats fed on Soy protein. Previous studies reported by Lee, (2006); Lu *et al.*, (2008) and Perez-Diaz *et al.*, (2013) also demonstrated the antioxidant effect of Soy protein diet. The later authors concluded that soybean diet attenuates the reduction oxidation (redox) changes and protects against morphological alterations induced by Cadmium in aorta. However, the hepatoprotective and nephroprotective activities of Soy protein diet in diabetic rats could be due to glycemic control by increase insulin secretion and because of high antioxidant renal protection.

In conclusion, the results denote that substitution of vegetable Soy protein for dietary animal protein casein increases weight gain and produces antidiabetic, hypolipidemic, hepatoprotective, nephroprotective, and antioxidant effects in alloxan-diabetic rats. The results suggest that consumption of Soy protein instead of casein

may be beneficial for diabetic patients as it prevents body weight loss, normalizes blood glucose and blood lipids and reduces the adverse effects of diabetes on the liver and kidneys. The study recommends that the diets containing vegetable Soy protein instead of animal protein casein or more soy protein and less casein may be beneficial for diabetics.

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