

Original Article

Red Cabbage (*Brassica oleracea*) Ameliorates Diabetic Nephropathy in Rats

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The protective action against oxidative stress of red cabbage (*Brassica oleracea*) extract was investigated. Diabetes was induced in male Wistar rats using streptozotocin (60 mg/kg body weight). Throughout the experimental period (60 days), diabetic rats exhibited many symptoms including loss of body weight, hyperglycemia, polyuria, polydipsia, renal enlargement and renal dysfunction. Significant increase in malondialdehyde, a lipid peroxidation marker, was observed in diabetic kidney. This was accompanied by a significant increase in reduced glutathione and superoxide dismutase activity and a decrease in catalase activity and in the total antioxidant capacity of the kidneys. Daily oral ingestion (1 g/kg body weight) of *B. oleracea* extract for 60 days reversed the adverse effect of diabetes in rats. *B. oleracea* extract lowered blood glucose levels and restored renal function and body weight loss. In addition, *B. oleracea* extract attenuated the adverse effect of diabetes on malondialdehyde, glutathione and superoxide dismutase activity as well as catalase activity and total antioxidant capacity of diabetic kidneys. In conclusion, the antioxidant and antihyperglycemic properties of *B. oleracea* extract may offer a potential therapeutic source for the treatment of diabetes.

Keywords: *Brassica oleracea*–diabetes–diabetic nephropathy–oxidative stress–red cabbage

Introduction

Microvascular complications of diabetes share a common pathophysiology that may be explained as a direct or indirect consequence of hyperglycemia-mediated overproduction of reactive oxygen species (ROS). Microvascular deterioration is preventable either by the inhibition of superoxide accumulation or by modulating the blood glucose levels, and among several microvascular disorders, nephropathy (1,2) can be improved by antioxidants (3,4). The antioxidant protection of natural plants is a promising therapeutic remedy for free radical pathologies (5). Among myriad natural plants, red cabbage (RC) (*Brassica oleracea var capitata*) and other *Brassica* vegetables, vegetables endemic to the Mediterranean region, have been found to have

antioxidant, antihyperglycemic (6–8), anticancer (9–11) and hypocholesterolemic (12) properties. RC extract has also prevented oxidative stress induced in livers and brains of animals exposed to paraquat (13) and *N*-methyl-D-aspartate (14).

The principle constituents of RC are isothiocyanates (glucosinolate), vitamins A, B, C and anthocyanins (11,15,16). Anthocyanins, a group of phenolic natural pigments present in RC, were found to have the strongest antioxidizing power of 150 flavonoids (17). Our objective was to investigate RC polar extract for antioxidant scavenging enzymes and oxidative stress in the kidney of streptozotocin-induced diabetic rats.

Methods

Chemicals

Fresh RCs were purchased from a local market at Al Ain, UAE. Reagent kits for urea and creatinine were

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acquired from BioMerieux (RCS Lyon, France). Thiobarbituric acid, reduced glutathione, 5,5-dithiobis (2-nitrobenzoic acid), Folin's reagent, epinephrine, superoxide dismutase (SOD) enzyme, H₂O₂, Streptozotocin (STZ) and bovine albumin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other standard chemicals came from common commercial suppliers.

Albino Rats

Male Wistar rats (130–180 g) were obtained from the Animal House at the Faculty of Medicine and Health Sciences, UAE-University, UAE. Rats were maintained on standard pellet diet and tap water *ad libitum*. They were kept in plastic cages under a 12 h light/dark cycle and room temperature 22–24°C and were acclimatized to the environment for 2 weeks prior to experimental use. Procedures for the care and use of research animals at UAE meet or exceed all applicable local, national and international laws and regulations.

Preparation of Red Cabbage Extract

RC leaves were sliced into small pieces and oven-dried at 50°C. Dried plants (800 g) were extracted in 8000 ml of 70% aqueous ethanol using ultrasonic treatment at an intensity of 70 W/cm² and oscillation frequency at 20 kHz for 5 min. The use of an ultrasound extraction method has been shown to diminish the danger of thermal degradation (18). Ultrasound also provides a greater penetration of solvent into cellular materials, via cavitation, and improves the release of cell contents into the bulk medium (18). Ultrasonic irradiation was applied by means of a Branson 450 digital sonifier (20 kHz, 450 W) equipped with a cylindrical titanium alloy probe (12.70 mm in diameter). The use of dry plants can be effective to minimize enzymatic degradation of phenolic compounds inside plant tissues. After overnight maceration, the extract was filtered through gauze and ethanol was evaporated under reduced pressure at 50°C by using a rotary evaporator. The remaining water extract was dried using a freeze dry system under reduced pressure. The dried extract was dissolved in distilled water to a concentration of 1 g/ml before administration to normal and diabetic rats. The extraction yield for RC was about 15%.

Induction of Experimental Diabetes

Diabetes was induced by injection of a single intraperitoneal dose of STZ (freshly prepared in 0.01 M citrate buffer, pH 4.5) as described previously (19). The control rats were only injected with citrate buffer. Diabetes was identified by polydipsia, polyuria (visual observations) and measuring blood glucose concentration 72 h after injection of STZ. Rats with a fasting blood glucose level

above 200 mg/dl were considered diabetic and were used in this study.

Experimental Groups

RC extract was dissolved in distilled water and given orally by gavage at a dose of 1 g/kg body weight and volume 5 ml/kg body weight. The daily dose of RC (1 g/kg body weight) was based on that previously shown to be effective in preventing the oxidative stress induced by another toxic agent in animals (14). Non-diabetic control rats as well as diabetic rats were each randomly assigned to two groups that ingested either vehicle or RC extracts at 1 g/kg body weight/day for 8 weeks, respectively. At the end of the experimental period, rats were anesthetized with diethyl ether after fasting for 12 h and blood and kidney samples were collected.

Sample Collection

Blood Sampling

For daily glucose analysis, a drop of blood was collected from the tail. Serum was extracted from blood collected from retro-orbital plexus (20) after rats were subject to light anesthesia. Serum urea and creatinine determined in fresh serum were indicators of kidney function.

Collection of Kidneys

At the end of the experimental period, rats were weighed and sacrificed. Kidneys were removed, weighed and kidney/body weight ratio was calculated. Data were expressed as relative organ weight of one kidney to 100 g of total body weight. The other kidney was excised and homogenized (1 g/10 ml ice-cold potassium chloride, 150 mM). The homogenate was then used for determination of the levels of reduced glutathione (GSH), lipid peroxidation (LP) product Malondialdehyde (MDA), total proteins and activities of SOD and catalase (CAT).

Biochemical Assays

Blood Glucose

The blood glucose level was determined by glucose oxidase method using a one touch basic plus glucometer (Lifescan Ltd., California, USA).

Reduced Glutathione

GSH content in the kidney homogenate was determined using the method described by Van Dooran *et al.* (21). The basis of the GSH determination method is the reaction of Ellman's reagent 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) with thiol group of

GSH at pH 8.0 to give yellow color of 5-thiol-2-nitrobenzoate anion.

Malondialdehyde

MDA is the most abundant individual aldehyde resulting from LP breakdown in biological systems. It is used as an indirect index of LP. The determination of MDA in biological materials, as described in Uchiyama and Mihara (22), is based on its reaction with thiobarbituric acid (TBA) to form a pink complex with absorption maximum at 535 nm.

Catalase and Superoxide Dismutase Enzymes

The activity of SOD enzyme in kidney homogenate was determined according to the method described by Sun and Zigman (23). This method is based on the ability of SOD to inhibit the auto-oxidation of epinephrine at alkaline pH to adrenochrome and other derivatives, which are easily monitored in the near-UV region of the absorption spectrum. CAT activity was determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed as units/mg of protein as described by Aebi (24).

Total Antioxidant Capacity

The total antioxidant capacity in the kidneys was evaluated using ferric reducing antioxidant power (FRAP) assay. The FRAP assay was determined as described by Benzie and Strain (25) and as developed in our laboratory. Briefly, the FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored ferrous-tripyridyltriazine complex from colorless oxidized ferric form by the action of electron donating antioxidants.

Creatinine and Urea

Creatinine and urea levels were determined in serum according to the method described by Houot (26) and Fawcett and Scott (27), respectively. The assay was performed using BioMerieux reagent kits. The total protein content in kidney was determined according to the Lowry's method and as modified by Peterson (28). Absorbance was recorded using a Shimadzu recording spectrophotometer UV-160A.

Statistical Analysis

The statistical analysis was carried out using SPSS version 10 statistical programs (SPSS Inc., Chicago, IL, USA). Differences among treatments within an experiment were analyzed by one-way analysis of variance (ANOVA). Significant differences between treatment means were determined by Dunnett *t*-test. The results are presented as mean \pm SEM of five independent experiments unless stated otherwise.

Results

Red Cabbage Ingestion Attenuated Symptoms of Diabetes

Intraperitoneal administration of STZ (60 mg/kg body weight) induced all characteristic symptoms of diabetes such as increased food and water intake (judged by the amount of food and water consumed per day), polyuria (data are not shown), failure to gain weight (Fig. 1). These symptoms were evident throughout the entire experimental period of 60 days. After 72 h of STZ injections, fasting blood glucose concentrations were increased as compared with non-diabetic control rats (Fig. 2). Blood glucose levels above 200 mg/dl were considered diabetic. Diabetic rats that daily ingested polar extract of RC (1 g/kg body weight) showed improvement in diabetic polyuria that almost disappeared by the end of the experimental period. Food and water consumption also declined when diabetic rats ingested

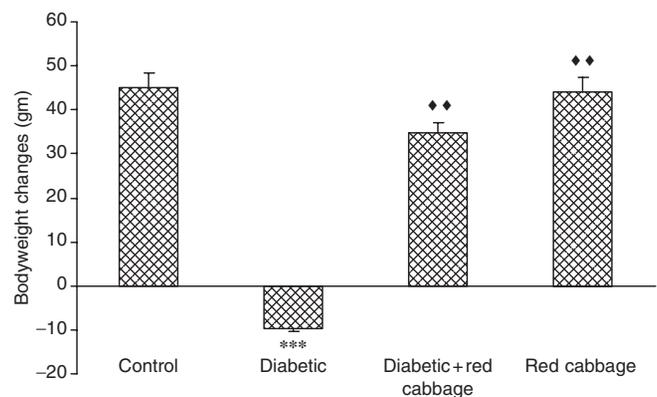


Figure 1. Body weight changes in rats that did not ingest (Control) or ingested RC extract (RC), and STZ-induced diabetes in rats that did not ingest (Diabetic) or ingested RC extract (Diabetic+RC), *** $P < 0.001$ as compared with control, ♦♦ $P < 0.01$ as compared with diabetic.

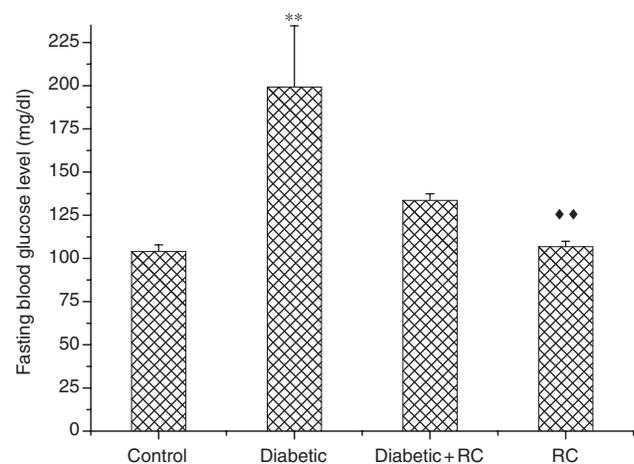


Figure 2. Fasting blood glucose levels, ** $P < 0.01$ as compared to control, ♦♦ $P < 0.01$ as compared with diabetic.

RC extract. RC extract also abridged the weight gain loss in diabetic rats (Fig. 1) as well as blood glucose levels (Fig. 2) at the end of the experimental period. Rats that ingested RC extract showed no significant changes as compared with control rats in any of the parameters considered.

Symptoms of Nephropathy Ameliorated in Diabetic Rats

At the end of the experimental period (60 days), diabetic rats exhibited significant ($P < 0.001$) renal enlargement as compared with non-diabetic control rats (Fig. 3). In diabetic rats, elevation in serum urea (Fig. 4) and serum creatinine concentrations (Fig. 5) indicated impairment in kidney function. Kidney nephropathy was improved when RC extract was ingested. Fig. 3 shows that kidney weight in diabetic rats that ingested RC extract was not different from control rats

($P > 0.05$). In addition, serum urea (Fig. 4) as well as serum creatinine (Fig. 5) concentrations were ameliorated with ingestion of RC extract.

Increase in Renal Malondialdehyde Abolished in Diabetic Rats

Severe LP in diabetic kidneys implicated oxidative stress. Fig. 6 indicates an increase ($P < 0.001$) in MDA concentrations. This increase was abolished when diabetic rats ingested RC extract for 60 days.

Red Cabbage Modulated Change in Renal Antioxidants

Total antioxidant capacity in kidney tissues was analyzed using FRAP assay. Kidneys of diabetic rats showed reduction ($P < 0.05$) in antioxidant capacity in comparison with control rats (Fig. 7).

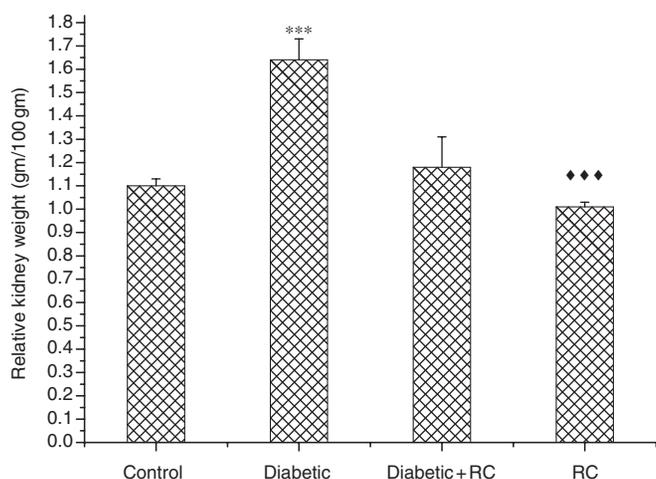


Figure 3. Relative kidney weights. *** $P < 0.001$, as compared with control, *** $P < 0.001$ as compared with diabetic.

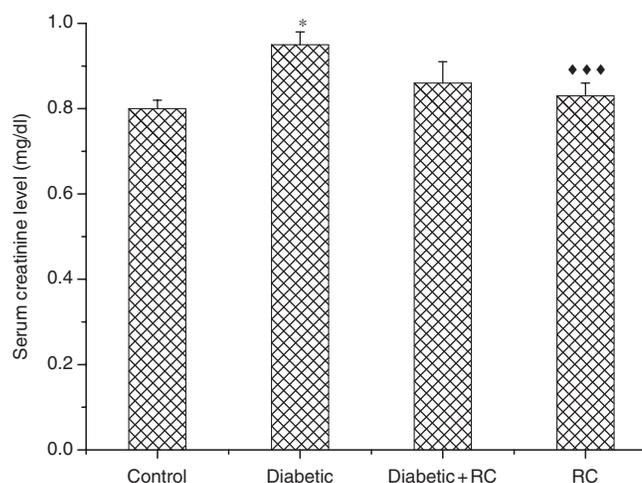


Figure 5. Serum creatinine concentrations, * $P < 0.05$, as compared with control, *** $P < 0.001$ as compared with diabetic.

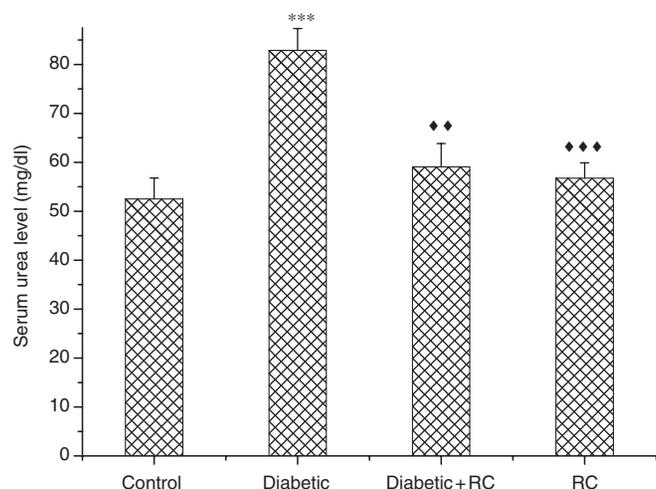


Figure 4. Serum urea concentrations. *** $P < 0.001$, as compared with control, ** $P < 0.01$; *** $P < 0.001$ as compared with diabetic.

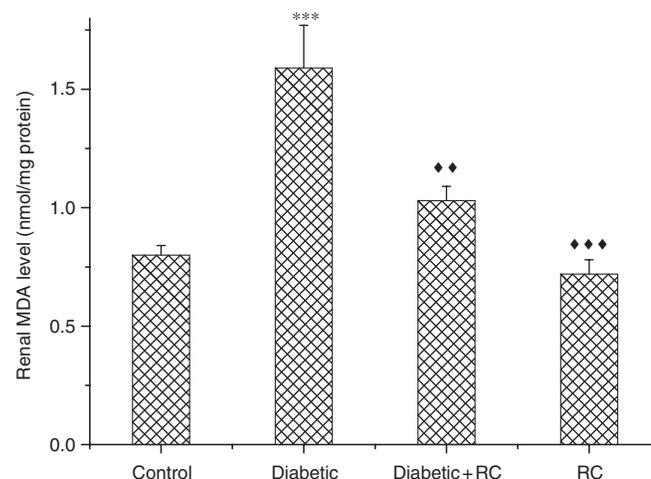


Figure 6. Renal level of MDA, *** $P < 0.001$, as compared with control, ** $P < 0.01$; *** $P < 0.001$ as compared with diabetic.

To understand the effect of RC extract on antioxidant eminence, we measured the activities of enzymic antioxidants SOD and CAT and non-enzymic antioxidant GSH. SOD activity showed an increase ($P < 0.05$) at the end of experimental period (Fig. 8). Meanwhile, a significant decline ($P < 0.01$) in CAT activity was observed in diabetic rats (Fig. 9). GSH activity increased ($P < 0.001$) in diabetic rats (Fig. 10). Both enzymic and non-enzymic antioxidants were ameliorated after ingesting RC extract.

Discussion

Tight control of blood glucose can reduce clinical complications in diabetic patients; however, alternative treatment strategies are needed to prevent oxidative stress complications and to optimize recovery (1). It is well documented that modulation of oxidative stress through treatment with antioxidants can effectively reduce diabetic snag. Oxidative stress induced by hyperglycemia in

diabetics is a major cause for development and progression of diabetic microvascular complications such as nephropathy (1,2).

We investigated the therapeutic value of RC against diabetic nephropathy. These plants, members of the *Cruciferae* and genus *Brassica* such as RC, broccoli, cauliflower, kale and Brussel sprouts, contain anthocyanin pigments that are described as free-radical scavenging and antioxidant agents (11,15,16). Anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoid provide protection against myriad physiological failures such as LP, decreasing capillary permeability and fragility and membrane strengthening (39). RC extract contains vitamins A, B and C (11,15,16), all of which have protective roles against oxidative damage.

Measuring FRAP in kidney tissues revealed that anti-oxidant potency of the STZ-diabetic kidneys was significantly reduced, indicating oxidative stress and

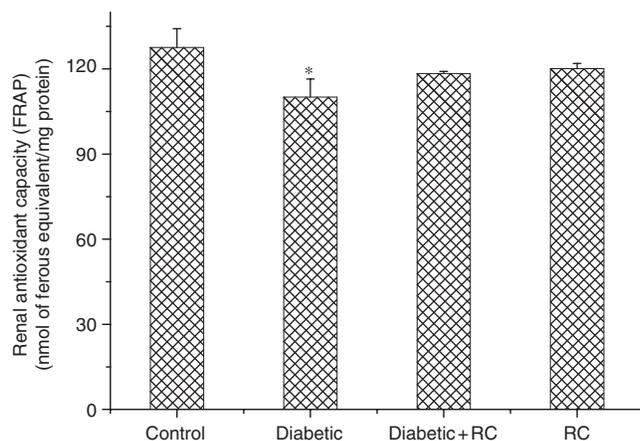


Figure 7. Kidney antioxidant capacity. * $P < 0.05$, as compared with control.

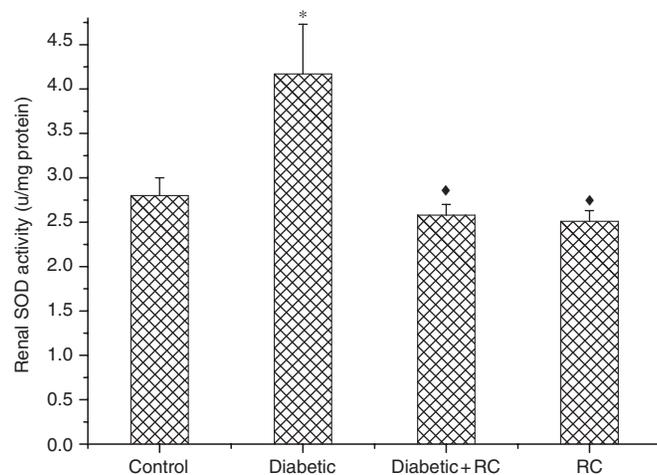


Figure 8. Kidney SOD activity. * $P < 0.05$, as compared with control, ♦ $P < 0.05$, as compared with diabetic.

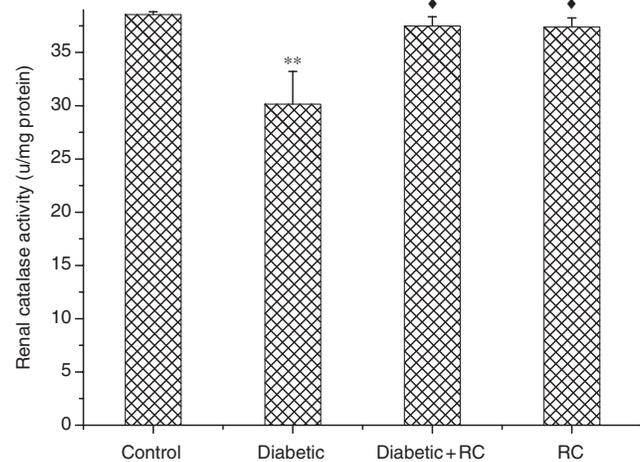


Figure 9. Kidney CAT activity. ** $P < 0.01$, as compared with control, ♦ $P < 0.05$, as compared with diabetic.

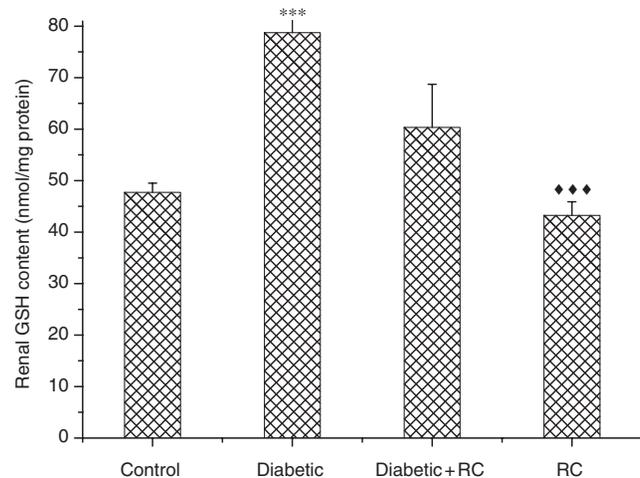


Figure 10. Kidney GSH concentrations. *** $P < 0.001$, as compared with control, ♦♦♦ $P < 0.001$ as compared with diabetic.

production of ROS. Generation of ROS by diabetic hyperglycemia is evident through many biochemical pathways such as glucose autooxidation and protein glycation (1,29). This oxidative stress was further assessed by the increase in renal MDA as an LP marker (22). These data confirmed previously published reports of a state of oxidative stress in diabetics (30–32). Our results, renal MDA concentrations correlated with blood hyperglycemia, which is in agreement with Ha *et al.* (33) who found that elevated glucose level *per se*, could cause oxidative stress in isolated rat glomeruli *in vitro*.

STZ-diabetic rats showed symptoms of renal nephropathy such as kidney hypertrophy that were attributed to an increase in blood glucose and polyuria. In addition, STZ-diabetic rats showed elevated serum urea and creatinine, as in previous reports (7,19,34). RC polar extract prevented renal enlargement and attenuated polyuria. Meanwhile, elevated serum urea and creatinine in STZ-diabetic rats were also normalized to control values after 60 days with ingestion of RC polar extract. This was consistent with other reports using chemical antioxidants (35,36), and diet of natural antioxidant plants (4,7,34) to normalize nephropathy in STZ-diabetic rats. Although STZ-diabetic rats showed elevated serum urea and creatinine and kidney hypertrophy, the measurement of other indicators of nephropathy such as glomerular filtration rate and histopathology is needed to further investigate our diabetic model.

Diabetic rats had elevated levels of enzymic antioxidants SOD, reduced CAT and increased concentrations of non-enzymic antioxidant GSH. RC extract ameliorated these changes. These elevations in GSH level and SOD activity may be compensatory mechanisms for the chronic overproduction of free radicals and oxidative stress. The upregulation of SOD and GSH in diabetic rats is consistent with a number of reports (35,38,39). Others, however, reported a reduction in both SOD and GSH levels (36,37). These discrepancies may be due to the nature of the tissue used, experimental period, the STZ dose and the severity of diabetic state as well as oxidative stress. The protective qualities of RC are quite consistent with its ability to protect against oxidative damage induced by toxic agents in other tissues of animals (13,14).

Other investigations have shown that acylated anthocyanins isolated from RC protect against paraquat-induced oxidative stress in rats (13). Our data showed that acylated anthocyanins were effective antioxidants against hepatic LP elevation and CAT depletion induced by paraquat in rats. Several vegetable aqueous extracts including *Brassica* vegetables such as white cabbage, RC, green mustard leaf, red mustard leaf, white turnip and red turnip were examined for their neuroprotective action against oxidative stress induced by *N*-methyl-D-aspartate in brains of mice (14). Only RC showed a markedly restored glutathione levels and prevented LP induced by

N-methyl-D-aspartate in the brains of mice at a dose of 1 gm/kg body weight. RC polar extract was more protective than white cabbage against amyloid protein induced neuronal damage (40). These data indicate that the neuronal cell protective capacities of RC might be due to high total phenolic contents and antioxidative activity. Among the three different cultivated forms of cabbage, RC has higher Vitamin C (24.38 mg/100 g), DL- α -tocopherol (0.261 mg/100 g) and phenolic content (101.30 mg/100 g) as compared with white and Savoy cabbage (16), all of which protect against oxidative damage.

RC and all other edible plants belonging to the family *Cruciferae* and genus *Brassica* (e.g. broccoli and cauliflower) contain substantial quantities of isothiocyanates (mostly in the form of their glucosinolate precursors) some of which (e.g. sulforaphane or 4-methylsulfinylbutyl isothiocyanate) are very potent antioxidants (11,15,16). Anthocyanin, which is a phenolic natural pigment present in RC, was demonstrated to have the antioxidizing power of 150 flavonoids (17). We report that RC polar extract has significant antihyperglycemic activity that may, at least in part, modulate the oxidative stress caused by hyperglycemia-induced generation free radicals. Natural health products of vegetable origin have shown promise for the prevention of chronic diseases (41).

Daily ingestion of RC polar extract (1 g/kg body weight) ameliorates oxidative stress and ameliorates diabetic nephropathy. The dose response of RC for the pharmacological benefits observed here is still to be determined and further study is needed to see whether a higher dose and different routes of administration can protect against diabetic nephropathy.

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