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Antihyperglycemic, Antihyperlipidemic and Antioxidant Effects of *Zizyphus spina christi* and *Zizyphus jujuba* in Alloxan Diabetic Rats

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Abstract: The present research aims to investigate the effects of methanol extracts of *Zizyphus spina christi* (ZSC) and *Zizyphus jujuba* (ZJ) roots for the treatment of alloxan diabetic rats. Sixty rats were included in the study; they were divided into six groups. The first group, non-diabetic control rats received distilled water. The 2nd and the third groups, non-diabetic control rats were given oral dose of ZSC and ZJ extracts (100 mg kg^{-1} body weight). The fourth group, diabetic control rats received distilled water. The fifth and sixth groups, diabetic rats were given an oral dose of ZSC and ZJ extracts (100 mg kg^{-1} body weight). Fasting serum glucose was measured every week and the period of the treatment continued for 2 weeks. Serum insulin, lipid profiles, liver and kidney functions were measured at the end of the experiment. In diabetic rats both extracts significantly reduced fasting serum glucose level ($p<0.001$) and markedly increased serum insulin level ($p<0.001$). ZJ significantly reduced serum total lipids (TL), triglycerides (TG), total cholesterol (TC) and lipid peroxides (LP) ($p<0.001$), low density lipoprotein cholesterol (LDL-C) ($p<0.05$), but no significant difference on high density lipoprotein cholesterol (HDL-C). Meanwhile, ZSC caused a noticeable decrease in TC, TG ($p<0.05$) and LP ($p<0.001$) compared with the untreated diabetic rats. ZJ significantly decreased alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin (TB) ($p<0.001$) in diabetic rats. Serum creatinine and urea showed significant reduction in diabetic rats treated with ZSC extract. Both extracts produced no significant changes in all studied parameters except for a significant reduction of serum lipid peroxides and urea by ZJ extract as compared to untreated diabetic control. Present data revealed that both extracts of ZSC and ZJ have beneficial effects on diabetic rats. They reduce hyperglycemia, hyperlipidemia and lipid peroxides that associate diabetes. Besides, they were safe towards liver and kidney functions. The effect of ZJ is more pronounced than that of ZSC.

Key words: Zizyphus *spina christi*, *Zizyphus jujuba*, glucose, insulin, lipids, lipid peroxides

INTRODUCTION

Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjunction with gross abnormalities in glucose homeostasis and lipid metabolism. Diabetic cases fall into two broad etiopathogenic categories: either the Insulin Dependent Diabetes Mellitus (IDDM) causes by deficiency of insulin secretion or Non Insulin Dependent Diabetes Mellitus (NIDDM) which results from combination of resistance to insulin action and an inadequate compensatory secretory response (Gavin *et al*., 1999). Diabetic patients suffer from hyperglycemia in addition to its long term complications such as atherosclerosis (Ng *et al*., 2005; Gaster and Hirsch, 1998), diabetic blindness (Reber *et al*., 2003) and nephropathy (Grazeszczak *et al*., 1997).

Currently, free radicals and oxidative stress are suggested as mechanisms underlying diabetes as well as diabetic complications (Baynes, 1991; Santini *et al*., 1997; Laaksonen *et al*., 2001). Reactive oxygen species oxidize nucleic acid, proteins and membrane lipids, thus cause direct cellular damage. Moreover, in diabetic patients, protein glycation and glucose autoxidation may generate excess free radicals (Wolff, 1993) which in turn catalyze lipid peroxidation (Bayness, 1991) and subsequently increase lipid peroxide levels (Miller *et al*., 2005; Feillet-Coudray *et al*., 1999).

Alloxan selectively destroys the islets of Langerhans by oxidant production. Current evidence suggests that alloxan cytotoxity is a function of three factors: efficient uptake, oxidant production by redox coupling of the drug with intracellular reductant (ascorbate and thiol) coupled with low levels of glutathione peroxidase in the islets (Malaisse, 1982). The latter affects prostaglandin metabolites including the peroxides which regulate insulin secretion (Metz, 1985). Effective blood glucose control

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and antioxidant actions are the keys to prevent or reverse diabetes and its complications (DeFronzo, 1999). Many herbal plants possess both effects and have been used in traditional medicine for the treatment of diabetes (Ajgaonkar, 1984; Lee *et al*., 2003; Diatewa *et al*., 2004).

In Egypt, some medicinal plants and herbs which exert hypoglycemic effect in patients with increased levels of serum glucose have been studied to develop new diabetes therapeutic agents for diabetic patients (Eskander *et al*., 1994). *Zizyphus* species (Rhamnaceae) are widely spread in Egypt which is used for various medicinal purposes. They are used as demulcent, depurative, analgesic and for treating insomnia. A survey of literature revealed that a number of cyclopeptides and isoquinoline alkaloids (Tschesche and Kaussmann, 1975) flavonoids, terpenoids and their glycosides (Byung Keun *et al*., 2002; Lee *et al*., 2003), have been found to occur in various amounts in most *Zizyphus* species. Besides, saponins and tannins have been found in *Zizyphus* leaves (Glombitza *et al*., 1994) and roots (Adzu *et al*., 2001).

Recently, some *Zizyphus* species leaves extracts were reported to have hypoglycemic effect (Glombitza *et al*., 1994; Cisse *et al*., 2000). Reviewing the current literature, nothing was reported concerning the hypoglycemic effects of *Zizyphus* roots. Therefore, the present study was undertaken to investigate the use of methanol root extracts of both ZSC and ZJ as hypoglycemic, hypolipidemic and antioxidant agents in alloxan diabetic rats with the aim of developing a natural antidiabetic drug.

MATERIALS AND METHODS

Preparation of extracts: The roots of the plants were separated and cleaned, then dried under shade and powdered. 500 and 450 g of dried powdered roots each of ZJ and ZSC, respectively were extracted in a continuous extraction apparatus then subjected to successive extractions using diethyl ether, chloroform and 70% methanol. For each organic solvent the extraction was continued till exhaustion. Also, for each extract the solvent was completely removed by distillation under reduced pressure in a rotary evaporator at a temperature not exceeding 40°C and dried to constant weight in vacuum desiccators over anhydrous calcium chloride. The yield was 59.4 and 42.3 w/w of the roots of jujuba and spina *Christi*, respectively. The residual extract was re-dissolved in water and used in the study.

Animals: Male Sprague-Dawely rats (150-200 g) bred in the Animal House of the National Research Centre. They were kept individually in stainless steel wire bottomed cages at room temperature (25±2°C) under 12 h dark-light cycle. Animals were fed standard pellets diet. The rats had free access to food and tap water.

Induction of diabetes in rats: Rats were fasted overnight, then intraperitoneally injected with alloxan monohydrate (Sigma) dissolved in sterile normal saline in a dose of 150 mg kg^{-1} body weight. Diabetes was identified by polydipsia, polyuria and by measuring blood glucose levels. After two weeks the rats with moderate hyperglycemia having blood glucose ranging between 200-280 mg/100 mL were included in the experiment.

Experimental design: Sixty rats were included in the study; they were divided into six groups, each of 10 rats. The first group, non-diabetic control rats received distilled water. The second and the third groups, non-diabetic rats were given oral dose of ZSC and ZJ extracts (100 mg kg^{-1}) (Glombitza *et al*., 1994). The fourth group, diabetic control rats received distilled water. The fifth and sixth groups, diabetic rats were given an oral dose of ZSC and ZJ extracts (100 mg kg^{-1}). The dose level used in this study was chosen taking into consideration the toxicity study of this plant as made by Adzu *et al*. (2001).

The fasting serum glucose was measured every week and the period of the treatment continued for 2 weeks. At the end of the experiment, the animals were fasted overnight and anesthetized using diethyl-ether. Blood samples were collected from sub-orbital vein and centrifuged after clotting at 3500 rpm for 15 min; serum was separated and frozen at 20°C until analysis.

Analytical determinations: Serum insulin was assayed by competitive immuno-assay method according to the manufacture's instructions (Axis-cheild As, Axis-insulin, Bickbeerngrund 4, D-29614 Soltau, Germany). Serum glucose was estimated according to Trinder (1969). Total lipids, total cholesterol and LDL-C were determined according to the methods of Knight *et al*. (1972), Richmond (1973) and Steinberg (1981), respectively as cited in Biosystem. Serum HDL-C was determined after precipitation of VLDL and LDL with phosphotungstenic acid and magnesium chloride (Burstein and Schoinick, 1973). as cited in Biosystem. Moreover, triglycerides were estimated enzymatically (Fossati and Prencipe, 1982). as cited in Biosystem. Serum lipid peroxides were determined as thiobarbituric acid reactive species (Satoh, 1978). In addition, liver function was evaluated by measuring both serum ALT and AST by the method of Reitman and Frankel (1957) and serum total bilirubin (Walter and Gerarade, 1970) as cited in randox. Serum urea and Cr concentrations were determined as a measure of kidney function according to Fawcett and Scatt (1960) and Larsen (1972), respectively as cited in Biosystem.

Statistical analysis: The data were expressed as means±SE. The significance of difference was determined by standard Student's t-tests. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Diabetes mellitus is a chronic disease characterized by high blood glucose level due to absolute or relative deficiency of circulating insulin level or insulin resistance. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increasing demand by patients to use the natural products with antidiabetic activity to overcome the side effects and toxicity of synthetic drugs. Herbal antidiabetic drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Jahodar, 1993). The aim of the present work was to confirm the effect of antidiabetic extracts of ZSC and ZJ and to evaluate the hypolipidemic and antioxidant potential of these extracts in alloxan diabetic rats.

The data showed that injected of rats with alloxan resulted in a significant increase in serum glucose level ($p<0.001$) and a marked decrease in serum insulin level ($p<0.001$) (Fig. 1 and 2). Alloxan selectively destroys the islets of langerhans thereby inducting its diabetogenicity Alloxan selectively destroys the islets of langerhans thereby inducting its diabetogenicity (Lenzen *et al*., 1996). Alloxan also increases the free radical production and causes pancreatic tissue injury (Wolff, 1993) with a consequent increase in lipid peroxidation which contributes to the increased rate of hepatic glucose production (Franssila-Kallunki and Group, 1992). Moreover, alloxan oxidize the pancreatic β-cell glucokinase with concomitant inactivation of the enzyme which couples changes in the serum glucose level that inhibits glucose induced insulin secretion and may ultimately lead to necrosis of the pancreatic β-cells (Lenzen and Panten, 1988).

Treatment of diabetic rats with each of *Zizyphus* extracts produced significant reduction in serum glucose level ($p<0.001$), while serum insulin level was significantly increased ($p<0.05$) as compared with those of untreated diabetic rats (Fig. 3 and 4). The increased insulin level due to treatment can be attributed to the stimulating effect or the activation of the extracts to the remaining intact pancreatic cells after alloxan injection. The obtained results are in agreement with somes (Glombitza *et al*., 1994; Cisse *et al*., 2000) who proved the antidiabetic effect of some *Zizyphus* species. Phytochemical screening of *Zizphus* roots revealed the presence of saponin and tannins (Adzu *et al*., 2001). Saponins have been reported to show a glucagon lowering effect which may enhance

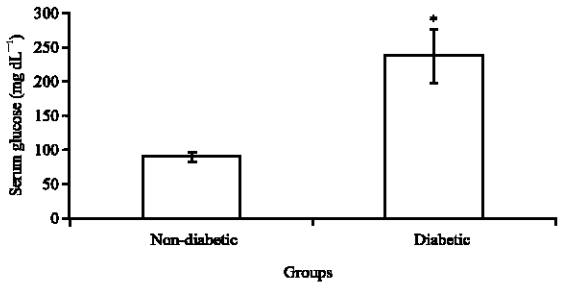

Fig. 1: Serum glucose level of non-diabetic and alloxan diabetic rats

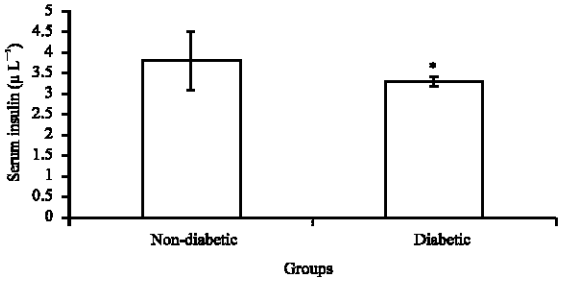

Fig. 2: Serum insulin level of non-diabetic and alloxan diabetic rats

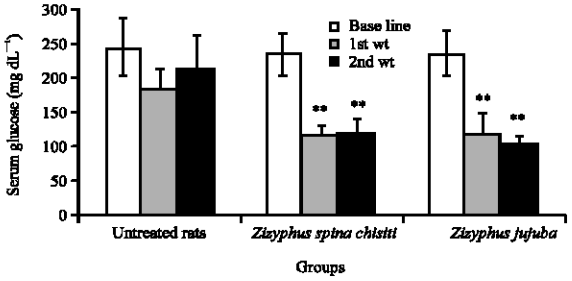

Fig. 3: Effect of *Zizyphus spina* christi and *Zizyphus jujuba* extracts on serum glucose level in alloxan diabetic rats

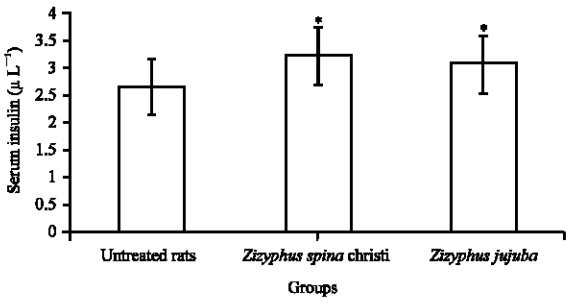

Fig. 4: Effect of *Zizyphus spina christi* and *Zizyphus jujuba* extracts on serum insulin level in alloxan diabetic rats

the glucose utilization, with consequent reduction in serum glucose levels in diabetics (Valette *et al*., 1984). Also, some saponins were found to simulate insulin release from isolated rat pancreatic islets (Norberg *et al*., 2004; Parie *et al*., 2002). It is worth mentioning that Catto *et al*. (2000), stated that glibenclamide exerts hypoglycemic action via stimulation of insulin secretion and inhibition of glucagon release. Considering that a large number of the pancreatic cells were destroyed by the action of injected alloxan, it is expected that the remaining cells are stimulated by the *zizyphus* root extract. Another possible interpretation might depend on the ability of saponin to inhibit both gastric emptying and intestinal glucose absorption (Matsuda *et al*., 1999).

Oxidative stress in diabetes and the increase of free radicals generation cause injury or destruction of pancreatic B cells which were repaired or regenerated by the action of potent antioxidant such as tannins. Tannins via their antioxidative actions increased the secretion of insulin and hence decreased the hyperglycemia in diabetic rats (Aviram *et al*., 2004; Latte and Kolodziej, 2004). Moreover, tannins may also increase insulin as a consequence of inhibition of insulin degradation, since compounds have a benzoic acid related molecules inhibited insulinase and enhanced insulin effects (Marles and Farns, 1995; Peungvicha *et al*., 1998). Green tea, a rich source of tannins, was reported to have a putative antidiabetic action (Mohamadin *et al*., 2003).

The alloxan diabetic rats developed a state of hypercholesterolemia and hypertriglycerodemia with a significant increase in LDL-C level ($p<0.001$) (Table 1). These results are in consistence with the data represented by others authors (Sharma *et al*., 1996; Pushparaj *et al*., 2000). The abnormalities in lipid metabolism in diabetes generally lead to elevation in the levels of serum lipids and lipoproteins that in turn plays an crucial role in the occurrence of premature and severe atherosclerosis (Mitra *et al*., 1996). Insulin deficiency in diabetes induces the synthesis of lipase which enhances lipolysis and increases the concentration of free fatty acids in plasma and liver. Glucagon level also increases in diabetes which enhances the release of fatty acids (Granner, 1985). Excess fatty acids in serum promote their conversion into cholesterol and TG with concomitant increase in LDL-C (Ghebremeskel *et al*., 2002; Bopanna *et al*., 1997). Moreover, insulin deficiency elevates LDL-C level and consequently the levels of cholesterol.

Treatment of diabetic rats with both extracts normalized the hyperlipidemia that occurred due to injection with alloxan, the effect of ZJ was more pronounced (Table 1). Serum total lipids, total cholesterol, TG and LDL-C were significantly ($p<0.001$) reduced except the latter at ($p<0.05$) for rats given ZJ extract when compared with values of diabetic control. Diabetic rats treated with ZSC showed significant reduction in serum total cholesterol and TG ($p<0.05$) as compared to those of diabetic control. It was demonstrated that the glycemic control of antidiabetic herbs was associated with their hypocholesterolemic and normolipidemic effects on the hyperlipidemia of alloxan induced diabetic rats (Newairy *et al*., 2002). Elevated serum insulin level in alloxan diabetic rats treated with the zizyphus extract, increased the clearance rate of both very low density lipoprotein and LDL-C consequently, it decreased the serum levels of TG and cholesterol, respectively. Moreover, insulin stimulates the lipogenesis in adipose tissue and induces lipoprotein lipase both decrease serum TG (Granner, 1985). Another possible mechanism, might depend on the presence of saponins in *Zizyphus* where saponins were reported to have a hypolipidemic effects such as decreasing total cholesterol, triglycerides and LDL-C levels in hyperlipidemic rats (Li *et al*., 2002; Zhang *et al*., 2004; Zhao *et al*., 2005) but not in normal lipidemic rats (Gupta *et al*., 2005). Saponins form insoluble complex with cholesterol, increase fecal lipid excretion (Zhao *et al*., 2005). They also increase the liver LDL receptor activity and decrease triglycerides synthesis (Yugarami *et al*., 1992). In addition, the presence of medium chain fatty acid triglycerides in ZJ may enhance the fat metabolism in diabetics (Guil-Guerrero *et al*., 2004).

Lipid peroxide was found to be significantly high in diabetic group as compared to normal group (Table 1). The present data are in accordance with those of Prakasam *et al*. (2003). The level of lipid peroxides in the cell is controlled by various cellular defense mechanisms consisting of enzymatic and non enzymatic scavenging

Table 1: The effect of *Zizyphus spina christi* and *Zizyphus jujube* extracts on lipid profile and lipid peroxidation in non-diabetic and alloxan-diabetic rats

Test groups parameters	TL (mg dL^{-1})	TC (mg dL^{-1})	LDL-C (mg dL^{-1})	HDL-C (mg dL^{-1})	TG (mg dL^{-1})	LP (μ mol L^{-1})
Non-diabetic untreated	231.5±9.650	76.2±2.17	23.6±1.63	37.5±0.26	54.9±5.61	2.2±0.12
ZSC	235.5±10.08	81.2±8.12	27.5±2.22	40.7±1.58	58.6±1.80	2.6±1.14
ZJ	239.5±14.52	82.7±6.07	25.5±3.52	38.6±2.67	51.8±4.23	1.4±0.15[a]
Diabetic rats untreated	306.8±6.290	105.5±5.91[a]	54.4±8.50[a]	39.8±1.30	282.1±9.30[a]	4.3±0.20[a]
ZSC	287.1±20.26	83.1±7.62[b]	36.4±7.06	34.6±4.42	58.8±5.43[b]	3.3±0.18[a]
ZJ	232.1±11.47[a]	73.6±5.88[a]	28.0±4.69[b]	33.6±2.86	41.6±4.45[a]	1.8±0.19

[a]TL: Total Lipid, TC: Total Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, TG: Triglycerides and LP: Lipid Peroxides. Values significantly differ from normal control[a], $p<0.001$., [b]$p<0.05$

Table 2: The effects of *Zizyphus spina christi* and *Zizyphus jujuba* extracts on liver and kidney functions in non-diabetic and alloxan-diabetic rats

	liver function				
Test groups parameters	TB (mg dL^{-1})	ALT (μL^{-1})	AST (μL^{-1})	Urea (mg dL^{-1})	Urea (mg dL^{-1})
Non-diabetic rats untreated	0.59±0.070	50.5±10.89	141.1±6.830	45.3±3.12	0.85±0.110
ZSC	0.46±0.080	53.5±3.48	147.9±4.440	56.4±4.50	0.84±0.040
ZJ	0.49±0.051	48.8±4.630	144.4±6.480	54.3±1.27	0.90±0.056
Diabetic rats untreated	0.75±0.210	79.6±8.310	206.0±21.01 [b]	59.2±7.45	0.98±0.060
ZSC	58.3±6.400 [b]	71.6±6.380	205.2±23.62	56.9±8.66	0.78±0.053 [a]
ZJ	0.41±0.040 [a]	58.3±6.400	154.8±10.14 [b]	61.8±6.48	0.81±0.044

[b]TB: Total Bilirubin, ALT: Alanine transaminase, AST: Aspartate transaminase, Cr: Creatinine, Values significantly differ from normal control, [a]$p<0.001$, [b]$p<0.05$

Table 3: The effect of *Zizyphus spina christi* and *Zizyphus jujuba* extracts on serum glucose and insulin in non-diabetic rats

	Serum glucose (mg dL^{-1})			
Parameters groups	Base line	1st week	2nd week	Serum insulin ($u L^{-1}$)
Non-diabetic untreated	90.0±6.30	95.8±5.50	92.7±4.5	3.76±0.71
ZSC	85.0±4.91	77.0±5.10	76.0±8.6	3.61±0.13
ZJ	90.5±6.80	84.5±7.00	89.6±7.0	3.71±0.35

systems (Halliwell and Gutteridge, 1994). The efficiency of these defense mechanisms is altered in diabetes (Wohaieb and Godin, 1987). In addition, oxidative stress is suggested as a mechanism underlying diabetes and diabetic complications (Halliwell *et al*., 1989) protein glycation and glucose autoxidation may generate free radicals which play a crucial role in catalyzing lipid peroxidation (Mullarkey *et al*., 1990; Baynes, 1991).

The level of lipid peroxides as determined by thiobarbituric acid reactive species was significantly ($p<0.001$) reduced in alloxan diabetic rats treated with both *Zizyphus* extracts (Table 2). It is suggested that the apparent antioxidant activities of extracts were due to the presence of tannins (Adzu *et al*., 2001) and carotenes (Guil-Guerrero *et al*., 2004) in some *Zizyphus* species as proved by phytochemical screening. Tannins which are water soluble polyphenols are considered as superior antioxidants, as their eventual oxidation may lead to oligomerization and a production of number of reactive sites (Rodrigo *et al*., 2006; Latte and Kolodzjed, 2004). Besides, Bors and Michel (2002) showed that tannins act as antioxidant via copper scavenging. They also protect protein against oxidation and glycation (Nakagawa *et al*., 2002). β-carotene has been shown to have the potential of acting as efficient antioxidant. Carotenoids were found to inhibit free radicals induced lipid peroxidation (Krinsky, 1989) and β-carotene is one of the most efficient quenchers of singlet oxygen (Burton and Ingold, 1984). It also may prevent lipid peroxidation by inhibiting the activity of lipooxygenase towards linoleate (Lomnitski *et al*., 1993).

Diabetic rats had significant increase in serum AST level ($p<0.05$), while serum total bilirubin, ALT, urea and Cr concentrations were slightly increased in diabetics when compared with values of non-diabetic rats (Table 2). Treatment of diabetic rats with ZJ extract significantly normalized the elevated values of ALT, AST, Cr ($p<0.05$) and total bilirubin ($p<0.001$) as compared to diabetic control. While ZSC significantly decreased Cr level ($p<0.001$) as compared to diabetic rats (Table 2).

Alloxan diabetic rats are characterized by considerable tissue damage in liver, kidney and pancreas which resulted from excessive free radicals production by alloxan (Halliwell and Gutteridge, 1989). The increase of AST level in diabetic rats is in agreement with similar result (Stanely *et al*., 2000) while the increase in the markers of the kidney function in alloxan diabetic rats is consistent with the data of Kumar *et al*. (2002). Liver and kidney dysfunction in diabetics may result in leaking out of their enzymes from the injured tissue and their migration into the blood stream. On the other hand, treatment with both extracts especially that of *jujuba* greatly reduced free radicals and consequently reduced oxidative stress with concomitant hepatic and renal protection. Moreover, Zhang *et al*. (2004) reported that saponins in herbs have a hepatoprotective effects.

There were no noticeable variations in serum glucose and insulin levels for non-diabetic rats when treated by both extracts as compared with untreated non-diabetic (Table 3). Also lipid profile, liver and kidney function tests showed no significant changes by administrating each of the two extracts for non-diabetic control rats (Table 1). On the other hand, lipid peroxide level was markedly reduced ($p<0.001$) in rats treated with ZJ as compared with non-diabetic control (Table 1). These results may be explained by the pronounced antioxidant activity of ZJ extract.

From the results of the present study, it can be concluded that methanol extracts of both ZSC and ZJ could be used as safe potential natural functional food ingredient or therapeutic drug in the treatment of diabetes. In addition, they are effective in reducing both hyperlipidemia and oxidative stress accompanying diabetes. ZJ extract has more pronounced effects than ZSC.

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