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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Beneficial Effects of Rutin and Vitamin C Coadministration in a Streptozotocin-Induced Diabetes Rat Model of Kidney Nephrotoxicity

Mohammed A. Alsaif

Department of Community Health Sciences, College of Applied Medical Sciences,
King Saud University, Riyadh, Saudi Arabia

Abstract: The aim of this study was to examine the possible antinephrotic activity of rutin (vitamin P) and vitamin C in kidney of streptozotocin (STZ)-induced diabetic rats. Oral administration on rutin (100 mg/kg), vitamin C (200 mg/day) and their combination (50 and 100 mg/kg) for 5 weeks on body and kidney weights and the levels of serum glucose, insulin and creatinine in normal and STZ-induced diabetic rats were evaluated. Reactive Oxygen Species (ROS), Malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG) concentrations and Glutathione Peroxide (GPx), Superoxide Dismutase (SOD) and Catalase (CAT) activities were estimated in kidney. Body weights decreased and kidney weights increased significantly ($p < 0.001$) in diabetic rats and those changes were more significantly reduced in the combined treatment group. Abnormal levels of glucose and insulin in diabetic rats were more normalized in the coadministered group. ROS and MDA concentrations were significantly ($p < 0.001$) increased in diabetic rats. The treatments with rutin, vitamin C and their combination to the diabetic rats significantly decreased the elevated ROS and MDA levels in kidney compared to diabetic control rats. The ratio of GSH/GSSG was significantly reduced in diabetic rats and these changes were rectified significantly ($p < 0.001$) by the combined vitamins treatments. GPx and CAT were decreased while SOD was increased in diabetic rats, however, these activities were bring back to normal in combined treated animals. In conclusion, long-term treatment with these vitamins particularly in combination as diabetic maintenance therapy may consequently control or prevent the development of diabetic complications especially diabetic nephropathy.

Key words: Streptozotocin, diabetes, kidney, oxidative stress, rutin, vitamin C

INTRODUCTION

Diabetes Mellitus (DM), characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves and blood vessels, is the most common endocrine disorder. Although the underlying mechanism of diabetic complications remains unclear, much attention has been focused on the role of oxidative stress. It has been suggested that oxidative stress may contribute to the pathogenesis of different diabetic complications (Ceriello, 2000). Diabetic experimental animal models have shown that oxidative stress causes persistent and chronic hyperglycemia, thereby depleting activities of the antioxidant defense system and otherwise promoting free radicals generations (Bhor *et al.*, 2004). Furthermore, an increase in oxidative stress in diabetes has been demonstrated to play a pivotal role in diabetic vascular complications and pathophysiology of diabetic nephropathy (Ugochukwu *et al.*, 2003 and Hayashi *et al.*, 2001).

Flavonoids, a large class of phenolic compounds widely distributed in plants and vegetables, have been reported to be strong antioxidants and radical scavengers (Janbaz *et al.*, 2002; Brenna and Pagliarini, 2001; Papadopoulou *et al.*, 2005). Rutin is a kind of flavonoids glycoside known as Vitamin P and has been extensively studied and is known to exhibit multiple

pharmacological activities including antibacterial and antiviral (Panasiak *et al.*, 1989), antiprotozoal (Iwu *et al.*, 1986), antitumor (Deschner *et al.*, 1991), antiallergic (Chen *et al.*, 2000), anti-inflammatory (Aleksandrov *et al.*, 1986) and antiplatelets (Swies *et al.*, 1984). Recently it has been conformed for anti-diabetic properties (Kamalakkannan and Prince, 2006a, 2006b; Prince and Kamalakkannan, 2006), which are the results of its high radical scavenging activity and antioxidant capacity (Nagai *et al.*, 2005; Kim *et al.*, 2002).

Vitamin C (ascorbic acid) is also a well-known natural antioxidant (Guo *et al.*, 2007 and Gil *et al.*, 2002). For example, vitamin C can recycle the lipid-soluble vitamin E by reducing alpha-tocopheroxyl radicals in membranes (Gramlich *et al.*, 2002). Besides its ability to scavenge various kinds of free radicals, synergistic antioxidant effects are also present in the combinations of vitamin C with other phenolic antioxidants (Liao and Yin, 2000). Thus, the co-application of vitamin C and Rutin may provide different protective effects against free-radical oxidation, which will be helpful for oxidation-related diseases prevention.

Streptozotocin (STZ) is widely used to induce diabetes in experimental animals by causing the selective destruction of pancreatic beta-cells that secrete insulin (Aksoy *et al.*, 2005). Hyperglycemia in diabetes results in

diabetes results in excessive protein glycation and the production of reactive oxidants, which leads to oxidative damage in organs (Brownlee, 2001). The present study was undertaken to evaluate the potential anti-oxidative activity of rutin and vitamin C particularly in combination of these vitamins in the kidney of STZ-induced diabetic rats.

MATERIALS AND METHODS

The present study was designed and studied in College of Applied Medical Sciences, King Saud University, Riyadh, KSA, during year 2008.

Animals: Adult male Wistar rats, similar in age (8-weeks), weighing 180 ± 20 g, obtained from the Experimental Animal Care Center, College of Pharmacy, Riyadh, were employed in the study. The animals were kept in an environmentally controlled breeding room (temperature: $22 \pm 2^\circ\text{C}$, humidity $55 \pm 5\%$, 12 h dark/light cycle). All rats had free access to tap water and rat chow. The handling of the animals was approved by the local Ethical Committee for the care and use of laboratory animals. The animals were injected with streptozotocin (70 mg/kg, i.p.). Five days after injection, the rats were fasted overnight, blood samples were collected through eyelids and drop of blood applied on a strip of ACCU-CHEK active Glucometer. Fasting glucose levels higher than 200 mg/dl considered diabetic were used for the experiments.

Experimental design: Forty-eight rats (24 diabetic and 24 normal rats) were used in the present study. The rats were randomly divided in to eight groups (6 rats in each group): [1] Normal Control (NC), [2] Rutin (RT; 100 mg/kg/day), [3] Vitamin C (VC; 200 mg/kg/day), [4] Rutin (50 mg/kg/day) + Vitamin C (100 mg/kg/day) (RTVC), [5] Diabetic Control (DC), [6] Rutin (100 mg/kg/day) to diabetic rats (RTD), [7] Vitamin C (200 mg/kg/day) to diabetic rats (VCD) and [8] Rutin (50 mg/kg/day) + Vitamin C (100 mg/kg/day) to diabetic rats (RTVCD). The treatments (by using intragastric feeding needle) were continued for 5 consecutive weeks. Body weights of all animals were recorded on 0-day before start the treatments and at the time of sacrifice. After 5 weeks animals were sacrificed by decapitation and the trunk blood was collected. The serum was separated after centrifugation at 3000 rpm for 15 min and stored at -20°C till analysis. Kidneys were dissected, weighed (calculated as g/100 g body weight) and dipped in liquid nitrogen for 1 min then preserved at -75°C (Ultra-low freezer, Environmental Equipment, Cincinnati, Ohio, USA) till analysis.

Biochemical assays in serum samples: Glucose and creatinine concentrations were estimated in serum by using commercially available diagnostic kits (Randox

diagnostic reagents, Randox Laboratories, USA). Serum insulin levels were measured by immunoenzymatic calorimetric method based on ELISA. The protocol used was according to the methods described for the kit (DIA. METRA, Italy).

Estimation of ROS in kidney: Reactive oxygen species (ROS) were determined according to methods previously described by LeBel *et al.* (1992). The weighed tissue samples were homogenized in 20 parts (w/v) of 0.32 mol/l sucrose solution and centrifuged at $1800 \times g$ for 10 min at 4°C . The supernatant was transferred to another centrifuge tube and centrifuged again at $31,500 \times g$ for 15 min at 4°C to yield a crude mitochondrial pellet. The pellet was resuspended in 2.5 parts (w/v) of 18:1 40 mmol/l Tris (pH 7.4); HEPES buffer (10 mmol/l HEPES, 120 mmol/l NaCl, 2.5 mmol/l KCl, 1.2 mmol/l NaH_2PO_4 , 0.1 mmol/l MgCl_2 , 5 mmol/l NaHCO_3 , 6 mmol/l glucose and 1 mmol/l CaCl_2). The ROS concentrations were measured via the formation of the fluorescent oxidized derivative 2,7-dichlorofluorescein using a Perkin Elmer luminescence spectrophotofluorometer LS50B (Wellesley, MA) with the instrument excitation wavelength set at 488 nm and the emission wavelength set at 525 nm. The readings were recorded at 15 and 75 min timed intervals with the samples maintained at 37°C .

Estimation of MDA in kidney: Renal MDA content was measured using the method described by Uchiyama and Mihara (1978) and modified by Sunderman *et al.* (1985) based on the Thiobarbituric Acid (TBA) reaction test. Weighed tissue samples were homogenized (1/10 w/v) in ice-cold 1.15% KCl solution and mixed with 0.1 ml of 8.1% acetic acid and 0.75 ml of 0.8% TBA solution. The mixture was made up to 2.0 ml with distilled water and heated at 95°C for 60 min. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of n-butanol/pyridine mixture (15:1 v/v) were added and the mixture shaken vigorously. The mixture was centrifuged at $4000 \times g$ for 10 min and the absorbance of the organic layer (upper layer) was measured at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard.

Estimation of GSH in kidney: Reduced glutathione (GSH) concentration was assayed using the method of Sedlak and Lindsay (1968). A cross sectional piece of kidney (200 mg) were dissected and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). Aliquots of 0.5 ml of the tissue homogenates were mixed with 0.2 M Tris buffer, pH 8.2 and 0.1 ml of 0.01 M Ellman's reagent, [5,5'-dithiobis-(2-nitro-benzoic acid)] (DTNB). Tubes were centrifuged at 3000 g at room temperature for 15 min. The absorbance of the clear supernatants was read in a spectrophotometer at 412

mn in 1 cm quartz cells. The concentrations were estimated by using the standard curve.

Estimation of Oxidized glutathione in kidney: The kidney samples were homogenized (1:20 w/v) in ice-cold 6% perchloric acid containing 20 mmol/l N-ethylmaleimide (NEM) and 2 mmol/l bathophenanthroline disulfonic acid (BPDS). The homogenate was centrifuged at 15,000 x g for 5 min at 4°C and the supernatant neutralized before assay. Oxidized glutathione was measured according to the method described by Aseni *et al.* (1999) based on the principle of glutathione reductase enzyme reducing GSSG to GSH with the concomitant oxidation of NADPH to NADP⁺. To 0.9 ml of 1.75 mol/l K₃PO₄ buffer (pH 7.0) containing 20 mmol/l NEM were added 0.05 ml of sample extract and 0.025 ml of 10 mg/ml of NADPH-Na solution. Absorbance at 340 nm was measured for 30 s immediately after addition of 0.025 ml of (10 mg/ml) Glutathione Reductase (GR) to the assay mixture.

Estimation of SOD activity in kidney: Superoxide Dismutase (SOD) activity in kidney was assayed spectrophotometrically (560 nm) by the method described Kakkar *et al.* (1984) using nitroblue tetrazolium as the indicator reagent. Briefly, the kidney tissues (200 mg) were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents: sodium pyrophosphate buffer 1.2 mL (0.052 M) pH 8.3, 0.1 ml phenazine methosulphate (186 µM), 0.3 mL nitro blue tetrazolium (300 µM) and 0.2 mL NADH (780 µM) were added to 0.1 mL of processed tissue sample. The mixture was then incubated for 90 min at 30°C. Four mL of n-butanol and one mL of acetic acid were then added. The mixture was shaken vigorously. Following centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 560 nm using a spectrophotometer (LKB-Pharmacia, Mark II, Ireland).

Estimation of CAT activity in kidney: The catalase (CAT) activity was measured by the method of Aebi (1983) using hydrogen peroxide as substrate. The disappearance of H₂O₂ was followed at 240 nm. The activity was expressed as µmole⁻¹min⁻¹mg⁻¹ protein using the extinction coefficient of 0.0436 mM⁻¹ mg⁻¹. To elaborate, CAT exerts a dual-function decomposition of H₂O₂ to give H₂O and O₂ and oxidation of H donors. In the ultraviolet range, H₂O₂ shows a continual increase in absorption with decreasing wavelength. The decomposition of H₂O₂ can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time is a measure of CAT activity. Kidney tissues (200 mg) were homogenized in eight mL of 0.05 M phosphate buffer at pH 7.0. The tissue homogenates were centrifuged at 4°C for 15 min at 1500g. The supernatants were removed into separate

test tubes and kept on ice until the enzyme assay. Sample was measured against a blank containing 2.8 mL (1:500 v/v) phosphate buffer instead of H₂O₂ (30 mM hydrogen peroxide) and 0.2 mL enzyme solution. The reaction was started by addition of H₂O₂. The initial absorbance should be A = 0.500 followed by the decrease in absorbance for about 30 seconds.

Estimation of GPx activity in kidney: *Glutathione Peroxidase* (GPx) activity was measured using Paglia and Valentine's method (1967). The reaction mixture contained 2.6 ml of 100 mmol/l phosphate buffer (pH 7.0) with 3 mmol/l EDTA, 0.05 ml of 10 mg/ml GSH solution, 0.1 ml GR (10 mg/ml), 0.05 (10 mg/ml) NADPH-Na salt, 0.1 ml 90 mmol/l hydrogen peroxide solution and 0.1 ml of tissue supernatant. The GPx activity was monitored by the decrease in absorbance due to the consumption of NADPH, Which absorbs at 340 nm on spectrophotometer.

Statistical analyses: The results were analyzed by one-way ANOVA test and Tukey-Kramer multi-comparison post-test using GraphPad Prism 5.0 computer software (San Diego, CA). The results were expressed as mean ± S.E.M., n = 6. p-Values <0.05, <0.01 and <0.001 were considered to be statistically significant.

RESULTS

Effects on body and kidney weights: Mean body weights (g) were significantly (p<0.001) increased in diabetic control group (49.67±9.78) of rats as compared to normal control rats (182.23±8.24). Treatment with rutin, vitamin C and their combination to diabetic rats showed significant (p<0.01, p<0.001 and p<0.001 respectively) increase in body weights (79.85±4.87, 96.74±4.76 and 115.16±8.41 respectively) while compared to diabetic control group of rats (Fig. 1a). In contrast, mean kidney weights (g/100 g body weight) were significantly (p<0.001) increased in diabetic control group (1.31±0.05) as compared to non-diabetic control rats (0.67±0.02 g). However, treatments with vitamin C alone (0.92±0.03) and in combination with rutin (0.81±0.02) to diabetic rats caused significant (p<0.01 and p<0.001) decrease in kidney weights compared to diabetic control rats respectively (Fig. 1b).

Effects on serum glucose, insulin and creatinine: Five weeks after the STZ injection the mean serum glucose concentrations reached 412.24±19.34 mg/dl in diabetic control rats compared to 148.65±12.11 mg/dl non-diabetic control rats. Daily rutin and vitamin C treatments significantly (p<0.05) decreased the glucose levels 412.24±19.34 and 405.74±21.56 mg/dl in diabetic rats compared to diabetic control rats respectively. However, the combined treatment reduced the glucose levels 378.57±18.76 mg/dl in diabetic rats that is more significantly (p<0.01) less than the diabetic control rats (Fig. 2a).

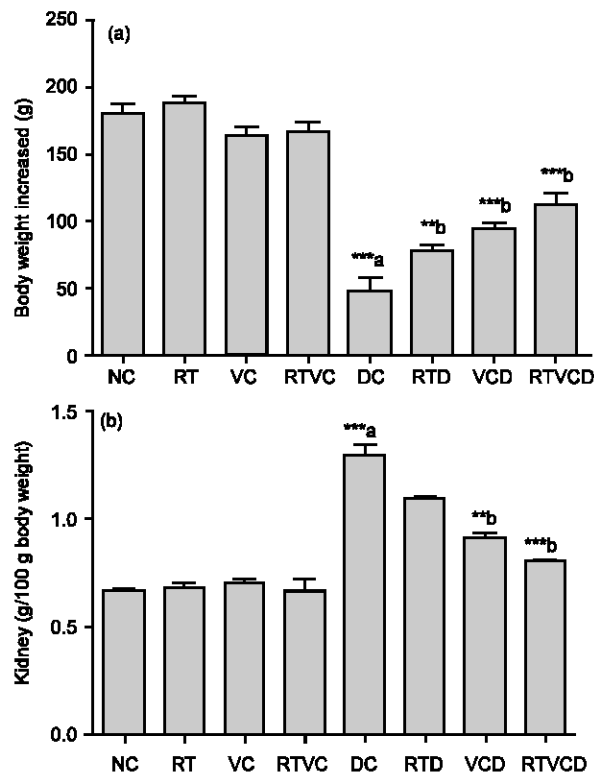


Fig. 1: Effect of rutin, vitamin C and their combination on (a) body and (b) kidney weights of normal and diabetic rats treated for 5 weeks, ^aNon-diabetic treated groups were compared to control group. ^bDrugs treated diabetic groups were compared to diabetic vehicle group. Values were expressed as Mean±S.E.M and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test where **p<0.01 and ***p<0.001. Six rats were used in each group

Mean insulin levels decreased significantly (p<0.001) in STZ-induced diabetic rats compared to non-diabetic rats. Treatments with rutin, vitamin C and their combination to diabetic rats significantly (p<0.01, p<0.001 and p<0.001 respectively) decreased the hyperinsulinogenic effect compared to diabetic control rats (Fig. 2b). Serum creatinine levels were not significantly altered in diabetic rats while statistically compared to non-diabetic controls. Treatments with antioxidant vitamins either to non-diabetic or diabetic rats did not alter the serum creatinine levels significantly when compared to their respective controls (Fig. 2c).

Effects on ROS concentration: The reactive oxygen species concentrations (84.62±4.15 nM) were significantly (p<0.001) increased in kidneys of diabetic control rats as compared to non-diabetic (37.14±2.54 nM) control rats (Fig. 3a). Five-week treatments with rutin, vitamin C and their combination to diabetic rats

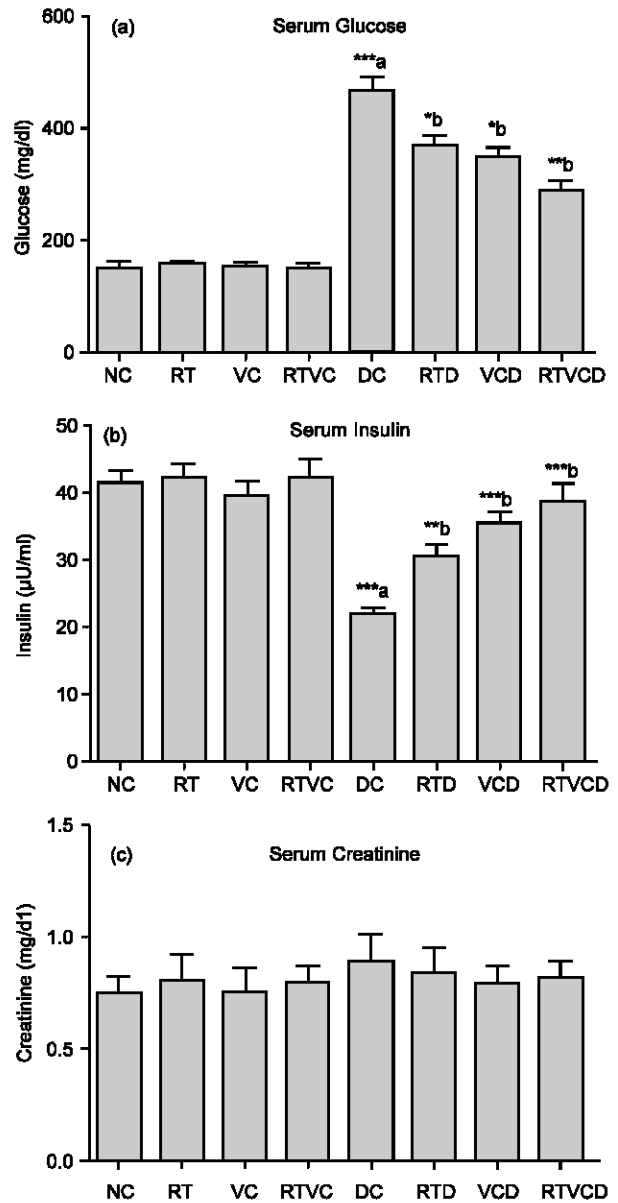


Fig. 2: Effect of rutin, vitamin C and their combination on serum (a) glucose, (b) insulin and (c) creatinine of normal and diabetic rats, ^aNon-diabetic treated groups were compared to control group. ^bDrugs treated diabetic groups were compared to diabetic vehicle group. Values were expressed as Mean±S.E.M and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test where *p<0.05, **p<0.01 and ***p<0.001. Six rats were used in each group

decreased the ROS values (62.15±3.67, 56.74±2.95 and 46.74±3.19 nM respectively) significantly (p<0.001) less compared to the diabetic rats (Fig. 3a). Thus it shows,

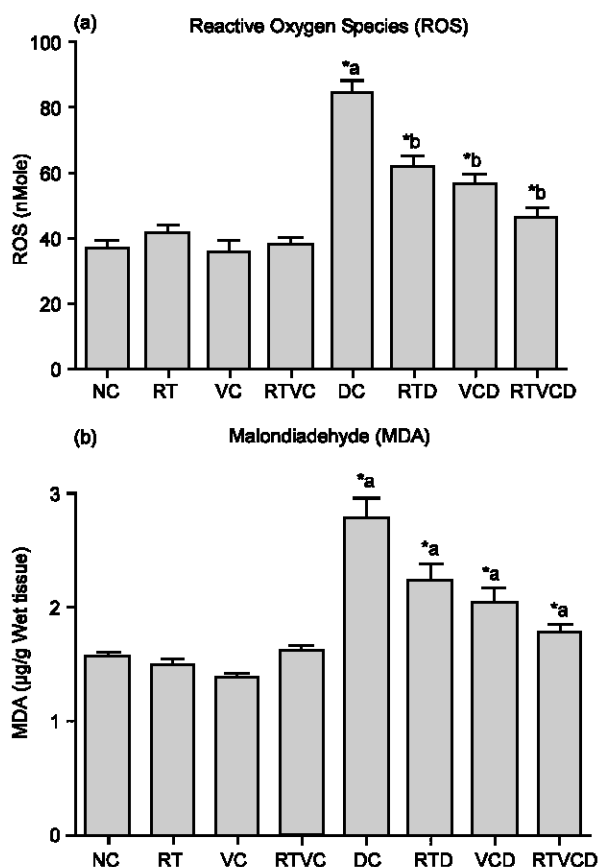


Fig. 3: Effect of rutin, vitamin C and their combination on kidney (a) ROS and (b) MDA concentrations of normal and diabetic rats, ^aNon-diabetic treated groups were compared to control group. ^bDrugs treated diabetic groups were compared to diabetic vehicle group. Values were expressed as Mean±S.E.M and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test where *p<0.001. Six rats were used in each group

combined treatment has more potential against the oxidative stress induced in kidneys of diabetic rats.

Effects on MDA concentration: Malondialdehyde (MDA) concentrations (2.78±0.18 µg/g) in kidneys were significantly (p<0.001) increased in diabetic control rats compared to the non-diabetic control rats (1.57±0.05 µg/g). Treatments with rutin, vitamin C and their combination for five consecutive weeks to the diabetic rats caused significant (p<0.001) decrease in kidney MDA levels (2.24±0.14, 2.04±0.13 and 1.78±0.08 µg/g respectively) while compared to diabetic control rats (Fig. 3b).

Effects on GSH concentration: Kidney reduced glutathione (GSH) levels (µg/g) found significantly less

(p<0.001) in diabetic rats (121.36±5.79) than non-diabetic rats (187.45±7.85). Rutin and vitamin C treatments to diabetic rats significantly (p<0.05 and p<0.001 respectively) increased the GSH levels (146.65±5.37 and 167.42±6.23) in kidneys compared to diabetic control rats. Combined treatment showed higher positive effect (p<0.001) against the oxidation-induced by STZ-injection in rats (Fig. 4a).

Effect on GSSG concentration: Oxidized glutathione (GSSG) levels (3.87±0.1 mg/g) significantly (p<0.001) increased in kidneys of diabetic rats as compared to non-diabetic controls (1.95±0.07 mg/g). Although the treatments with rutin, vitamin C and their combinations to diabetic rats significantly (p<0.001) decreased the GSSG levels (2.74±0.06, 2.57±0.09 and 2.04±0.06 mg/g respectively) as compared to diabetic control rats, however, the protection against increase found higher in combined treatment group (Fig. 4b).

Effects on GSH/GSSG ratio: Ratios of reduce glutathione and oxidized glutathione in kidney was found significantly (p<0.001) low in diabetic rats (31.47±3.75) compared to non-diabetic control rats (96.17±6.24). Rutin treatment to diabetic rats could not significantly alter the ratio (37.89±2.74) between GSH and GSSG compared to diabetic control rats. However, in vitamin C and the combination of rutin and vitamin C treated diabetic rats, the values of GSH/GSSG (65.21±3.04 and 87.56±4.36) were significantly p<0.01 and p<0.001 increased while compared to diabetic controls respectively (Fig. 4c).

Effects on GPx activity: Glutathione Peroxide (GPx) activity (85.62±4.67 µmole/min/mg protein) in the diabetic rats was significantly (p<0.001) decreased as compared to normal control rats 155.74±9.78 µmole/min/mg protein). The diabetic rats treated with rutin, vitamin C and their combination for five consecutive weeks showed significant (p<0.05, p<0.01 and p<0.001 respectively) increase in GPx activity (119.23±5.67, 128.78±6.24 and 141.47±5.93 µmole/min/mg protein respectively) while compared to diabetic control (Fig. 5a).

Effect on SOD activity: Superoxide Dismutase (SOD) activity (Units/mg of protein) in kidney of non-diabetic rats either treated or untreated was remain unchanged. Streptozotocin-induced diabetic rats significantly (p<0.001) increased the SOD activity (7.87±0.28) in kidneys compared to non-diabetic control group of rats (4.56±0.28). Five consecutive week treatments with rutin and vitamin C and their combination to the diabetic rats showed significant (p<0.05, p<0.05 and p<0.001 respectively) decrease in SOD activity as compared to diabetic control rats (Fig. 5b).

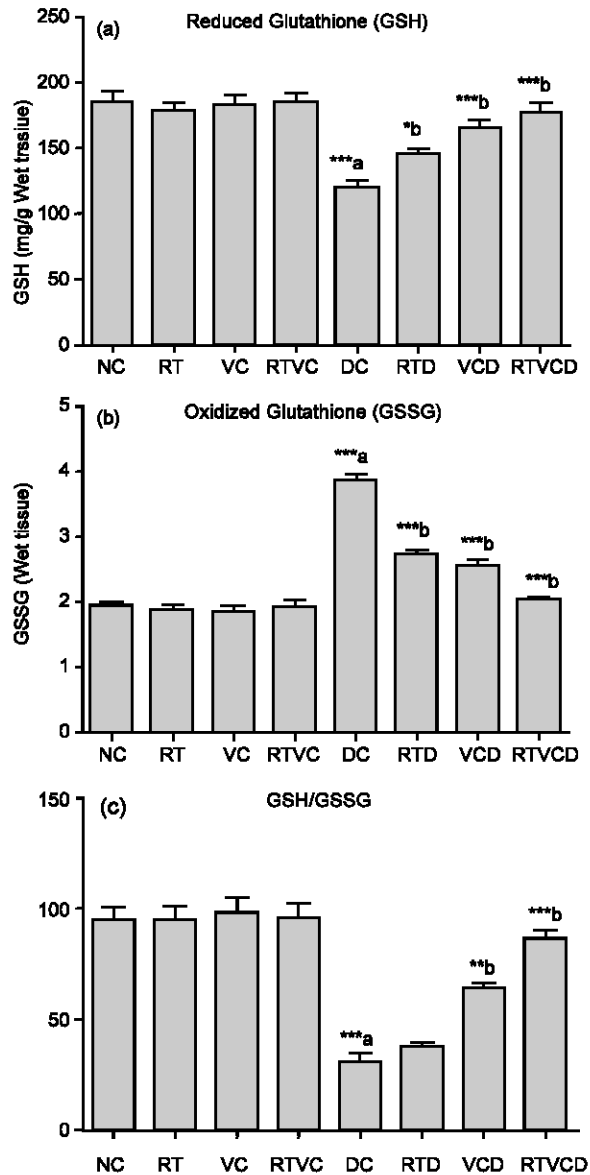


Fig. 4: Effect of rutin, vitamin C and their combination on kidney (a) GSH, (b) GSSG concentrations and (c) GSH/GSSG ratio of normal and diabetic rats, ^aNon-diabetic treated groups were compared to control group. ^bDrugs treated diabetic groups were compared to diabetic vehicle group. Values were expressed as Mean \pm S.E.M and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test where * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Six rats were used in each group

Effects on CAT activity: The catalase activity (CAT) values ($387.46 \pm 18.46 \mu\text{M}/\text{mg}/\text{g}$) in diabetic rats was significantly ($p < 0.001$) decreased as compared to

diabetic controls ($546.61 \pm 24.51 \mu\text{M}/\text{mg}/\text{g}$). Rutin treatments to diabetic rats could not increase the CAT activity significantly in kidneys while vitamin C treatment showed significant ($p < 0.05$) increase in that activity ($485.62 \pm 14.23 \mu\text{M}/\text{mg}/\text{g}$) when compared to diabetic control rats. However, more significant ($p < 0.01$) elevation was seen in CAT activity ($514.35 \pm 17.64 \mu\text{M}/\text{mg}/\text{g}$) after combined (rutin and vitamin C) treatments to the diabetic rats while compared to diabetic controls (Fig. 5c).

DISCUSSION

Streptozotocin is a commonly employed compound for the induction of diabetes in rats (Tomlinson *et al.*, 1992). It causes diabetes by the rapid depletion of beta-cells, which leads to a reduction in the insulin release. An insufficient release of insulin causes hyperglycemia which results in oxidative damage by generation of ROS (Mohamed *et al.*, 1999) and the development of diabetic complications (Aydin *et al.*, 2001) including diabetic nephropathy (Kedziora-Kornatowska *et al.*, 2002). The highly ROS generation is toxic to the cell, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides (Kesavulu *et al.*, 2001). However, endogenous antioxidant enzymes (SOD, CAT and GPx) are responsible for the detoxification of the deleterious oxygen species (Aydin *et al.*, 2001).

The destruction of beta-cells during diabetes ultimately causes physico-metabolic abnormalities such as a decrease in body weight gain and increase in food and water intake (Rodriguez *et al.*, 1997). In addition, diabetic rats showed a clear muscle atrophy involving a decrease in both skeletal muscle mass and protein content. This was accompanied by a marked loss of total carcass nitrogen. These changes were related to important alterations in protein turnover in skeletal muscle (Pepato *et al.*, 1996). Hence, a notable decrease in the body weight change observed in the diabetic group of rats might be the result of protein wasting due to the unavailability of carbohydrates for energy metabolism and loss of degradation of structural proteins (Brody, 1998). The improvement in body weight gain in diabetic rats supplemented with rutin and vitamin C and their combination highlight the body glucose homeostasis which in turn promotes the body weight gain. The observation that diabetes is associated with bilateral renal enlargement was made in 1841 (Rayer, 1841), the mechanisms underlying its development have only recently begun to be unraveled, with growth factors suggested to have a key role (Wassef *et al.*, 2004; Zhu *et al.*, 2005). Kidney growth in diabetes includes cell hyperplasia (Rasch and Norgaard, 1983) as well as hypertrophy (Seyer-Hansen *et al.*, 1980) and the accumulation of extracellular matrix material (Osterby and Gundersen, 1975). Present results are in agreement with earlier reports that, kidney

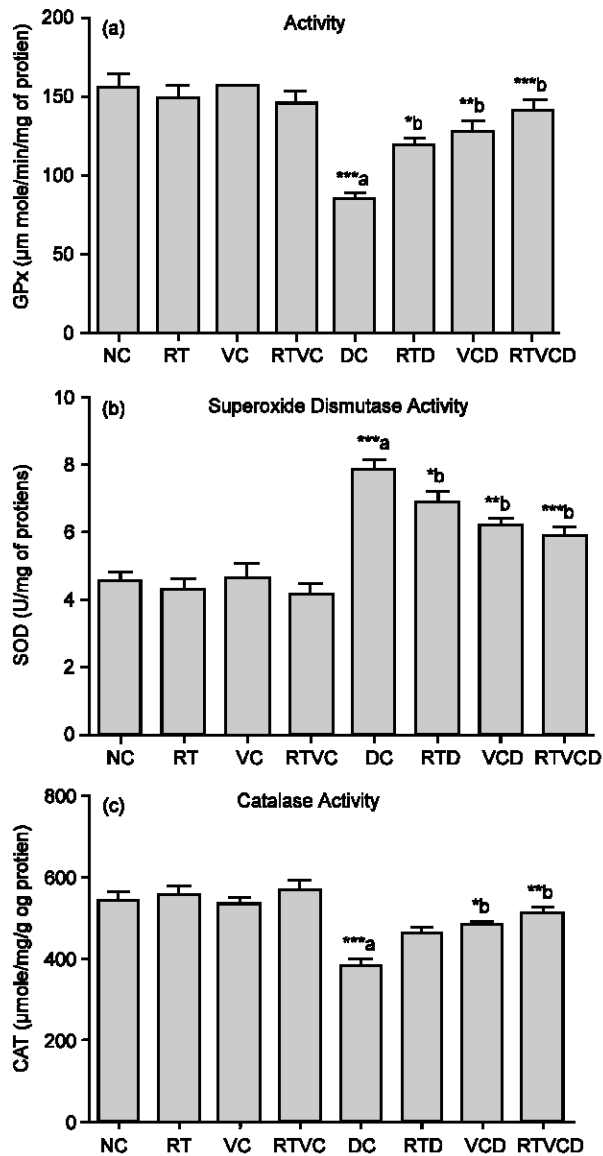


Fig. 5: Effect of rutin, vitamin C and their combination on kidney (a) GPx, (b) SOD and (c) CAT activity in normal and diabetic rats, ^aNon-diabetic treated groups were compared to control group. ^bDrugs treated diabetic groups were compared to diabetic vehicle group. Values were expressed as Mean \pm S.E.M and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Six rats were used in each group

weights significantly ($p < 0.001$) increased in STZ-induced diabetic rats as compared to normal rats. The renal enlargement in diabetic rats were significantly ($p < 0.001$) reduced in combined (rutin + vitamin C) treatment group while rutin alone did not show any significant change.

In agreement with the present results, the individual hypoglycemic effect of rutin and vitamin C has been demonstrated in experimentally induced diabetic rats (Kamalakkannan and Prince, 2006a,b; Prince and Kamalakkannan, 2006; Aksoy *et al.*, 2005; Hamden *et al.*, 2008). Rutin or vitamin C or their combined treatments for 5-weeks to diabetic rats caused decrease in increased glucose levels and increase in decreased insulin concentrations respectively as compared diabetic control group or rats. However, the effect was more pronounced in the group of rats treated with combined vitamins (C and P). Earlier studies, recommended the combined treatments with antioxidant vitamin against diabetes mellitus (Hamden *et al.*, 2008; Aksoy *et al.*, 2005).

The concentration of ROS in the kidney were measured and found to be significantly ($p < 0.001$) higher in the diabetic control rats compared to the non-diabetic control rats at the end of the 5-weeks in present study. Several studies, clinical (Aguirre *et al.*, 1998; Orié *et al.*, 1999) and experimental (Ugochukwu and Cobourne, 2003; Jung *et al.*, 2005) have also shown increased concentration ROS and oxidative stress in NIDDM subjects and STZ-induced diabetic rats. Rutin and vitamin C treatments to the diabetic rats well mitigated the increased values of MDA and ROS in kidneys. This could be due to the ability of these antioxidant vitamins that transfer electrons, free radicals, chelate metals catalays (Prince and Kamalakkannan, 2006; Guo *et al.*, 2007) and activate antioxidant enzymes (Kamalakkannan and Prince, 2006a,b; Nagai *et al.*, 2005). However, the combine treatment of rutin and vitamin C to the diabetic rats showed more beneficial effect against STZ-induced diabetic rats than the individual vitamin treatments.

Glutathione provide a first line of defense against ROS, as it can scavenge free radicals and reduce H_2O_2 . The decreased concentration of GSH in kidney might be due to NADPH depletion or GSH consumption in the removal of peroxide (Gumieniczek, 2005). The ration of reduced glutathione/oxidized glutathione is one of many cellular redox couples that directly contribute to redox status. Depletion of reduced GSH either by conjugation and removal from the cell or oxidation to GSSG could significantly affect the overall redox potential of the cell (Yadav *et al.*, 1997). GSH-dependent enzymes provide a second line of defense as they primarily detoxify noxious byproducts generated by ROS and also help to prevent propagation free radicals (Gumieniczek, 2005). GPx serves to detoxify peroxides by reacting them with GSH (Sen, 1997). In this study, GSH/GSSG rations were found to be lowest in the diabetic control group of animals. Some workers (Obrosova *et al.*, 2003; Lee *et al.*, 2000) reported that the concentrations in the diabetic kidney were found to be significantly reduced, suggesting that the reduced GSH concentrations may play a role in the

development of diabetic complications. In the present study, GSH/GSSG ratio in the diabetic rats treated with rutin and vitamin C together found significant ($p < 0.001$) increase compared to diabetic control group of rats. This increase is responsible for the reduced oxidative stress in the diabetic rats treated with antioxidant vitamins.

Oxidative stress has been known to induce the production of ROS scavenging enzymes (Niskanen *et al.*, 1995; Nourooz-Zadeh *et al.*, 1997). From the results, these enzymes are observed to respond in compensatory mechanism, increasing enzyme activity in the diabetic rats, in an attempt to maintain homeostasis. SOD activity increased in the diabetic control rats compared to that of the non-diabetic control rats. The higher SOD activity is believed to be due to increased dismutation of Superoxide anions due to their increased production (Cho *et al.*, 2002). However, the diabetic rats treated with rutin and vitamin C showed reduced activity. The activity for the diabetic rats treated with combination of rutin and vitamin C was shown to be closer to that of the non-diabetic rats. This could mean that there was an initial increase in SOD activity due to production of superoxide anions at the onset of diabetes but the administration of antioxidant vitamins would reduce further production of ROS with resulting decrease in SOD activity.

The current findings suggest that the increase in oxidative stress in the kidney of STZ-induced diabetic rats was effectively reduced and controlled via the administration of antioxidant vitamins. Previous study (Alsaif, 2009) done under the same research project showed that the combined treatment of rutin and vitamin C to diabetic rats more beneficial than individual supplementations against the hepatic damage caused by the STZ-induced diabetic in rats. Accordingly, these data imply that long-term treatment with antioxidants vitamins (rutin and vitamin C) in combination as diabetic maintenance therapy may consequently control or prevent the development of diabetic complications that are due to increased oxidative stress and lipid peroxidation, especially diabetic nephropathy.

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