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Effect of Flax Seeds and Date Palm Leaves Extracts on Serum Concentrations of Glucose and Lipids in Alloxan Diabetic Rats

Abuelgassim O. Abuelgassim
Department of Biochemistry, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

Abstract: The aim of the present investigation was to examine the effect of Flax Seeds (FS) and Date Palm Leaves (DPL) extracts on the concentration of serum glucose and lipids in alloxan diabetic rats. Rats were divided into six groups, normal control rats (without treatment with either FS or DPL), normal control rats treated with either FS or DPL extract for four weeks, diabetic control rats and diabetic rats treated with either FS or DPL extract for four weeks. The concentration of glucose in diabetic rats treated with FS (D+FS) for four weeks was significantly decreased from 17.20±2.33 to 8.14±0.54 mmol L^{-1} ($p<0.001$). Serum total cholesterol concentration (TC) of diabetic rats treated with either FS or DPL extract showed significant decrease by 40 and 31% respectively in the fourth week. Also, serum low density lipoprotein cholesterol (LDL-C) concentration was significantly decreased after two weeks of treatment ($p<0.001$ and $p<0.01$, respectively). However, no significant changes were obtained in serum high density lipoprotein cholesterol (HDL-C) concentration. Results of the present study showed that FS extract has a hypoglycaemic effect against alloxan diabetic rats. Present findings also showed that treatment of alloxan diabetic rats with either FS or DPL extract significantly decreased serum concentration of TC and LDL-C. Present findings suggest that both of FS and DPL extracts could have a protective effect against diabetes complications as well as against hyperlipidemia through improvement of lipid profile.

Key words: Flax seeds, date palm leaves, diabetes, alloxan, lipids, serum

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease that characterized by impairment of glucose metabolism and utilization. The prevalence of the disease all over the world is in a continuous increase expecting to reach 221 millions by 2010 (Carter, 2004; Wild *et al*., 2004). Most of diabetic patients have health complications or risk factors such as high blood pressure and cholesterol that increase one's risk for heart disease and stroke. When combined with DM, these risk factors add up to big trouble, however, vascular complications are the most common causes of morbidity and mortality in diabetic patients (Guo *et al*., 2005).

An intensive research work is conducted in herbs that could have a protective effect against DM due to the side effects associated with the oral hypoglycemic agents for the treatment of the disease (Kim *et al*., 2006). Although, Subash Babu *et al*. (2007) demonstrates that cinnamaldehyde, isolated from cinnamon, possesses hypoglycemic and hypolipidemic effects in streptozotocin-induced diabetic rats, however, an opposing findings reported that cinnamon has no effect on adolescents with type 1 diabetes (Altschuler *et al*., 2007). A survey study conducted in south-eastern Morocco, concluded that 78% of the patients regularly use medicinal plants, *Phoenix dactylifera* was among the most frequently used plants to treat diabetes and hypertension (Tahraoui *et al*., 2007). Haliga *et al*. (2007) reported that dietary flax seed supplementation improved serum lipid concentrations and reduced diabetic macro vascular complications in streptozotocin diabetic hamster. Several investigators reported the benefits of flax seeds administration in the reduction of the risk factors of atherosclerosis (Prasad, 2005, 2008; Mandasescu *et al*., 2005). Antioxidant activities were reported in many plants, among them is date palm fruit (*Phoenix dactylifera*) which possesses a potential antioxidant compounds that capable of scavenging free radicals (Vayalil, 2002; Mansouri *et al*., 2005; Biglari *et al*., 2008).

The present investigation attempts to study the effect flax seeds (*Linum usitatissimum*) and date palm (*Phoenix dactylifera*) leaves extracts on serum concentration of glucose and lipids in alloxan diabetic rats.

MATERIALS AND METHODS

This study was conducted between November 20, 2008 and March 12, 2009.

Chemicals and reagents: Alloxan for diabetes induction was obtained from CDH laboratory reagent (New Delhi, India). Methanol and other chemicals were of analytical grade and obtained from BDH (Poole, United Kingdom).

Kits for the assay of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (TG) and glucose were purchased from Human GmbH (Wiesbaden, Germany).

Plant material and extraction: Date palm leaves (DPL) were collected from Riyadh city. At first, DPL were washed from dust with tap water and then rinsed with distilled water and distributed in a clean benches and allowed to stand until dryness at room temperature. Dry DPL were cut into small pieces and immersed in liquid N_2.

Flax Seeds (FS) were purchased from Riyadh local market. One hundred grams powder of each plant were prepared by using a blender (FS powder was prepared without immersion of seeds in liquid N_2 prior blending). The powdered samples were then extracted with 300 mL methanol-water (4:1, v/v) at room temperature for overnight using an orbital shaker. Filtrates were collected through glass wools and residues were extracted again with the same above mentioned protocol. Pooled filtrates were collected and centrifuged at 8000 rpm for 10 min and the supernatants were concentrated under reduced pressure at 40°C using a rotary evaporator. Yields of DPL and FS were 8.4 and 6.2 g, respectively.

Animal groups and dosage: Male Wistar rats (180-205 g) were obtained from the animal house, Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia. All animals were housed in cages with 12/12 h light/dark cycles at 22°C. Animals were given rat chow and water ad libitum. Diabetes was induced by an injection of alloxan (150 mg kg^{-1}, i.p., dissolved in physiologic saline). The animals were kept under observation for one week prior to the start of treatment. All animal experiments were carried out in accordance with King Saud University Ethical Committee Acts. Extracts of FS and DPL were administered orally (0.2 mL) at a concentration of 100 mg KBW^{-1} by using oral gavage. Treatment with extracts was continued on regular daily basis over a period of 30 days.

Rats were divided into 6 groups (10:14 rats/each). Group 1 consisted of normal control rats (NC). Group 2 consisted of normal control rats treated with FS (NC+ FS). Group 3 consisted of normal control rats treated with DPL (NC+DPL). Group 4 consisted of diabetic control rats (DC). Group 5 consisted of diabetic rats treated with FS (D+FS). Group 6 consisted of diabetic rats treated with DPL (D+DPL).

Body weights and food intake of controls and diabetic treated rats were recorded daily. Aliquots of blood samples from all groups were collected weekly in plain tubes. Serum samples were separated from cells by centrifugation at 5000 g in a refrigerated centrifuge.

Biochemical assays: Glucose, Total Cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were estimated in serum samples by standard enzymatic methods using Human kits (Bartham and Trinder, 1972; Richmond, 1973; Schettler and Nussel, 1975; Izawa *et al*., 1997; Okada *et al*., 1998), respectively. The concentrations of these parameters were measured in a Humalyzer model 3000.

Data analysis: The data presented in this investigation are expressed as means±standard error of the mean (SEM) and comparison between experimental and corresponding control rats was made by using student's t-tests. The probability value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Body weight and food intake of control and diabetic rats treated with FS and DPL extracts: Table 1 shows that gain in body weight and food intake in alloxan diabetic rats was markedly reduced ($p<0.001$) compared to their matched control rats. However, normal control and alloxan diabetic rats that were treated with FS or DPL did not show any significant changes in the same parameters.

Effect of FS and DPL extracts on serum glucose and serum lipids concentration of normal control (NC) rats: Table 2 shows that serum glucose concentration of NC rats after two weeks treatment with FS or DPL was not significantly different from that of NC rats without treatment. However, after four weeks treatment with FS extract, NC rats showed significant decrease in serum glucose concentration ($p<0.03$). Treatment of NC rats with FS extract reduced TC significantly by 37 and 55% after two and four weeks of treatments, respectively. This finding coincided with the report of Bhathena *et al*. (2002). On the other hand, DPL extract showed significant decrease in serum TC concentration of NC rats after four weeks of treatment ($p<0.05$). The concentration of TG in both FS and DPL groups were not significantly changed from their matched NC group. Although, present results showed that FS had no significant effect on serum TG concentration is contrasting with the report of (Bhathena *et al*., 2002), however, this could be due to the used concentration of FS. Serum concentration of LDL-C was significantly decreased after four weeks treatment of NC rats with either FS or DPL ($p<0.02$ and 0.01), this decrease was 21% and 29% respectively. A significant increase ($p<0.02$) was observed in serum HDL-C concentration when NC rats were treated for four weeks with DPL extract as the concentration increased from 0.33 to 0.56 mmol L^{-1}.

Table 1: General characteristics of control and diabetic rats treated with flax seeds and date palm leaves extracts

Characteristics	NC	NC+FS	NC+DPL	DC	D+FS	D+DPL
Initial body wt. (g)	184±2.3 (12)	188±2.5 (10)	182±2.6 (12)	196±2.1 (12)	203±2.6 (9)	206±2.7(10)
Final body wt. (g)	329±3.1 (12)	331±2.7 (10)	326±2.2 (10)	311±2.7(10)*	336±3.6 (9)	341±2.8(10)
Food intake (g day^{-1})	22.7±0.67 (10)	22.2±0.47 (10)	23.3±0.55 (9)	18.2±0.62 (10)*	20.8±0.57 (9)	21.9±0.45(10)

Data are expressed as means±SEM, with number of rats given in parentheses. As compared with matched groups, *p<0.001 (student t test)

Table 2: Serum concentrations of glucose, Total Cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein -cholesterol (HDL-C) in control rats treated with flax seeds and date palm leaves extracts

Groups	Weeks	Glucose (mmol L^{-1})	TC (mmol L^{-1})	TG (mmol L^{-1})	LDL-C (mmol L^{-1})	HDL-C (mmol L^{-1})
NC	-	7.47±0.25 (14)	1.71±0.27 (14)	1.28±0.21 (10)	0.72±0.06 (10)	0.33±0.05 (9)
NC+FS	2 Weeks	7.14±0.47 (14)	1.08±0.08 (12)‡	1.26±0.07 (11)	0.76±0.28 (10)	0.28±0.03 (9)
	4 Weeks	6.57±0.64 (12)†	0.77±0.13 (12)*	1.12±0.08 (10)	0.57±0.18 (10)**	0.39±0.05 (8)
NC+DPL	2 Weeks	6.97±0.34 (14)	1.36±0.27 (12)	1.40±0.16 (10)	0.70±0.08 (9)	0.41±0.11 (9)
	4 Weeks	6.88±0.54 (14)	1.15±0.22 (12)‡	1.26±0.07 (10)	0.51±0.12 (9)*	0.56±0.08 (9)**

Data are expressed as means±SEM, with number of rats given in parentheses. As compared with matched groups, *p<0.01, **p<0.02, †p<0.03, ‡p < 0.05 (student t test)

Table 3: Serum concentrations of glucose, Total Cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein -cholesterol (HDL-C) in diabetic rats treated with flax seeds and date palm leaves extracts

Groups	Weeks	Glucose (mmol L^{-1})	TC (mmol L^{-1})	TG (mmol L^{-1})	LDL-C (mmol L^{-1})	HDL-C (mmol L^{-1})
DC	-	17.20±2.33 (14)	2.05±0.12 (12)	1.90±0.18 (12)	0.69±0.03 (12)	0.35±0.08 (12)
D+FS	I Week	15.61±0.84 (11)	0.80±0.12 (10)‡	0.87±0.13 (10)‡	0.70±0.10 (11)	0.37±0.09 (11)
	2 Weeks	12.02±0.64 (10)†	0.82±0.21 (10)‡	0.91±0.22 (9)‡	0.52±0.16 (10)‡	0.41±0.11 (9)
	3 Weeks	11.41±0.60 (9)**	0.96±0.20 (9)‡	1.00±0.12 (9)‡	0.55±0.11 (9)‡	0.44±0.10 (9)
	4 Weeks	8.14±0.54 (10)‡	1.23±0.06 (10)‡	1.14±0.09 (9)*	0.48±0.07 (10)‡	0.43±0.07 (10)
D+DPL	I Week	17.43±0.76 (11)	1.08±0.22 (9)‡‡	1.09±0.07 (9)‡	0.72±0.16 (9)	0.40±0.06 (9)
	2 Weeks	17.33±0.81 (10)	1.54±0.18 (10)**	0.94±0.14 (10)‡	0.60±0.11 (9)*	0.42±0.05 (10)
	3 Weeks	15.06±0.68 (10)	1.07±0.19 (10)‡	1.18±0.20 (12)*	0.52±0.05 (10)‡	0.39±0.08 (11)
	4 Weeks	16.77±0.28 (12)	1.41±0.19 (11)*	1.15±0.08 (10)*	0.50±0.09 (11)‡	0.42±0.05 (10)

Data are expressed as means±SEM, with number of rats given in parentheses. As compared with matched groups, *p<0.01, **p<0.02, †p<0.03, ‡‡p<0.003, ‡p<0.001 (student t test)

Effect of FS and DPL extracts on serum glucose and serum lipids concentration of alloxan diabetic rats: Table 3 shows that serum glucose concentration of diabetic rats treated with FS extract for 2, 3 and 4 weeks was significantly decreased reaching a value of 8.14 mmol L^{-1} at fourth week ($p<0.001$). In contrast, no any significant changes were noticed in the same parameters of diabetic rats treated with DPL extract throughout the study period. Serum TC concentration of diabetic rats treated with either FS or DPL extract showed significant decrease throughout the weeks of study. Our results obtained with DPL extract agreed with Al-Maiman (2005) who reported that date seeds fiber at a concentration of 1.5% significantly reduced plasma TC in rats. Although, present finding in that FS extract decreases serum TC concentration of diabetic rats was coincided with that reported by Haliga *et al.* (2007) however, the degree of this reduction was different, this could be due to many factors include species differences, diabetes induction procedure, dose concentration as well as the way of FS administration.

Both of FS and DPL extracts were significantly reduced serum TG levels of diabetic rats (Table 3). With regard to FS, our finding contrasted with those reported by Haliga *et al.* (2007) who stated that FS supplementation had no significant effect on serum TG concentrations of diabetic hamsters. Treatment of diabetic rats for two weeks with either FS or DPL extract resulted in a significant reduction of serum LDL-C concentration ($p<0.001$).

The decrease in serum LDL-C level after treating diabetic rats with FS extract could be accounted to the presence of Secoisolariciresinol diglucoside (SDG), a compound of an antioxidant activity, isolated from FS as reported by Prasad (1999). Although, Chaira *et al.* (2007) reported that flesh and pit extracts of date palm fruit have free radical scavenging activities, however, the significant effect ($p<0.001$) (third and fourth weeks) of DPL extract on serum LDL-C level could be attributed to the antioxidant potentials of DPL extract as it was investigated in our laboratory (unpublished work). Another rationale explanation for the effect of DPL extract on LDL-C concentration in diabetic rats could be due to prevention of oxidative stress, from this view, present findings support the report of Saafi *et al.* (2010).

Both of FS and DPL extracts have no significant effect on serum HDL-C concentration of diabetic rats during the study (Table 3). We have no explanation for these results at present, however, we can propose that if the study duration is extended, a positive increase in serum HDL-C concentrations could be obtained to associate the significant decrease in LDL-C concentration.

The findings of the present investigation showed that FS had a significant decrease of both serum

concentration of TC and LDL-C, from this view, our findings supported the suggestions reported by several investigators that FS has a hypocholesterolaemic effect (Lucas *et al*., 2004; Yang *et al*., 2005). DPL extract also showed a significant reduction of serum concentration of TC and LDL cholesterol in diabetic rats (Table 3).

CONCLUSION

The current investigation concludes that both of FS and DPL extracts have a hypocholesterolaemic effect and could have a protective effect against diabetes complications as well as hyperlipidemia through improvement of lipid profile.

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