Antihyperglycaemic and antioxidant effects of broccoli extract, n-butanol in STZ-induced diabetic rats

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ABSTRACT

This research was aimed at investigating the beneficial effects of broccoli (*Brassica olearacea*) on lipid peroxidation (LPO) and the antioxidant status of streptozotocin-induced diabetic rats. Four classes were allocated to thirty-two mature male rats of either non-diabetic and intake integrated feed per 28 days or three diabetic categories also and intake integrated feed per 28 days. Streptozotocin single injection diabetes blood glucose rats obtained 60 mg/kg bw, ip. Diabetic classes (II, III and IV) were drenched for 28 days including water, n-butanol extract and intake integrated feed per 28 days (60 mg/kg bw) and insulin injection (4 IU/animal). Weight gain was observed on day 29 and male rats were sacrificed, contained alanine aminotransferase, aminotransferase aspartate, catalase enzyme, superoxide dismutaseenzyme, glutathione (GSH)-transferaseenzyme, peroxidaseenzyme, reductaseenzyme, melondialdehyde and glutathioneenzyme. Diabetic rats (D) showed increased blood weight gain. There was increased ALT activity, SOD activity, CAT activity, GSH-transferase concentration and GSH-reductase concentration but still normal AST activity function was significantly decreased. Team (III), band (IV) maintained normal blood glucose. There was increased weight gain and regulated most activity of the antioxidant enzymes. N-butanol extract from celery seed also has an important role to play in alleviating complications caused by celery seed.

Keywords: Broccoli; antioxidant capacity; diabetes mellitus; LPO; liver enzyme

INTRODUCTION

Diabetes mellitus is induced by impaired insulin secretion or increased insulin resistance in adipose tissues. The International Diabetes Federation (IDF) recorded (Cai et al 2016) about 425 million people worldwide to have diabetes by 2017 with 693 million predicted to have diabetes by 2045. IGT among adults between the ages of 20 and 79 was 7.3 per cent in 2017 and is expected to reach 8.3 per cent by 2045. In 2017 four million people died of diabetes and its causes accounting for 10.7 per million of all deaths worldwide. Patients with diabetes having multiple symptoms such as increased urinary development, tiredness, weight loss and fatigue would not have a good standard of living like others (Subedi et al 2019). Indeed in 2017 several trillions of dollars were spent on health care for diabetes and its complications worldwide. Diabetes not only poses a serious threat to human health, it also imposes a heavy financial strain on individuals, families and society. Scientific research has focused on hypoglycemia and diabetes prevention and complications. Nutritional therapies can be modified and evolved in many factors affecting diabetes development compared to factors such as genetics (Grosso 2018). Several studies showed that broccoli has major hypoglycemic effects as a recommended whole grain (Alsaedi et al 2019). Bao and others reported in 2014 through 15 randomized trials (from the USA, Canada and Europe) that the daily intake of more than 3 mg of broccoli β-glucan (equivalent to more than 60 g of broccoli) in relation to foods such as wheat would substantially reduce the rate of fasting blood glucose, blood glucose and glycosylated hemoglobin for more than eight weeks (Zhang and Hamauzu 2004). Therefore it shows that other components of broccoli may play a key role in controlling blood glucose. In recent years it has been noted that bioactive peptides have many remarkable physiological components and activities (Gliszczyñska-Swiglo et al 2006). Bioactive peptides have simpler structures compared with proteins, higher stability and

diminished immunogenicity or numerous peptides with hypoglycemic function have been isolated by national and international scholars from natural plants and animals ((Subedi et al 2019). The protein content in broccoli is 10–20 per cent (Chen et al 2020). That is approximately twice as much as rice, wheat and corn. Broccoli protein contains 18 types of amino acids and the essential amino acid composition is relatively balanced and detailed. For example lysine is as high as 0.75 g/100 g which is lower than other grain crops (Grosso 2018). Therefore the broccoli protein is considered a better protein for grain (Casajus et al 2019) and also a raw material for preparations for high quality OOPs. Nevertheless little work on the effect of broccoli oligopeptides on blood glucose is currently under way while the estimation of the micturition frequency in diabetic rats is not yet under investigation. This work focuses on improving the micturition frequency with OOPs in diabetic rats.

MATERIAL and METHODS

Experimental animal: In the experiment mature male rats (Sprague-Dawley) were used. Male rats were accustomed to the environment of the animal house for a week before beginning the experiment. They were fed all and drinking water ad libitum. Room temperature was kept at 23 ± 2°C.

Preparation of n-butanol: Broccoli (*Brassica olearacea*) was taken from the local store and graded by the State Seed Testing and Identification Board. Ministry of Agriculture supplied 1 kg of celery plant, methanol extract dried, rotavaporated (40°C, 50 to 60 rpm) and lyophilized by a dry freezer. The dried extract was measured and put under extreme conditions of congelation. Three forms of polarity dependent solvents were found (Tsi and Tan 2000).

Induction of diabetes in rats: Twenty-four mature rats weighing between 240 and 250 g (56 days old) were selected for diabetes injection (Mansford and Opie 1968). Rats were injected with dissolved STZ (60 mg/kg bw/ip) in 1 M sodium citrate buffer (pH 4.5). STZ induces DM within the Langerhans islet pancreas for around 3 to 5 days by killing beta cells (Kayali et al 2006).

Experimental design: The rats were accordingly divided into five sections. Eighteen animals were fed on each group [Group I: Normal control, rats 28 days, fed on standard animal feed for laboratory use; Group II: Diabetic rats control, received single dose STZ (60 mg/kg bw, ip); Group III: Frequent drenche, rats were anesthetic twenty-four hours after the last pentobarbital injection (35 mg/kg bw, ip) Group IV: 28-day insulin]. Diabetic rats obtained insulin after STZ treatment (4 IU/rat, subcutaneous) and continued for 28 days at a single dose of 60 mg/kg for groups II, III and IV. Three days after STZ diagnosis the development of diabetes was confirmed by measuring blood glucose level, sacrificed and liver sub-cellular fluid ALT, AST, GSH, SOD, GPx, catalase, GSH-transferase and GSH-reductase tests were obtained.

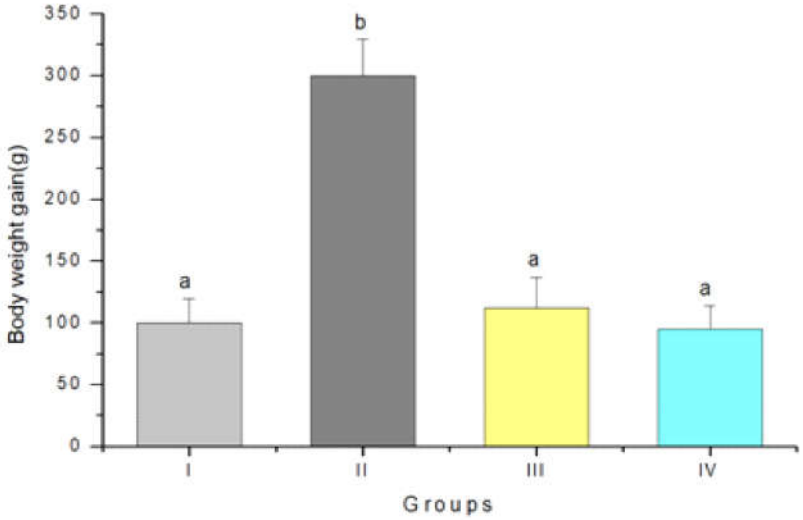
Preparation of sub-cellular fluid: Loaded with distilled water, the liver tissues were perfused to a pink colour and homogenized in a ground-glass tissue grinder by about 20 strokes up and down. Particulate fractions were homogenized, washed and re-suspended with sucrose (0.88 M). Homogenates were solubilized with cooled ultracentrifuge to obtain the sub-cellular fluid (Okado-Matsumoto and Fridovich 2002).

The blood glucose levels were measured using oxidase from glucose (Braham and Trinder 1972); for evaluation of ALT and AST activity, victimisation was performed in the evaluation of quantitative chemical analysis methods (Reitman and Frankel 1957). LPO was determined by the thiobarbituric acid (TBA) reaction of malondialdehyde (MDA) estimated by the method of Dillard et al (1982); GSH by the method of Burtis and Ashwood (1999); SOD activity according to the method of Winterbourn et al (1975); CAT activity by the method of Aebi (1974); GPx activity according to the method of Ren et al (2009); GR evaluation according to the method of Carlberg and Mannervik (1975); glutathione-transferase activity by the method of Lowry et al (1951) and Habig et al (1974).

Statistical analysis: All the clustered data were analyzed using one-way variance analysis followed by multiple range testing of Duncan using the SPSS software package, version 9.05. To each group of 8 rats P-value (<0.05) was considered and included in the analysis as being important.

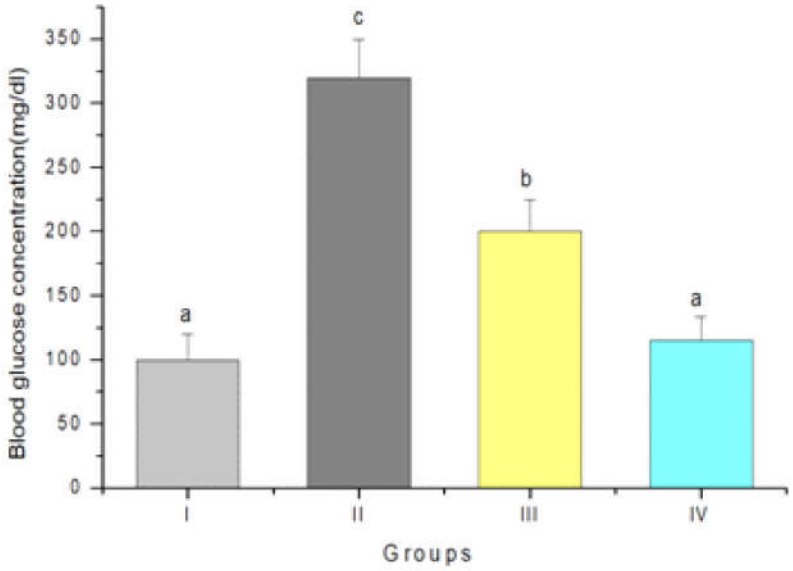
RESULTS

Body weight gain: Rats' body weight at the start of the research was comparable. Untreated diabetic rodents (Group II) had a considerably higher weight gain ($p < 0.05$) (Fig 1) compared to rats in groups I, III and IV.



The mean values are ±SD ($p < 0.05$)

Fig 1. Effect of n-butanol extract from broccoli (*Brassica olearacea*) on bodyweight in STZ-induced mature male rats with diabetics

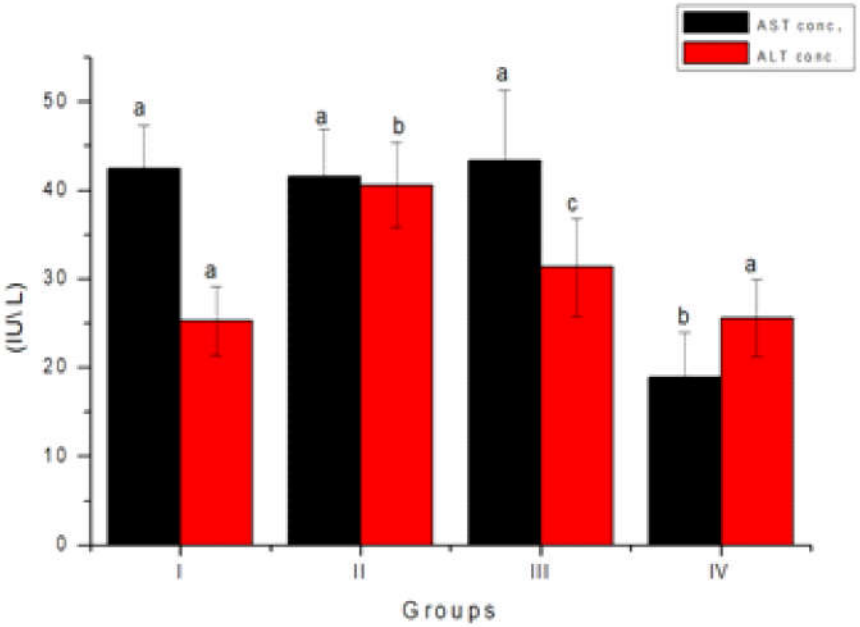


The mean values are ±SD ($p < 0.05$)

Fig 2. Effect of n-butanol extract from broccoli (*Brassica olearacea*) on blood sugar in STZ-induced mature male rats with diabetics

Blood glucose: The blood glucose levels in the II group were significantly higher at the end of the experiment ($p < 0.05$) than in other groups (Fig 2). No big divergences between groups I and IV were found but the group III was significantly lower than the group II.

Sub-cellular ALT and AST concentrations: As shown in Fig 3 in the diabetic community there was a significant increase in ALT concentration ($p < 0.05$) and normalized concentration of AST in the liver tissue while both enzymes were normalized by fractionation of the n-butanol seed extract and insulin therapy.



The mean values are ±SD ($p < 0.05$)

Fig 3. Effect of n-butanol extract from broccoli (*Brassica olearacea*) on sub-cellular concentrations of ALT and AST in STZ-induced mature male rats with diabetics

Table 1. Effect of broccoli (*Brassica olearacea*) extract on levels of serum_antioxidants in mature male rats induced by STZ

Antioxidant capacity parameter	Group			
	I	II	III	IV
GSH concentration (nmol/Mg protein)	891.18 ± 0.57^{a}	751.07 ± 0.67^{b}	811.00 ± 0.96^{a}	812.78 ± 0.71^{a}
MDA (nmol/g tissue)	6.72 ± 0.98^{c}	60.65 ± 0.89^{a}	16.15 ± 0.88^{b}	19.66 ± 1.08^{b}
GST (U/mg Hb protein)	0.5 ± 0.62^{d}	0.78 ± 0.75^{a}	0.46 ± 0.73^{b}	0.44 ± 0.47^{c}
GPx (U/g Hb)	15.28 ± 0.88^{d}	39.27 ± 0.84^{a}	19.5 ± 1.35^{b}	23.62 ± 1.13^{c}
GR (µmol/mg protein)	50.62 ± 0.55^{a}	42.56 ± 1.12^{b}	44.16 ± 0.58^{b}	46.44 ± 0.73^{b}
CAT (U/L tissue)	0.048 ± 0.83^{b}	0.766 ± 0.98^{a}	0.552 ± 1.35^{b}	0.594 ± 0.74^{b}
SOD activity (U/L tissue)	5.703 ± 0.96^{b}	6.098 ± 2.99^{a}	4.942 ± 1.89^{b}	5.941 ± 2.79^{a}

Figures are ±SD means; Figures carrying same alphabet in a column do not differ significantly at 5% level of significance

Anti-oxidant activity: The diabetic group showed significantly increased concentrations of GSH, MDA, SOD, CAT, GPx, GR and GSH-transferase and decreased activity of GSH-reductase ($p < 0.05$). The N-butanol extract and insulin treatment had all regulated antioxidant enzyme activity (Table 1).

DISCUSSION

This research was aimed at assessing the function of antioxidants of *Brassica olearacea* seed extract in male rats caused by STZ dose. The use of this compound is for diabetes (Panhwar et al 2018) and oxidative stress (Junod et al 1969). It has been found that STZ possesses the characteristics of diabetes by destroying beta-pancreatic cells. In the laboratory gaskets recent studies have shown that broccoli seed extract works to lower blood lipids and antioxidants because there are free radicals (Zhang et al 2010). The hypoglycemic effect of broccoli seed extract is due to antioxidants of phenolic compounds in broccoli seeds (Singh and Handa 1995) or it may

suggest high standards of alkaloids and flavonoids in broccoli seed-butanol extract (Haring and Vinck 2000). It has been suggested that strong lipid peroxide by modified collagen gene expression can be linked between tissue injury and cirrhosis (Poli et al 1993). Pure oxygen in diabetes due to hyperglycemia generates free radicals after auto-oxidation of the disease. Oxygen-free radical production was shown by STZ (Ivorra et al 1989). It has been shown that there is an increase in lipid peroxide in the liver of diabetic and kidney in rats (Hussein et al 2012). The rats treated with STZ sub-cellular cell concentration in ALT were able to strengthen themselves while rats group that treated with n-butanol and insulin reduced level of the same enzyme. On the other hand (Venkateswaran and Pari 2002) broccoli extract was found to have no side effects, as shown by sub-cellular ALT and AST activity, nor weight loss or damaged liver function.

The enzymes with antioxidants are SOD, CAT, Gr, GH-transferase and GSH-reductase (Alvarez et al 2019). The content of sub-cellular GSH in the liver of rats treated with n-butanol and insulin associated with lipid fracturing activation of these organsand non-enzymatic (GSH) is studied here. GSH plays an important role in the cellular antioxidant mechanism as there are free radicals and other metabolic reactive oxygen forms (Darre et al 2017). During diabetes a significant reduction in GSH liver was observed (II group). Reduced growth hormone levels in the body are associated with increased use due to reactive oxidative stress (Baynes and Thorpe 1999). GSH concentrations in the liver may reduce antioxidant activity such as GSH as the mainstay of this vital activity (Anuradha and Selvam 1993). The level of MDA concentration SOD, GPx, GR, CAT and GSH-transferase concentration is determined in rat induced by diabetic STZ in response to oxidative pressure in both lipid peroxide. The depletion of GSH in the liver reduces the metabolism of antioxidants such as GSH as a base material for this activity (Winterbourn et al 1975).

The concentration of MDA and the antioxidant protection mechanism imagined by SOD, GR, CAT and GSH-transfer are determined in rat caused by STZ diabetes in response to the oxidative forces in both lipid peroxides. SOD and CAT are main enzymes that contain free radicals from the toxins from it. Previous experiments revealed a low level of diabetes in SOD (Vucic et al 1997).

Absolute intake of broccoli meals has been active in controlling free radicals where n-butanol is rich in flavonoids, an antioxidant known throughout the world (Middleton et al 2000). This free radical scan CAT is an enzyme that converts toxins into hydrogen peroxide in water. The concentration of CAT in malignant species was reduced and SOD could be improved in n-BF treated rat due to improved GSH ratio restored. This intervention may include cellular metabolic processes. Some studies have identified the relationship between the efficacy of high xanthine oxidase (XOD) and oxygen root formation for diabetes (Subedi et al 2019). XOD inhibitors are recognized that reduce oxidative stress in diabetes. XOD inhibitors in oxidative stress in diabetes are known to be reduced in clinical practice. Broccoli-n-butanol crop extract can interact with the active subtypes of Cl3OO making the activity of XOD, SOD and CAT enzymes. For positive results on flavonoids, N-BF broccoli seed extract can be extracted with Cl3OO subtypes making the activity of XOD, SOD and CAT enzymes. In rats treated with thioacetamide in addition to insulin an effect was observed on the liver of broccoli (Bachiega et al 2016). Inner flavonoids can interfere with metabolism by free radical scavenging or by weakening the microenvironment enzyme necessary for this metabolism.

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