Research in Pharmaceutical Biotechnology Vol. 4(2), pp. 24-29, November 2012
Available online at http://www.academicjournals.org/RPB
DOI: 10.5897/RPB12.002
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Full Length Research Paper

Antioxidant activity of n-butanol extract of celery (*Apium graveolens*) seed in streptozotocin-induced diabetic male rats

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Accepted 20 November, 2012

The potent of n-butanol extract of celery (*Apium graveolens*) seed in ameliorating the lipid peroxidation and antioxidant status were investigated in streptozotocin-induced diabetic rats. Thirty two mature male rats were assigned to four groups, non-diabetic control and three diabetic groups. Diabetes was induced by single injection with streptozotocin (60 mg/kg b.w., *i.p.*). Rats ≥ 200 mg/dl of blood glucose were used as diabetic. Diabetic groups (D, B, and I) were drenched with drinking water, n-butanol extract (60 mg/kg, b.w.), or injected with insulin (4 IU/animal), respectively for 21 days. On day 22, body weight gain was registered and male rats were sacrificed. Blood and liver subcellular fluid was obtained to assess blood glucose level and subcellular activity of Alanine aminotranferease (ALT), Aspartate aminotransferase (AST), catalase, Super oxide dismutase (SOD), Glutathione (GSH)-transferase and -reductase, and assessment of Malondialdehyde (MDA) and glutathione concentrations. Diabetic rats (D) showed marked increased blood glucose, decreased weight gain, increased activity of ALT, SOD, CAT, GSH-transferase, decreased GSH-reductase and normal AST. N-butanol extract of celery seed (B) or insulin (I) therapy moderated blood glucose within normal range, enhanced body weight gain and normalized the activities of all antioxidant enzymes. In conclusion, n-butanol extract of celery seed have potent role in ameliorating stressful complications accompanied by diabetes mellitus.

Key words: Celery, *Apium graveolens*, diabetes mellitus, antioxidants.

INTRODUCTION

Diabetes mellitus is the most important disease involving the endocrine pancreas. Its major manifestations include disordered metabolism and inappropriate hyperglycemia. Recent studies illustrated that uncontrolled hyperglycemia in rats was associated with oxidative stress (Koo et al., 2002, 2003). During diabetes, persistent hyperglycemia increases the production of reducing oxygen species (ROS) through glucose autoxidation (Hunt et al., 1990; Wolff et al., 1991). It is well known that in diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defense system. In addition, there is a relationship between diabetes and between diabetes and impairment of lipid metabolism (Sharpe et al., 1998).

Although almost all organisms possess antioxidant defense and repair systems to protect them from oxidative damage, in some cases, these systems are insufficient to entirely prevent such damage. Currently, there is a trend towards replacement of the widely used synthetic antioxidants with antioxidants from natural sources to extend the shelf life of foods and to improve health conditions.

Flavonoids have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the body from free radicals and against oxidative stress (Bors et al., 1996). Flavonoids cannot be produced by the human body and are taken in through the daily diet. The evidence reported that flavornoids play a vital biological role, including the function of scavenging reactive oxygen species (Pietta and Simonetti, 1998). On the other hand, it has been shown

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that phenolics from edible fruits and vegetables are also effective antioxidants (Karadeniz et al., 2005). The antioxidative properties of phenolics arose from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions (Rice-Evans et al., 1997). Hence, there is a strong interest to search for potential antioxidant agents derived from natural products.

A number of plants have shown a free radical scavenging activity in experimental animals, and one of it is the celery. Celery fruit (seed) extracts are extensively used as flavoring ingredients in many food products, including meat products, soups, frozen dairy desserts, candies, baked goods, gelatins, puddings, condiments and relishes, snack foods, alcoholic and non-alcoholic beverages and others (Momin and Nair, 2001). In the present study, we shall assess the activity of n-butanol extract of celery seed as antioxidant agent.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague-Dawley rats have been used in the experiment. Male rats were allowed one week to acclimatize to the animal house environment before beginning of experiment. Animals were fed on the standard chow and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at 22 ± 2°C, the light-dark cycle was on a 12:12 h with light on at 06:00 a.m and off at 06:00 p.m throughout the experimental period.

Preparation of n-butanol extract

Celery (*A. graveolens*) seed was purchased from the local market and classified by State Board for Seed Testing and Classification, Agriculture Ministry, Iraq (SBSTC). N-butanol extract of celery seed has been prepared from methanolic extract according to Harborne (1984) using Soxhlet apparatus. Using 1 kg of celery seed, methanolic extract was prepared, rotavaporated (40°C and 50 to 60 rpm), and lyophilized by dry freezer. Dried extract was weighted and stored in deep freeze. According to the polarity, three types of solvents have been used to separate different fractions of the crude extract; ethyl acetate, n-butanol, and distilled water, using a separating funnel in order to obtain the high, middle, and low polar fractions of the seed. N-butanol fraction of the celery seed has been evaporated, lyophilized, and kept at -4°C until use (Tsi and Benny, 1999).

Induction of diabetes in rats

Twenty four adult Sprague-Dawley rats weighting 240 to 251 g (56 days old) were used for inducing diabetes. Streptozotocin (STZ) was used to create animal models of type I diabetes (Mansford and Opie, 1968). The animals were injected by STZ (60 mg/kg b.w., i.p.) dissolved in 1 M of sodium citrate buffer (pH 4.5) STZ induces DM within 3 to 5 days by destroying the beta cells of Langerhans islets in the pancreas. The rats with plasma glucose ≥ 200 mg/dL were considered as diabetic rats and used for experiment (Cakatay and Kayali, 2006).

Experimental design

Eight non-diabetic and twenty four STZ-induced diabetic male rats were randomly assigned to 4 equal groups treated for three weeks as follows: non-diabetic control (C): daily drenched with 1 ml of drinking water and injected with 0.1 ml of normal saline; diabetic-control (D): daily drenched with 1 ml of drinking water and injected with 0.1 ml of normal saline; n-butanol treated (B): daily drenched with drinking water contains (60 mg/kg b.w) of n-butanol extract of celery seed and injected with 0.1 ml of normal saline and; insulin treated (I): daily drenched with drinking water and injected with single dose of insulin (4 IU/rat). Twenty four hours after the last treatment, rats were anaesthetized with pentobarbital (35 mg/kg b.w. ip), sacrificed, and liver subcellular fluid were obtained for evaluation of ALT, AST, GSH, SOD, Catalase, GSH-transferase and GSH-reductase.

Preparation of subcellular fluid

Liver tissues were perfused with distilled water until a pink color appeared. Tissues were homogenized by about 20 up and down strokes in a ground-glass tissue grinder. Sucrose (0.88 M) was used for homogenization, washing and re-suspension of the particulate fractions. Using cooled ultracentrifuge, homogenates were fractionated for obtaining subcellular fluid (Ayako and Fridovich, 2002).

Assessment of subcellular ALT and AST enzymes activity

Assessment has been performed by using the colorimetric method of Reitman and Frankel (1957).

Assessment of total GSH

The absorbance of the reduced chromagen was measured at 412 nm and was directly proportional to the GSH concentration (Burtis and Ashwood, 1999).

Assessment of superoxide dismutase (SOD) activity in liver subcellular fluid

By using the modified photochemical Nitroblue tetrazolium (NBT) method in utilizing sodium cyanide as peroxidase inhibitor, SOD levels were assessed (Winterbourn et al., 1975).

Determination of catalase (CAT) activity in liver subcellular fluid

According to Aebi (1974) and Kakkar et al. (1984), CAT activity was assessed by measuring the degradation rate of H_2O_2. The rate of disappearance of H_2O_2 was monitored spectrophotometrically at 230 nm.

Estimation of lipid peroxidation

The level of peroxidation product; Malondialdehyde (MDA) was measured according to Dillard and Kunnert (1982).

Glutathione reductase activity

This was measured by the method of Carlberg and Mannervik

(1975).

Glutathione-transferase activity

This was measured by the method of Habig et al. (1974). Protein was estimated by the method of Lowry et al. (1951).

Statistical analysis

All the values are expressed as mean ± SD. Data of the experiment were analyzed using one way analysis of variance (ANOVA 1), using F-test (Shiefler, 1980). Least significant difference (LSD) was carried out to estimate the significance of difference between individual groups. P value less than 0.05 was considered significant.

RESULTS

Body weight gain

The body weight of the rats at beginning of the study was similar in all groups. At the end of the treatment (after 21 days), all rats gained weight (Figure 1) but non-treated diabetic rats had a significantly ($p < 0.05$) lesser weight gain (20.5 ± 1.56 g) when compared with the rats in other three groups (control: 119.2 ± 4.63 g; B: 88.7 ± 3.55 g; and I: 92.9 ± 3.84 g).

Blood glucose

The blood glucose level of D group was significantly higher ($p < 0.05$) at the end of the experiment as compared with other groups (Figure 2). No significant differences were observed between B and I groups but control rats recorded the significant lesser level among the experimental groups.

Sub-cellular ALT and AST concentrations

As illustrated in Table 1, diabetic animals showed significant increased ($p < 0.05$) subcellular ALT concentration and normalized subcellular AST concentration in liver tissues, while n-butanol fraction of celery seed extract and insulin therapy normalized the two enzymes.

Sub-cellular anti-oxidant activity

Diabetic animals showed marked increased ($p < 0.05$) of MDA and GSH concentrations, and SOD CAT and GSH-transferase activity, and decreased ($p < 0.05$) GSH-reductase activity. N-butanol extract of celery seed and insulin therapy normalized the activities of all antioxidant enzymes (Table 2).

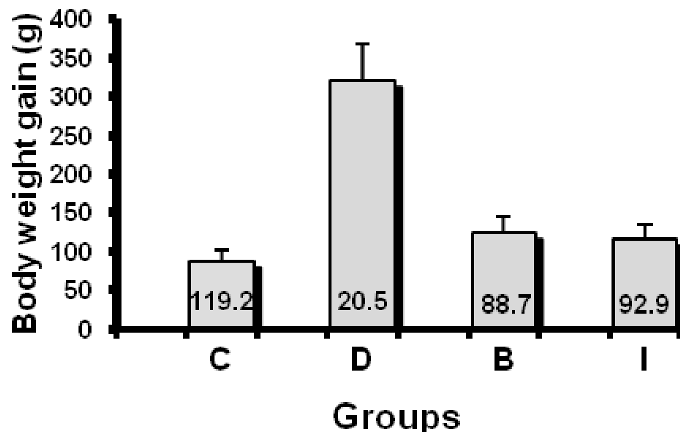

Figure 1. Effect of n-butanol extract of celery seed (*Apium graveolens*) on body weight gain (g) in STZ- induced diabetic mature male rats. C: non-diabetic control, D: diabetic control, B: diabetic treated with n-butanol extract, I: diabetic treated with insulin.

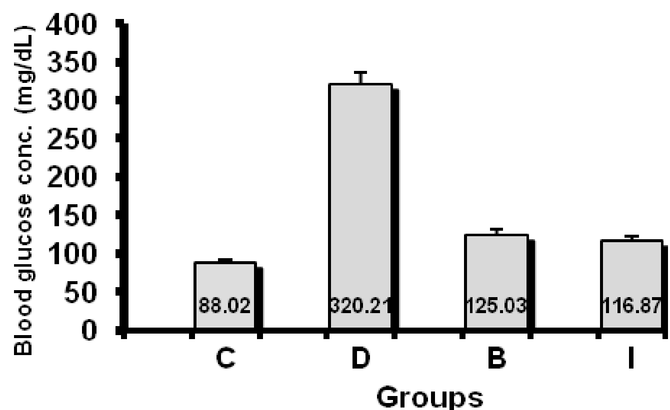

Figure 2. Role of n-butanol extract of celery seed (*Apium graveolens*) on blood glucose concentration in STZ- induced diabetic mature male rats. C: non-diabetic control, D: diabetic control, B: diabetic treated with n-butanol extract, I: diabetic treated with insulin.

DISCUSSION

The present experiment aimed to evaluate the antioxidant activity of n-butanol extract of celery (*A. graveolens*) seed in STZ-induced mature male rats. Since the finding that STZ possesses diabetogenic properties material by pancreatic beta cell destruction, this compound has been widely used to induce diabetes (Junod et al., 1969) and oxidative stress (Wright et al., 1999) in experimental animals. Recent studies evidenced that celery seed extract act as hypolipidemic and antioxidant as it has a scavenger to the free radicals (Cheng et al., 2008). Our results also registered hypoglycaemic effect of n-B extract of celery seed which may be attributed to the strong antioxidant of the phenolic compound in celery

Table 1. Effect of n-butanol extract of celery seed on subcellular concentration of ALT and AST in STZ-induced diabetic mature male rats.

Parameter	Group			
	C	D	B	I
ALT concentration (IU/L)	25.30 ± 3.92^{C}	40.62 ± 4.77^{A}	31.35 ± 5.52^{B}	25.61 ± 4.34^{C}
AST concentration (IU/L)	41.44 ± 4.92^{A}	41.55 ± 5.38^{A}	43.41 ± 13.94^{A}	18.93 ± 5.04^{B}

Numbers represent mean ± SD. Superscript letters represent the presence of significances between groups ($p < 0.05$). C: non-diabetic control, D: diabetic control, B: diabetic treated with n-butanol extract, I: diabetic treated with insulin.

Table 2. Effect of n-butanol extract of celery (*A. graveolens*) seed on serum antioxidants concentrations in STZ-induced diabetic mature male rats.

Parameter	Group			
	C	D	B	I
GSH concentration (nmol/mg protein)	842.2 ± 90.500^{A}	740.3 ± 20.960^{B}	847.6 ± 70.330^{A}	839.9 ± 40.750^{A}
SOD activity (U/L)	4.743 ± 0.205^{B}	6.003 ± 0.313^{A}	4.673 ± 0.398^{B}	5.968 ± 0.425^{A}
CAT activity (U/L)	0.039 ± 0.002^{B}	0.107 ± 0.008^{A}	0.052 ± 0.006^{B}	0.060 ± 0.009^{B}
MDA concentration (nmol/g tissue)	6.331 ± 0.506^{C}	55.898 ± 6.001^{A}	14.376 ± 1.722^{B}	18.187 ± 1.709^{B}
GSH-transferase activity (Unit/mg protein per min)	0.498 ± 0.091^{B}	0.776 ± 0.098^{A}	0.521 ± 0.073^{B}	0.593 ± 0.088^{A}
GSH-reductase activity (µmol of NADPH/mg protein)	49.328 ± 3.779^{A}	41.539 ± 3.281^{B}	43.632 ± 4.718^{B}	45.387 ± 4.899^{B}

Numbers represent mean ± SD. Superscript letters represent the presence of significances between groups ($p < 0.05$). C: non-diabetic control, D: diabetic control, B: diabetic treated with n-butanol extract, I: diabetic treated with insulin.

seed (Singh and Handa, 1995) or it can be suggested that the presence of alkaloids and flavonoids in high concentration in n-B extract of celery seed (Middeton et al., 2000) may be responsible for the oral hypoglycaemic effect registered in the present study.

It has been suggested that the lipid peroxidation may be a link between tissue injury and liver fibrosis by modulatory collagen gene expression (Parola et al., 1993). Our results show increased lipid peroxidation in non-treated diabetic group. The increase in oxygen free radicals in diabetes could be due to hyperglycemia, which upon auto-oxidation generate free radicals. STZ has been shown to produce oxygen free radicals (Ivorra et al., 1989). Lipid peroxide mediated tissue damages have been observed in the development of type I and type 2 diabetes mellitus (Feillet-Coudary et al., 1999). Previous studies have reported that there was an increased lipid peroxidation in liver and kidney of diabetic rats (Pari and Latha, 2002; VenKateswaran and Pari, 2002).

STZ-treated rats registered increased liver subcellular concentration of ALT, whereas n-butanol and insulin-treated rats showed decreased concentrations of this enzymes. On the other hand, the present study revealed that the n-BF of celery seed extract at the given dose has no harmful effect on liver function in normal rats. On the other hand, Tsi and Benny (1999) found that aqueous extract of celery did not produce any undesirable side effects such as weight loss or liver dysfunction as demonstrated by similar levels of subcellular ALT and AST in both the control and treated rats.

The antioxidant defense system, both enzymatic (SOD, CAT, GSH-transferase and GSH-reductase) and non enzymatic (GSH), has been studied here. The increased subcellular GSH

content in liver of rats treated with n-BF and insulin may be one factor responsible for inhibition of lipid peroxidation. GSH, a major non protein thiol, involving many aspects of cellular metabolism and regulation, plays a crucial role in the cellular antioxidant defense system by scavenging free radicals and other reactive oxygen species (Wu et al., 2004). Under *in vivo* conditions, GSH acts as an antioxidant and its decrease was reported in diabetes mellitus (Baynes and Thorpe, 1999). We have observed significant decrease in GSH levels in liver during diabetes (D group). The decrease in GSH levels represent increased utilization due to oxidative stress (Anuradha and Selvam, 1993). The depletion of GSH content of liver may lower the antioxidant activity, as GSH is required as a substrate for this activity (Rathore et al., 2000).

Our results evidenced a parallel increase in both lipid peroxidation as determined by MDA concentration and antioxidant defense system represented by SOD, CAT and GSH-transferase in STZ-induced diabetic rats in response to oxidative stress. These results were reversed with n-BF and insulin treatment to a level comparable to that recorded with control rats. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals. Previous studies have reported that the activity of SOD is low in diabetes mellitus (Vucic et al., 1997). Administration of n-BF increased the activity of these enzymes and may help to control free radical, as n-BF has been reported to be rich in flavonoids, a well known antioxidant (Middleton et al., 2000) which scavenges the free radical generated during diabetes.

CAT is a haem containing ubiquities enzyme, detoxify the hydrogen peroxide into water and oxygen. The elevated level of CAT in diabetic non-treated male rats was reduced and it was improved by the n-BF of celery seed treatment. However, improvement of SOD and CAT activities in the n-BF treatment rats could be due to the restoration of GSH. The result of antioxidant enzymes activity led to suggestions that n-BF of celery seed extract contains free radical scavenging activity which could exert a beneficial action against pathological alteration caused by the presence of free radicals. This action could involve mechanisms related to scavenging activity.

Some studies pointed to the relationship between the elevated xanthin oxidase (XOD) activity and production of oxygen radicals in diabetes (Swei et al., 1998; Desco et al., 2002). XOD inhibitor is known in clinical practice to reduce oxidative stress in diabetes. Thus, it can be said that active constituents of the n-BF of celery seed interact with the peroxy species Cl_3OO, thus reducing the activities of XOD, SOD and CAT enzymes. Our results demonstrated that n-BF of celery seed showed protective effect, probably to the presence of flavonoids. A hepatoprotective effect of celery has been observed in rats treated with paracetamol and thioacetamide (Singh and Handa, 1995). Flavonoids can act in the initiation stage of peroxidation interfering with the metabolism of oxidative agent either by scavenging the free radicals or by impairing the microsomal enzymatic system needed for this metabolism.

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