EFFECT OF AQUEOUS FIG LEAVES EXTRACT AS HYPOGLYCEMIC AGENT

Allam, Sahar O.*; Kh. M. Nematalla and Ensaf M. Khalil****

*** Dept. of Crops Technology Research, FTRI, ARC, Giza.**

**** Dept. of Special Food and Nutrition, FTRI, ARC, Giza.**

ABESTRACT

Diabetes is a chronic metabolic disorder characterized by altered carbohydrate, fat and protein metabolism, and an increased risk of multiple complications. Effect of aqueous fig leaves extract at doses of 20, 40 and 60 mg on alloxan-induced diabetic rats were studied. Sprauge-Dawley albino rats (30 male) weighing 155 to 165 g were divided into 5 groups and administered aqueous fig leaves extract daily for 28 days. Blood samples were taken from each rat and tested for blood glucose, total cholesterol, low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL), high density lipoprotein cholesterol (HDL), triglycerides, urea, uric acid and creatinine levels, serum total protein, albumin, globulin and liver enzymes activities. Also, phytochemical screening of fig leaves including total phenols, flavonoids, tannins and saponins contents were determined.

In alloxan-induced diabetic rats, blood glucose, triglycerides, total cholesterol, LDL, VLDL, urea, uric acid, creatinine and liver enzymes activities (AST and ALT) were significantly increased. While. HDL, serum total protein, albumin and globulin were significantly decreased compared with the negative control rats. Treating diabetic rats with 20, 40 and 60 mg aqueous fig leaves extract caused a significant improvement in these biochemical measures and the best results were achieved by using 60 mg fig leaves extract followed by 40 and 20 mg aqueous extract, respectively.

It could be concluded from these results that, aqueous fig leaves extract which was found to be rich in total phenols and total flavonoids which considered powerful antioxidants should be used in manufacture processes of the natural products with anti-diabetic activity as hypoglycemic agent.

Keywords: Alloxan-induced diabetic rats; Fig leaves extract; Blood glucose; Total phenols; Total flavonoids; Hypoglycemic agent.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia caused by defective insulin secretion and/or action, resulting in long term multi-organ complications (Caughron and Smith, 2002). Chronic hyperglycemia cause damage to the eye, heart, kidneys, nerves and blood vessels (Lebovitz, 2001). High glucose level was found to increase the production of free radicals, as determined by cell damage markers. Increased oxidative stress has been implicated in the pathogenesis of diabetic complications and reduced levels of antioxidants are found in blood and tissue in both human and experimental diabetes (Cuncio *et al.,* 1995 and Baynes and Thorpe, 1999).

Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use the natural products with anti-diabetic activity (Venkatesh *et al.,* 2003). One such plant expected to have anti-diabetic activity is *Ficus carica* (fig plant).

Several studies in animal models with diabetes have shown that both short and long term hypoglycemic effects of fig leaves were proven. Its mechanism for lowering glucose levels is unknown, however, some studies suggest facilitation of glucose uptake peripherally (Torres *et al.,* 1993 and Perez *et al.,* 1996). Potential hypolipidemic effects in diabetic rats have also been shown with fig leaves extract (Campillo *et al.,* 1991).

Aqueous fig leaves extract was used in small crossover clinical trial study for 4 weeks in patients with type I diabetes (n=10). There was a significant decrease in post-prandial glycemia during supplementation with fig leaves (293.7±45.0 mg/dl in control diabetic patients against 156.6±75.9 mg/dl in diabetic patients treated with fig leaves extract). Average insulin dose was 12% lower during fig leaves treatment in the total group. This result supporting a non-insulin-mediated effect and no adverse effects were reported (Serraclara *et al.,* 1998).

The administration of aqueous extract obtained from a decoction of fig leaves to streptozotocininduced diabetic rats led to a decline in the levels of total cholesterol and a decrease in the total cholesterol/HDL- cholesterol ratio with respect to the control group, together with a reduction of the hyperglycemia (Canal *et al.,* 2000).

The safety of fig leaves has been investigated in several studies. For instance, a decoction of the leaf has been established to exhibit significant reduction in intestinal motility in addition to its anti-ulcer activity with no sign of toxicity (Gamaniel *et al.,* 1997).

A number of data showed that the presence of phenolics in foods is particularly important for their oxidative stability and anti-microbial protection. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify gene expression (Marinova and Atanassova, 2005). Phenols, especially those with multiple phenolic groups, are better antioxidants than the well-known antioxidant vitamins (Vinson *et al,* 1995a).

The antioxidant capacities of the extracts prepared from fig leaves are consistent with total flavonoids and phenolic contents (Konyalioglu *et al.,* 2005). The aqueous extract of fig stem bark showed the presence of gallic tannins, saponins, reducing sugars and flavone aglycones. It was concluded that the extract contains important constituents for pharmacological activities (Sandabe *et al.,* 2006). Recoveries above 85% were obtained for chlorogenic acid, rutin and psoralen from fig leaves extract by using the sea sand extraction method (Teixeira *et al.,* 2006).

The aim of this study is to examine the effect of aqueous extract from fig leaves in various doses (20 mg, 40 mg and 60 mg) on serum glucose, lipid profiles, liver and kidney functions in alloxan-induced diabetic rats. Also, the phytochemical screening including: total phenols, flavonoids, tannins and saponins were determined in the aqueous fig leaves extract.

MATERIALS AND METHODS

Materials: Fig leaves *(Ficus carica* L.) were collected from the fields surrounding the Cairo-Alexandria desert road and carefully washed with tap water and left to dry in the dark at room temperature. The air-dried leaves were converted to powder form using an electric machine. The air-dried powdered leaves (100 g) were macerated for 24 hr. with distilled water (1 L), then filtered to obtain the water extract. The extract was allowed to dry under vacuum using rotary evaporator and the residue was stored at -10^{0} C until used. The residue was dissolved in adequate water to obtain 20, 40 and 60 mg/ml as concentration.

Animals and experimental diets: Thirty male Sprague-Dawley albino rats weighing 155 to 165 g were obtained from animal house of Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. All rats were fed on basal diet for one week (adaptation period). The basal diet consisted of casein (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%), corn oil (10%) and corn starch (70%) according to Lane Peter and Pearson (1971). After the adaptation period, diabetes was induced by intraperitoneally injection of 150 mg/kg body weight of alloxan monohydrate dissolved in 0.9 % w/v of NaCl according to the method described by Porchezian *et al.,* (2000). Blood samples were collected after 48 hr. of injection and glucose levels were determined. Rats with blood glucose level higher than 250 mg/dl were considered to be diabetic. Five groups of rats (6 rats each) were studied according to the following scheme for 28 days: (1) negative control (non diabetic rats), (2) positive control (untreated diabetic rats), (3) diabetic rats orally dosed with 20 mg fig leaves extract, (4) diabetic rats orally dosed with 40 mg fig leaves extract and (5) diabetic rats orally dosed with 60 mg fig leaves extract. The aqueous extract of fig leaves was administered for each rat once daily using a stomach tube. Blood samples were collected from orbital plexus venous into centrifuge tubes and the serum was separated and stored at -20^{0} C for analysis.

Biochemical analysis: Serum glucose levels were determined according to the method described by Trinder (1969). Serum total cholesterol, (high-density lipoprotein cholesterol and low-density lipoprotein cholesterol), very low density lipoprotein cholesterol and triglycerides were determined according to the methods of Roeschlau *et al.,* (1974); Assmann (1979); Hatch and Lees (1968) and Uwajima *et al.,* (1984), respectively. Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were colorimetrically determined according to the method of Bergmeyer and Harder (1986). Serum urea was measured by the method of Wybenga *et al.,* (1971). serum creatinine was estimated by the method of Tomas (1998). Uric acid was determined according to the method described by Fossati *et al.,* (1980) using spectrophotometer. Serum total protein content was estimated by the method of Lowry *et al.,* (1951) using bovine serum albumin as standard. Albumin was determined by an enzyme-linked immunosorbent assay as described by *Borcea et al.,* (1999). Globulin was calculated by subtracting albumin from serum total protein content.

Phytochemical analysis: The total phenolic content was determined by using the Folin-Ciocalteu assay as described by Vinson *et al.,* (2001b). Total phenolic content was expressed as gallic acid equivalents. Total flavonoid content was determined by using the aluminium chloride method mentioned by Harbone (1978). Tannins were determined by Folin Denis Spectrometric method as described by Pearson (1976). Saponins were measured according to Evans (1989).

Statistical analysis: The standard analysis of variance procedure in a completely randomized design was applied for the present data according to Gomez and Gomes (1984). Least significant difference (LSD) and Duncan's test were done to compare a pair of group means. The level of statistical significance was set at $p< 0.05$.

RESULTS AND DISCUSSION

Effect of aqueous fig leaves extract on body weight and blood glucose levels in diabetic rats are shown in Table (1). Alloxan-induced diabetic rats gained on average less body weight than the negative control rats over the whole period of the study. Final body weight of the negative control rats was significantly higher than initial body weight and all the diabetic groups. The reduction in body weight for the three doses of fig leaves extract was significantly lower than the control rats. Reduction in body weight due to administration of 40 and 60 mg fig leaves extract was not significantly different. These results are in agreement with the results mentioned by Hassan *et al.,* (2007) who found a significant decrease in body weights of the rats administered aqueous ethanol extracts of fig stem bark compared with control group.

Table (1): Effect of aqueous fig leaves extract on body weight and blood glucose levels in diabetic rats.

Time / Groups	Body weight (gm)		Blood glucose levels (mg/dl)	
	Initial	Final	At zero time	At the end
Negative control	A 158.33	B 166.55	D 139.39	D 142.33
Positive control	A 156.55	C 135.33	A 253.26	A 259.60
Fig leaves extract (20mg)	A 155.67	D 125.67	A 255.19	B 175.58
Fig leaves extract (40mg)	A 158.00	E 120.33	A 257.33	C 160.11
Fig leaves extract (60mg)	A 160.33	E 118.55	A 254.49	D 144.33
L.S.D.	4.93		15.34	

From Table (l), blood glucose levels increased by 1.8 folds in diabetic rats compared with the negative control rats. The significant increase in the levels of blood glucose in alloxan-induced diabetic rats could be due to it is a beta cytotoxic induces chemical diabetes through damaging insulin-secreting cells (Hecht *et al.,* 1973). At the end of the experiment, blood glucose levels in diabetic rats dosed with 20, 40 and 60 mg aqueous fig leaves extract

decreased significantly than those of the diabetic control rats. The decrease in blood glucose levels as a result of treatments was 31.20% , 37.78% and 43.29% for 20, 40 and 60 mg aqueous fig leaves extract,respectively. The dose of 60mg fig leaves extract is the best for controlling blood glucose level which was not significantly different than the negative control rats.

In this respect, Edwin *et al.,* (2008) found that the bark and aerial roots extracts of fig significantly lowered the blood sugar level of hyperglycemic rats. Also, Campillo *et al.,* (1991) observed that aqueous extract from fig leaves showed a clear hypoglycemic effect in diabetic rats. The glucose lowering activity observed in the diabetic animals may be due to the stimulation of β cells of pancreatic islets, also may be mediated through stimulation of insulin release resembling the oral hypoglycemic drugs or peripheral glucose utilization (Esmaeili and Yazdanparast, 2004). This may be due to the presence of some hypoglycemic principles in fig leaves extract which were similar to insulin or oral hypoglycemic drugs.

Table (2) represents the effect of aqueous fig leaves extract on serum triglycerides levels and lipid profiles in diabetic rats. Diabetes resulted in a significant increase in triglycerides levels compared with the non diabetic rats. Treating diabetic rats with 20, 40 and 60 mg aqueous fig leaves extract showed a significant reduction in serum triglycerides levels compared with the control rats. Fig leaves extract (60 mg/ml) was not significantly different when compared with the negative control rats for triglycerides levels. Serum triglycerides levels reduced from 110.47 mg/dl to 68.60 mg/dl (37.90%) by using 60 mg fig leaves extract. From data in Table (2), it could be observed that total cholesterol, LDL-cholesterol and VLDL-cholesterol levels increased significantly in diabetic rats compared with the negative control rats as a result of diabetes. When diabetic rats administered with 20, 40 and 60 mg fig leaves extract the levels of lipid profiles were significantly reduced compared with the positive control rats. The highest reduction was achieved by using 60 mg aqueous fig leaves extract followed by 40 mg and 20 mg fig leaves extract, respectively, for the three lipid profiles. Diabetes caused a significant decrease in HDL-cholesterol level when compared with the negative control rats. Treating diabetic rats with 20, 40 and 60 mg aqueous fig leaves extract caused a significant increase in the levels of HDL-cholesterol compared with the untreated diabetic rats. The highest increase in HDL-cholesterol level was achieved by using 40 and 60mg aqueous fig leaves extract which was not significantly different from each other .

Nimenibo-Uadia (2003) reported that administration of aqueous extract of fig leaves resulted in decreased plasma triacylglycerol and butyrate levels in alloxan treated rats. Carmen *et al.,* (1999) found a significant decrease in triglycerides levels after the intraperitoneal injection of aqueous extract of fig leaves in hypertriglyceridemic rats, while the plasma total cholesterol levels showed no significant difference in relation to baseline levels. These results suggest the presence of some compounds in the aqueous fig leaves extract that influence lipid catabolism. In another study reported by Rimi *et al.,* (2004), treatment with water extract of the bark of fig plant decreased the serum cholesterol level by 59%, triacylglycerol by 54% and LDL+VLDL-cholesterol by 60% compared with control rats.

Data presented in Table (3) show the effect of aqueous fig leaves extract on kidney functions in diabetic rats. Serum urea, uric acid and creatinine levels (mg/dl) which were the bio-chemical parameters that are related to kidney functions increased significantly in diabetic control rats compared with the negative control rats as a result of diabetes. This may be due to the hyperglycemia which caused damage to kidneys (Lebovitz, 2001). Treating diabetic rats with aqueous fig leaves extract caused a significant reduction in serum urea, uric acid and creatinine levels compared with diabetic control rats. The highest reduction was achieved by using 60 mg aqueous fig leaves extract followed by 40 mg and 20 mg fig leaves extract, respectively.

The significant increase in serum urea, uric acid and creatinine levels suggests renal malfunction. Creatinine levels are indicators of renal functions, with increased levels appearing in the event of significant impairment (Tietz, 1982 and Chessbrough, 1991). There is considerable evidence that increased oxidative stress may participate in the pathogenesis of diabetic complications, including nephropathy. This shows that with significant increase in the levels of kidney markers, about 75% of the nephrons might have been damaged (Boyd, 1983 and Baynes and Thorpe, 1999). From the above mentioned data, it could be concluded that, all tested diabetic groups which were administered aqueous fig leaves extract with different doses (20mg, 40mg and 60 mg) improved their renal functions.

Table (4) shows the effect of aqueous fig leaves extract on liver functions in diabetic rats. Diabetes resulted in a significant increase in liver enzymes aspartate amino transferase (AST) and alanine amino transferase (ALT) activities compared with the negative control rats as a result of diabetes and oxidative stress which reduced liver functions. Significant increases of AST and ALT as shown in the results suggest possible necrotic injury of the liver or cholestasis with hepatocellular necrosis (Van Hoof and De Broe, 1994). Liver enzymes activities were decreased to the normal levels found in the negative control rats after treatment with 20, 40 and 60 mg aqueous fig leaves extract.

From Table (4), it could be observed that, diabetes caused a significant decrease in total protein, albumin and globulin levels (g/dl) in serum of diabetic rats. Treating diabetic rats with aqueous fig leaves extract caused significant increase in total protein and albumin levels compared with untreated diabetic rats. Measurement of the activities of various enzymes and non-enzymatic indices in tissues and body fluids play a significant and well-known aid in disease investigation and diagnosis. Tissue damage is usually associated with the release of enzymes to the affected organ or tissue into circulation (Malomo, 2000). The significant decrease in total protein and albumin with diabetes are indication of compromised liver excretory function and impairment of the liver synthetic function, which improved by treating with aqueous fig leaves extract used in this study. Dey *et al.,* (1998) found that, serum total protein, albumin, globulin, AST and ALT levels were improved by feeding lambs on dietary inclusion of fig leaves.

Table (5) represents the phytochemical constituents which found in aqueous extract of fig leaves. Fig leaves extract (60 mg) contained higher contents of total phenols and flavonoids than the other doses which were found to be 9.14 **±** 0.09 and 0.67 ± 0.03 mg/g extract for total phenols and total flavonoids, respectively. Tannins and saponins were detected in aqueous fig leaves extract with higher contents found in 60 mg leaves extract. From these data, it could be observed that fig leaves are rich in total phenols and flavonoids. The values of tannins and saponins found in leaves extract are within the acceptable limits.

Table (5): Phytochemical constituents of fig leaves extract

Extract	Total phenols (mg/g extract)	Total flavonoids (mg/g extract)	Tannins (%)	Saponins (%)
Fig leaves extract (20mg)	3.05 **±** 0.02	0.22 **±** 0.03	1.88 **±** 0.02	0.09 **±** 0.01
Fig leaves extract (40mg)	6.10 **±** 0.06	0.45 **±** 0.05	2.81 **±** 0.02	0.12 **±** 0.01
Fig leaves extract (60mg)	9.14 **±** 0.09	0.67 **±** 0.03	3.94 **±** 0.02	0.17 **±** 0.01

Ethanolic fractions of plant materials usually extract tannins, polyphenols, flavonoids, terpenes, alkaloids and sterols if they are present. The fraction of fig leaves used in the study of Irene and Ukweni, (2007) seem to be rich in alkaloids, flavonoids and tannins, but not saponins. These suggest that the medicinal properties attributed to fig leaves could be based on the antioxidant and antimicrobial effects of these phytochemicals (Cowman, 1999).

Phenolic compounds can play an important role in preventing body cells from injuries by hydrogen peroxide, preventing cells and the organs of man from damage by lipid peroxides and absorbing and neutralizing free radicals (Sroka and Cisowski, 2003). It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular disease (Cai *et al.,* 2004).

Our findings were in accordance with the results mentioned by Buniyamin *et al.,* (2007) who found that the aqueous extract of fig leaves showed the presence of flavonoids, saponins and tannins, with no traces of alkaloids or anthraquinones. It was found that high total phenols content increases antioxidant activity and there was a linear correlation between phenolic content and antioxidant activity in fig leaves extract (Changwei *et al.,* 2008).

From the results obtained in this study, it could be observed that aqueous fig leaves extract had powerful antioxidants activity because of its high total phenols and total flavonoids contents. These antioxidant activities may be the factor responsible for lowering diabetic complications observed in the alloxan-induced diabetic rats and, therefore, this extract should be added to the natural products used for treating diabetes as hypoglycemic agent.

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تأثير المستخلص المائي لأوراق التين كعامل خافض للسكر.

سحر عثمان علام * ، خالد محمد نعمة الله ** و إنصاف محمد خليل **

*** قسم بحوث تكنولوجيا المحاصيل ـ معهد بحوث تكنولوجيا الأغذية ـ مركز البحوث الزراعية – الجيزة**

**** قسم الأغذية الخاصة والتغذية ـ معهد بحوث تكنولوجيا الأغذية ، مركز البحوث الزراعية – الجيزة .**

يرتبط مرض السكر بنقص معدل التمثيل الغذائي المزمن ويتميز بانخفاض تمثيل الكربوهيدرات والبروتينات والدهون وبزيادة خطر الإصابة بالمضاعفات المتعددة ، وقد أجريت هذة الدراسة لاختبار تأثير استخدام المستخلص المائي لأوراق التين بجرعات مختلفة هي ٢٠ ، ٤٠ ، ٦٠ ملجم علي الفئران المصابة بالسكر .

تم في هذة الدراسة استخدام عدد ٣٠ فأر تتراوح أوزانهم ما بين ١٥٥ ألي ١٦٥ جم مقسمة ألي خمسة مجموعات وحصلت علي المستخلص المائي لأوراق التين لمدة ٢٨ يوما وتم جمع عينات الدم من الفئران وأجريت عليها الاختبارات البيوكيميائيه لقياس مستوى جلوكوز الدم ، الكوليستيرول الكلي ، الليبوبروتينات منخفضة وشديدة الانخفاض وعالية الكثافة ، التراي جليسريدات ، تركيزات اليوريا وحامض اليوريك والكرياتينين في السيرم وكذلك محتوي السيرم من البروتين الكلي والألبيومين والجلوبيولين وإنزيمات الكبد ، كما تم تقدير المركبات الفيتوكميائية في المستخلص المائي لأوراق التين وتشمل الفينولات الكلية ، الفلافونات الكلية، التانين والسابونين.

وجد في الفئران المصابة بالسكر أن مستوي جلوكوز الدم ، التراي جلسريدات ، الكوليستيرول الكلي ، الليبوبروتينات منخفضة وشديدة الانخفاض في الكثافة ، اليوريا ، حامض اليوريك ، الكرياتينين وانزيمات الكبد قد زادت كنتيجة مباشرة للإصابة بالسكر ، بينما وجد أن الليبو بروتينات عالية الكثافة ، محتوي البروتين الكلي في السيرم ، الألبيومين والجلوبيولين قد انخفضت بالمقارنة بالفئران السليمة غير المصابة بالسكر.

معالجة الفئران المصابة بالسكر باستخدام ٢٠ ، ٤٠ ، ٦٠ ملجم من المستخلص المائي لأوراق التين أدت ألي حدوث تحسن ملحوظ في القياسات البيوكميائية وقد تم الحصول علي أفضل النتائج باستخدام المستخلص المائي