Global Journal of Biotechnology & Biochemistry 10 (1): 16-21, 2015
ISSN 2078-466X

DOI: 10.5829/idosi.gjbb.2015.10.01.1115

Effect of Egyptian Radish and Clover Sprouts on Blood Sugar and Lipid Metabolisms in Diabetic Rats

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Abstract: No information is available about the effect of Egyptian radish (*Raphanus sativus*) and clover (*Trifolium alexandrinum*), sprouts (ERS and ECS) on diabetic animals. To clarify the effects, the influence of ERS and ECS on blood sugar and lipid metabolisms was investigated in normal and streptozotocin-induced (STZ) diabetic rats. Rats were fed a semi-modified diet containing 10% of ERS or ECS *ad-libitum* for 6 weeks. Diabetic rats showed significant increase in the levels of blood glucose, triglycerides (TG), total cholesterol, (TC), LDL-c; and VLDL-c. The addition of ERS or ECS at 10% to diabetic rats diet as semi-modified diet resulted in significant decrease in blood glucose, TG, TC, LDL-c and VLDL-c without changes in the HDL-c parameter. Histopathological examination of pancreas tissues revealed significant changes attributable to the STZ- induced diabetic rats, while ERS and ECS semi-modified diet rats showed recovery of STZ effects on pancreas tissues. These results showed that ERS and ECS had a hypoglycemic activity in diabetic rats and partly improved lipid metabolism in the experimental animals. ERS and ECS have the potential to alleviate hyperglycemia in cases where diabetes is present and to serve in the primary prevention of diabetes mellitus. Also ERS and ECS semi-modified diet appears to be essentially and non-toxic in doses given to rats in this study over 6 weeks period.

Key words: Egyptian Radish · Clover · Sprouts · Streptozotocin · Diabetic · Lipid Metabolism · Cholesterol · Pancreatic Histopathology

INTODUCTION

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells don't respond to the insulin that is produced. Due to high blood sugar this produces the classical symptoms of polyuria (frequent urination), polydipsio (increased thirst) and polyphagio (increased hunger) [1].

Injection of 60 mg/kg dose of STZ into adult Wister rats induced experimental diabetes mellitus in the 2-4 days [2-4]. In diabetes mellitus, the abnormalities of carbohydrate, lipid and protein metabolism are the result of the insufficient presence of or a response to insulin in the peripheral tissues [5]. In carbohydrate metabolism, the role of insulin is to stimulate the disposal of ingested glucose into skeletal muscle and adipose tissue and to reduce hepatic glucose production by decreasing gluconeogenesis and glycogenolysis [6]. In lipid metabolism, insulin suppresses the release of free fatty acids from adipose tissue by inhibiting lipolysis [6].

Hence, the insufficient presence of or a response to insulin causes clinical diabetic conditions such as hyperglycemia. In addition, chronic hyperglycemia is a major initiator of diabetic microvascular complications including retinopathy, neuropathy and nephropathy [7]. Natural products have been reported to have beneficial effects on diabetes mellitus. For instance, green tea has been reported to decrease the plasma levels of blood glucose and insulin in rats because of the increase of insulin sensitivity caused by green tea antioxidant (polyphenol) [8]. Vitamin E was also reported to improve insulin sensitivity in rats fed a high fructose diet [9]. Takaya *et al*. [10] reported that a methanol extract of Japanese radish sprout (JRS) had a higher scavenger activity than L-ascorbic acid and that antioxidants including flavonoids and sinapinic acid esters were responsible for the activity. Indeed, it was shown that flavonoids in some natural products had hypoglycemic effects [11]. Amer *et al*. [12] also suggested that flavonoids in extracts from *Trifolium alexandrinum* might result in a hypoglycemic effect in STZ-induced diabetic

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rats. Antioxidants, such as flavonoids, in JRS improved insulin sensitivity and thereby induced the hypoglycemic effect [13].

Concerning seed sprout effects, dietary broccoli sprout at 10g per day (4 weeks) improved insulin resistance among type 2 diabetic human patients by significantly improved blood lipid profile, decreased blood serum triglycerides and increased HDL-c concentration among human patients [14]. White radish sprout improved plasma metabolites and lipid metabolism [15, 13]. Chickpea sprouts improved lipid metabolism [16]. Five weeks dietary intake of mung bean sprout lowered blood glucose, cholesterol and triglyccride [17]. Wheat sprout is therapeutic for diabetes mellitus because wheat sprouts stimulate insulin secretion [18].

Many authors reported that all lipid parameters expect HDL-c significantly elevated in diabetic rat as compared to normal control rat [19-22]. Diabetes caused lipid peroxidation that mediated tissues damage in pancreas [23, 22].

The present study investigated the influence of organic ERS and ECS as semi-modified diet on blood sugar and lipid metabolites as well as histopathological effects on pancreas in normal and STZ-induced diabetic rats.

MATERIALS AND METHODS

Materials: Seeds of Egyptian radish and Egyptian clover were obtained from private farm in Kalubia Governorate. The seeds were cleaned from all impurities for sprouting using the methods described by Abdallah [24]. The harvested sprouts were washed and sun-air dried for 3 days according to Dzowela *et al*. [25] before crushed into powder for rat diet. Streptozotocin (STZ) was obtained from Biomedicals, LLC.

Animals and Diets: Male Wister rats {obtained from Egyptian Organization of Biological Products and Vaccines} were individually housed in cages in animal experimental room. They were allowed free access to water and standard diet for 7 days for acclimazation, half of the rats were injected intraperitoneally with STZ (60 mg/kg body weight) dissolved in 0.09 M citrate buffer (pH 4.5) to induce a diabetic status according to Aly-Tahany [4]. One week after the injection, hyperglycemia was confirmed with a blood glucose test. Only rats with at least blood glucose values of 350 mg/dl or above were used in this study. After the acclimatization, the normal and diabetic rats were each divided into three group (n=6/group) and fed diets containing 0.0 and 10% of Egyptian radish sprout (ERS) or 10% of Egyptian clover sprout (ECS) at the expense of cornstarch *ad-libitum* according to Kumar *et al.* [26] for 6 weeks. After that, blood and the pancreas were collected from anesthetized rats that had been deprived of food for overnight. The food and water intake and body weight of individual rats were also recorded throughout the experiment.

Analytical Methods: The serum levels triglyceride (TG) total cholesterol (TC). Low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol(HDL-c), were measured with an automatic analyzer using a diagnostic kit for each according to Fossati *et al*. [27];Allain *et al*. [28]; Wieland *et al*. [29] and Burstein *et al*. [30] respectively. While glucose was measured according to Hugget and Nixon [31]. Very low-density lipoprotein-cholesterol (VLDL-c). by subdivision (TG/5).

Histopathological Examination of Pancreas: Sample of rat pancreas was immersed in 10% formalin until fixed in Bouins fixative embedded in paraffin wax. Detailed microscopic examination for pancreas sample section (5µm) was carried out on all pancreas of each group according to Carleton *et al*. [32].

Statistical Analysis: Data were statistically analyzed as Complete Randomize Design (ANOVA) according to Snedecor and Cochran [33] using package for social science SPSS [34] program version17.0.0. SPSS Inc., Chicago, Significant differences between the groups (treatments) were evaluated using Duncan's multiple range test according to Waller and Duncan [35].

RESULTS AND DISCUSIONS

There have been many reports on the beneficial effects of natural products in addition to Japanese radish sprouts (JRS) on diabetes mellitus but no information is available on the antidiabetic effect for Egyptian radish sprout (ERS) and clover sprout (ECS).Although, Egyptian radish and Egyptian clover were first cultivated thousands of years ago in Egypt before pyramid, building [36, 37].

The present study investigated the effect of ERS and ECS on blood sugar and lipid metabolites in normal and STZ-treated rats, which become insulin-penically diabetic because of the destruction of pancreatic β-cells [38]. Experimental animals showed changes in the body weight gain in the normal and diabetic rats fed semi-modified diets ERS and ECS, as reported by Aly Tahany *et al*. [4].

Table 1: Effect of ERS and ECS semi-modified diets on blood glucose and daily food, water intake and body weight (BW) of experimental normal and STZ-diabetic albino rats

Treatments (Rat's Group)	Food intake (g/24 h)	%	Water intake (ml/24 h)	%	Fasting Blood Glucose (mg/dl)	%	B.W (g)
G1-normal control	19.0 c	100	30.7 c	100	141.5 b	100	274.3 a
G2-diabetic control(+STZ)	21.0 a	111	93.6 a	305	543.5 a	384	204.5 b
G3- normal ERS	20.8 ab	109	29.7 c	97	141.0 b	100	270.0 a
G4- diabetic ERS(+STZ)	19.3 c	102	50.9 b	166	171.7 b	121	281.2 a
G5-normal ECS	19.7 c	104	32.8 c	107	142.3 b	101	301.3 a
G6- diabetic ECS(+STZ)	19.9 bc	105	56.7 b	185	177.7 b	126	296.8 a

Means in each column followed by the same letter (s) are not significantly different at the 5% level.

In the normal rats, ERS and ECS intake did not change the body weight throughout the present experiment. In the diabetic rats, ERS and ECS intake increased significantly the body weight throughout these experiments. The body weight increment was about 37.5% and 45.1% respectively for ERS and ECS groups more than STZ-diabetic control (Table 1) Also, normal control rats body weight increased about 34.1% more than STZ-diabetic control (Table 1). In addition it had significant influence on food and water intake between the normal control rats(19.0 g/day/rat and 30ml/day/rat) and STZ-diabetic rats (21.0 g/day/rat and 93/ml/day/rat). In the diabetic rats, the feeding of semi-modified diet ERS and ECS decreased significantly food intake (19.3 g and 19.9 g/day/rat respectively) and water intake (51 ml and 56 ml/day/rat respectively) when compared with the STZ diabetic rat (Table 1).

Concerning blood glucose levels, the diabetic rats fed ERS and ECS showed significantly lower levels than corresponding STZ-diabetic control group (92% and 91% reduction respectively).

In the normal rats, ERS and ECS intake did not change the blood glucose level throughout the experimental period. On the other hand STZ-diabetic control increased blood glucose level about 284% compared to the normal control (141mg/dl) at the end of the experiment (Table 1).

However, the increase in blood glucose levels and the effect of the diabetogenic agent streptozotocin, enhance the increase in oxygen free radicals in diabetes [39]. Also previous studies demonstrated that sprouts and radish extracts and their active constituent have proven free radical scavenging by antioxidant activities [40, 10, 41]. The present results showed that ERS and ECS had a hypoglycemic effect in diabetic status.

Table (2) shows the effect of sprouting on ERS and ECS content of sugars, total phenols; and flavonoids. ERS and ECS contained higher total phenols and flavonoids and lower total and non soluble sugar percentage of dry weight basis compared with radish and clover seeds. These results agree with those reported by Takaya *et al*. [10] that Japanese radish sprout had higher flavonoids and *Trifolium alexandrinum* extracts had also higher flavonoids [12].

Based on above mentioned reports and data in table(2), the present study suggested that the mechanism of action by ERS and ECS in decreasing blood glucose level could be related to antioxidant that aid to recover from impaired metabolism of glucose as antiglycemic agents.

These results agree with those obtained by Wu *et al*. [8] and Taniguchil *et al.* [13] who found that green tea polyphenol and Japanese radish sprouts, had been show to decrease the plasma levels glucose and insulin as well as to increase insulin sensitivity in rats. Vitamin E was also reported to improve insulin sensitivity in rats, fed a high-fructose diet [9].

It was also shown that flavonoids in some natural products and radish sprout and Egyptian clover had hypoglycemic effect [11, 10, 12]. Thus there is the possibility that antioxidants (such as flavonoids, phenols and vit. C) in ERS and ECS improved insulin sensitivity and thereby induced the hypoglycemic effect. Another component plausibly responsible for the hypoglycemic effect is dietary fiber [4].

Table (3) shows plasma lipid profile (TC, TG, LDL-c, HDL-c and VLDL-c) in the normal and diabetic rat. Serum levels of TC, TG, LDL-c and VLDL-c, were increased- in STZ induced diabetic control group when compared with normal control. The ERS and ECS semi-modified diets fed STZ- diabetic rats showed lower or approximately equal TC,TG, LDL-c,vLDL-c levels to control group (in the same table). Percentage of recovery in the serum level of LDL-c was about 68% and 95% by feeding on ERS and ECS semi-modified diets respectively in respect to diabetic control. The recovery percentage % of VLDL-c was 80% and 98% for both ERS and ECS semi-modified diets respectively. HDL-c level showed no statistical significant between groups (Table 3).

Table 2: Effect of seed sprouting on chemical analysis of organic Egyptian radish sprout (ERS) and clover sprout (ECS)

		Total phenols (mg/g)	Total flavonoids (mg/g)	Sugars % Total (%)	Soluble (%)	Non-Soluble (%)
Egyptian radish	Seeds	1.04 c	0.09 d	18.33 c	9.84 b	8.49 c
ERS (8 days old)	sprout	1.63 a	0.37 a	17.58 c	9.44 b	8.14 c
Egyptian clover	Seeds	1.43 b	0.13 c	64.36 a	13.04 a	51.32 a
ECS (3 days old)	Sprout	1.65 a	0.16 b	22.67 b	12.72 a	9.95 b

Table 3: Mean values of lipid profile of different experimental animals

Treatments (Rat's Group)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	vLDL-c (mg/dl)
G1-normal control	76.0 bc	48 b	45.2 a	22.5 b	9.6 b
G2-diabetic control(+STZ)	98.2 a	97 b	43.2 a	36.6 a	19.4 a
G3-normal ERS	75.8 bc	51 b	43.5 a	23.4 b	10.2 b
G4-diabetic ERS(+S TZ)	81.8 b	b 58	45.8 a	27.0 b	11.6 b
G5-normal ECS	69.8 c	b 39	43.0 a	20.4 b	7.8 b
G6-diabetic ECS(+STZ)	77.8 bc	49 a	46.2 a	23.1 b	9.8 b

Means in each column followed by the same letter (s) are not significantly different at the 5% level

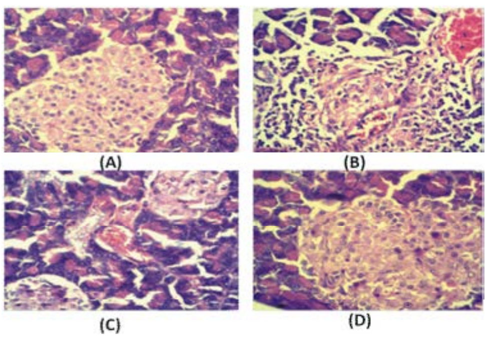

Fig. 1: Histopathological changes of pancreas in various groups

(A) Normal pancreatic section (normal control), (B) Pancreatic section in STZ-diabetic rats (diabetic control), (C) Pancreatic section in diabetic rats feeding by ERS semi-modified diets and (D) Pancreatic section in diabetic rats feeding by ECS semi-modified diets

However the results of increased levels of serum TC,TG, LDL-c and VLDL-c in STZ-induced diabetic rat, are in agreement with those obtained by Ravi *et al.* [19]; Al-logmani and Zari [20] and Alnahdi [21].

The abnormal high concentration of serum lipids in diabetic animals are due mainly to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone- sensitive lipase as reported by Pushparaj *et al.* [42]. Excess fatty acids in serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins [43]. The present study showed that ERS and ECS had favorably modified serum lipid profile in rats with decrease in triglyceride (TG) total cholesterol, (TC), LDL-cholesterol, VLDL-cholesterol. On the other hand, the effect was more with ECS than ERS. From these results, it may be stated that ERS and ECS may be due to the regeneration of β-cells of the pancreas and potentiating of insulin secretion from surviving β-cells. The increase in insulin secretion and consequent decrease in blood glucose level may lead to inhibition of lipid per oxidation and control of lipolytic hormones. This result suggested that ERS and ECS could effectively prevent hypercholesterolemia as lipotropic factors.

Histopathology: Form the histopathological examination of the rat pancreas section under study as shown in Fig. (1), the section of the pancreas of normal rats (normal control) showed normal appearance of langerhans and acinar cells (Fig. 1A). The rats from STZ-induced diabetic (Fig. 1B) group showed necrosis of cells of islets of langerhans with pyknosis of the nucleus of some cells. Also some section showed complete lysis of epithelial lining of islets of langerhans with infiltration of monoclear cells mainly lymphocytes and macrophages. The rats STZ- induced diabetes which fed on diet supplemented with ERS and ECS status, their pancreas sections appeared normal (Fig. 1 C and D respectively).

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