



# Journal of Medical Sciences

ISSN 1682-4474

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*JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.*

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## **Combined Treatment of Rutin and Vitamin C Improves the Antioxidant Status in Streptozotocin-Induced Diabetic Rats**

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The aim of the present study is to determine if a combination of rutin (vitamin-P) and vitamin C has any advantage on antioxidant status in streptozotocin (STZ)-induced diabetes in rats. Oral administration on rutin (100 mg kg<sup>-1</sup>), vitamin C (200 mg day<sup>-1</sup>) and their combination (50 and 100 mg kg<sup>-1</sup>) for 5 weeks on the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), glucose, insulin, Total Cholesterol (TC) and triglycerides (TG) in normal and STZ-induced diabetic rats were evaluated. Reduced glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations were estimated in liver. Histopathological changes were screened in liver. Body weight significantly ( $p < 0.001$ ) drops although liver and kidney weights were increased in diabetic rats. Hepatic enzymes (AST, ALT and ALP), cytokines (TNF- $\alpha$  and IL-6) and lipids (TC and TG) were significantly elevated in diabetic rats. Treatments with rutin and vitamin C significantly lowered the elevated values in diabetic rats while it found higher in combined treated group. Liver MDA increased, GSH and SOD levels decreased significantly ( $p < 0.001$ ) in diabetic rats. The treatment with rutin and vitamin C lowered MDA and increased the antioxidant levels to near control values. The results verify the presence of oxidative stress in diabetes and suggest beneficial effects of rutin and vitamin C combinations in combating the oxidative stress in this disease.

**Key words:** Streptozotocin, diabetes, liver, oxidative stress, rutin, vitamin C

## INTRODUCTION

In clinical and experimental research, attention is paid to the role of antioxidant defense systems in the prevention of human diseases such as cancer, diabetes mellitus and cardiovascular pathologies (Vural *et al.*, 2000, 2001; Sabuncu *et al.*, 2001). During the progression of these diseases, oxidative stress events occur and free radicals and Reactive Oxygen Species (ROS) are generated from the molecular oxygen. These free radicals and ROS are thought to contribute to lipid peroxidation and protein degradation (Aksoy *et al.*, 2005). Host survival depends upon the ability of cells and tissues to adapt to or resist the stress and repair or remove damaged molecules and cells. Multiple enzymatic and non-enzymatic antioxidant defense systems present in cells in activate those free radicals and reduce the amount of cellular oxidative damage they cause. While synthetic antioxidants have potential health hazards, the search of natural radical scavengers (antioxidants) is of great interest among scientists (Bastianetto and Quirian, 2002; Guo *et al.*, 2007).

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 Paliarini, 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), anti-allergic (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, b; 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; 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 is one of the most commonly used substances to induce diabetes in the rat. This toxin

causes the death of pancreatic  $\beta$ -cells by alkylation of DNA resulting in reduced synthesis and release of insulin. Furthermore, it has been shown to be involved in the fragmentation of DNA by means of production of reactive oxygen species (Montilla *et al.*, 2004). Little information is available on the endogenous levels of vitamins C and P (Rutin), particularly in the state of chronic oxidative stress associated with the development of diabetes. The present study was conducted to describe the effects of these vitamins on the oxidative-antioxidative system in diabetic rats.

## MATERIALS AND METHODS

**Animals:** The present study was designed and studied in College of Applied Medical Sciences, King Saud University, Riyadh, KSA during 2008. Adult male Wistar rats, weighing  $180 \pm 20$  g and 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 ( $70 \text{ mg kg}^{-1}$ , i.p.). Five days after injection, the rats with fasting blood glucose higher than  $200 \text{ mg dL}^{-1}$  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] -Ve Control (normal rats, vehicle), [2] Rutin ( $100 \text{ mg/kg/day}$ ), [3] Vitamin C ( $200 \text{ mg/kg/day}$ ), [4] Rutin ( $50 \text{ mg/kg/day}$ ) +Vitamin C ( $100 \text{ mg/kg/day}$ ), [5] +Ve Control (diabetic rats, vehicle), [6] Rutin (diabetic rats,  $100 \text{ mg/kg/day}$ ), [7] Vitamin C (diabetic rats,  $200 \text{ mg/kg/day}$ ) and [8] Rutin ( $50 \text{ mg/kg/day}$ ) + Vitamin C ( $100 \text{ mg/kg/day}$ ) to diabetic rats. 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. After 5 weeks animals were sacrificed by decapitation and the trunk blood was collected. The serum was prepared by centrifugation ( $1500 \text{ g}$ ,  $15 \text{ min}$ ,  $4^\circ\text{C}$ ) and stored at  $-20^\circ\text{C}$  till analysis. Organs (liver, heart, kidneys and spleen) were dissected, weighed and calculated as  $\text{g}/100 \text{ g}$  body weight. Part of liver from each rat were dipped in liquid nitrogen for 1 min and preserved at  $-75^\circ\text{C}$ . Another part of livers were fixed in 10% formaldehyde solution for histopathological screening.

**Biochemical assays in serum samples:** AST, ALT, ALP, glucose, TC and triglyceride 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).

Serum TNF- $\alpha$  concentration was estimated by using the CYTELISA rat TNF- $\alpha$  obtained from CYTIMMUNE Sciences Inc., Maryland, USA. All samples were assayed in duplicate. The intra assay variation was 6.7%. To avoid inter assay variation all samples were run at one time. Optical density of each well was determined by using a microplate reader (Thermo Labsystems, Finland).

Serum IL-6 was estimated by using the ELISA Quantikine rat IL-6 immunoassay kit obtained from R and D Systems Inc., Minneapolis, MN, USA. The samples were assayed in duplicate. The intra assay variation was 5.5%. To avoid inter assay variation all samples were run at one time. Optical density of each well was determined by using a microplate reader (Thermo Labsystems, Finland).

**Estimation of MDA in liver:** The method described by Ohkawa *et al.* (1979) was used. Malondialdehyde (MDA) was measured as an indicator of lipid peroxidation. Liver tissues were homogenized in KCl solution and incubated with thiobarbituric acid. After centrifugation the pink clear layer was read at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard.

**Estimation of GSH in liver:** Glutathione concentration was assayed using the method of Sedlak and Lindsay (1968). A cross sectional piece of liver tissues (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 nm in 1 cm quartz cells. The concentrations were estimated by using the standard curve.

**Estimation of SOD activity in liver:** Superoxide dismutase activity in liver was assayed spectrophotometrically (560 nm) by the method described Kakkar *et al.* (1984) using nitroblue tetrazolium as the indicator reagent.

**Histopathological assessment:** The liver samples preserved in 10% natural buffered formalin and processed

for routine paraffin block preparation. Using an American optical rotary microtome, sections of thickness about 3  $\mu$ m were cut and stained with hematoxyline and eosin (Culling, 1974). The slides were then examined under a microscope for pathomorphological changes as congestion, hemorrhage, edema, erosions and fatty bodies using an arbitrary scale for the assessment of severity of these changes: Within normal (0-1), mild injury (2-3), moderate injury (4-5) and severe injury (6-7).

**Statistical analysis:** All data are presented as the Mean $\pm$ SE. The data were evaluated by a one-way ANOVA using SPSS program and the differences between the means assessed using student's t-test.

## RESULTS

**Effect on body and organ weights:** Body growth was similar in all control and treated non-diabetic rats. After the experimental period (5 weeks), there was significant ( $p < 0.001$ ) decrease in body weight of diabetic rats compared with that of control rats (the body weight increased in control rats 176.83 $\pm$ 8.37 g while in diabetic rats 53.00 $\pm$ 7.34 g). However, this significant weight loss is prevented in diabetic rats orally treated with rutin ( $p < 0.05$ ), vitamin C ( $p < 0.05$ ) and combination of rutin and vitamin C ( $p < 0.01$ ). This suggests that combined treatment of these vitamins can prevent more than individuals the body weight loss in diabetes. Liver and kidney weights were also significantly ( $p < 0.001$ ) increased in untreated diabetic group of animals compared to controls. Treatments with rutin ( $p < 0.05$ ), vitamin C ( $p < 0.01$ ) and their combination ( $p < 0.001$ ) decreased the liver weights compared to control diabetic rats (the values are 3.64 $\pm$ 0.19, 3.45 $\pm$ 0.28, 3.30 $\pm$ 0.12 and 4.53 $\pm$ 0.12 g, respectively). Here, also the combined treatment with vitamins showed more potent against the diabetic effect on liver weight increase. Similar treatments affect were found on kidney weights increase in diabetic rats (Table 1).

**Effect on serum enzymes and cytokines:** The activities of ALT, AST and ALP were increased significantly ( $p < 0.001$ ) in serum of diabetic rats when compared to controls. Oral treatment of rutin, vitamin C and their combination normalized the activities of AST and ALP enzymes to near normalcy when compared to control group of rats although the protective effect found higher in co-treated group. Serum TNF- $\alpha$  and IL-6 activities were increased significantly ( $p < 0.001$ ) in diabetic rats compared to the controls. Only the combined treatment group showed protection against the increase in TNF- $\alpha$  ( $p < 0.05$ ) and IL-6 ( $p < 0.001$ ) levels (Table 2).

Table 1: Effect on rutin and vitamin C on body growth and organs weight of normal and diabetic rats treated for 5 weeks

Treatment (mg/kg/day)	Body weight increased (g) in 5 weeks	Organs weight (g/100 g body weight)			
		Liver	Heart	Spleen	Kidney
Control (Vehicle)	176.83±8.37	3.63±0.07	0.32±0.01	0.37±0.01	0.58±0.01
Rutin (100)	172.17±4.71	3.66±0.043	0.33±0.02	0.39±0.02	0.59±0.01
Vitamin C (200)	163.83±7.64	3.69±0.07	0.32±0.01	0.37±0.03	0.60±0.01
Vitamin C and Rutin (100 and 50)	178.33±7.39	3.82±0.10	0.38±0.03	0.36±0.03	0.68±0.07
Diabetic (vehicle)	53.00±7.34****	4.53±0.12****	0.36±0.02	0.47±0.07	1.12±0.07****
Rutin (Diab.) (100)	83.00±6.36 <sup>b*</sup>	3.64±0.19 <sup>b*</sup>	0.35±0.02	0.37±0.02	0.92±0.05
Vitamin C (Diab.) (200)	84.67±5.85 <sup>b*</sup>	3.45±0.28 <sup>b**</sup>	0.36±0.01	0.36±0.02	0.87±0.06 <sup>b*</sup>
Rutin+Vitamin C (Diab.) (100 and 50)	92.17±9.36 <sup>b**</sup>	3.30±0.12 <sup>b***</sup>	0.36±0.01	0.30±0.02	0.68±0.03 <sup>b***</sup>

<sup>a</sup>Non-diabetic treated and diabetic groups were compared to control group. <sup>b</sup>Drugs treated diabetic groups were compared to diabetic vehicle group. \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Six rats were used in each group

Table 2: Effect on rutin and vitamin C on serum intracellular enzymes (ALT, AST and ALP) and interleukins activities of normal and diabetic rats

Treatment (mg/kg/day)	ALT (U L <sup>-1</sup> )	AST (U L <sup>-1</sup> )	ALP (U L <sup>-1</sup> )	TNF-α (pg mL <sup>-1</sup> )	IL-6 (pg mL <sup>-1</sup> )
Control (vehicle)	26.53±2.31	55.16±2.63	309.53±24.35	72.15±2.81	176.74±9.15
Rutin (100)	22.94±1.78	50.59±2.98	276.16±20.45	69.99±3.32	181.01±3.49
Vitamin C (200)	24.75±1.89	48.03±4.51	281.69±28.30	66.70±4.49	186.07±7.36
Vitamin C and Rutin (100 and 50)	25.18±1.18	53.85±3.03	293.75±11.27	70.91±5.06	176.04±3.49
Diabetic (vehicle)	63.66±5.11****	78.66±5.41 <sup>***</sup>	581.06±20.68****	122.79±10.37****	240.38±14.13****
Rutin (Diab.) (100)	54.95±5.97	58.04±2.98 <sup>b*</sup>	528.63±25.12	111.22±5.67	215.83±5.68
Vitamin C (Diab.) (200)	46.50±5.03	56.79±5.92 <sup>b**</sup>	465.89±16.87 <sup>b*</sup>	102.69±5.51	198.44±12.31
Vitamin C+Rutin (Diab.) (100 and 50)	44.29±3.29	54.23±3.58 <sup>b***</sup>	452.99±35.60 <sup>b*</sup>	90.79±8.98 <sup>b*</sup>	193.99±8.81 <sup>b***</sup>

<sup>a</sup>Non-diabetic treated and diabetic groups were compared to control group. <sup>b</sup>Drugs treated diabetic groups were compared to diabetic vehicle group. \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Six rats were used in each group

Table 3: Effect on rutin and vitamin C on serum glucose, insulin, total cholesterol and triglycerides of normal and diabetic rats

Treatment (mg/kg/day)	Glucose (mg dL <sup>-1</sup> )	Insulin (μU mL <sup>-1</sup> )	Total cholesterol (mg dL <sup>-1</sup> )	Triglycerides (mg dL <sup>-1</sup> )
Control (vehicle)	155.26±14.22	36.57±1.96	52.21±2.65	162.84±13.07
Rutin (100)	163.37±12.65	39.94±3.78	49.84±3.66	148.49±10.95
Vitamin C (200)	167.82±10.80	37.70±4.16	46.61±3.37	145.81±17.31
Vitamin C and Rutin (100 and 50)	157.34±10.09	36.67±2.16	46.93±3.50	146.90±15.11
Diabetic (vehicle)	481.49±31.64****	18.24±1.21****	88.28±10.65****	629.32±19.45****
Rutin (Diab.) (100)	391.93±18.01 <sup>b*</sup>	26.48±1.33	61.61±3.08 <sup>b*</sup>	435.26±21.52 <sup>b**</sup>
Vitamin C (Diab.) (200)	401.03±20.69 <sup>b*</sup>	27.67±1.50	57.04±8.32 <sup>b*</sup>	454.78±29.32 <sup>b**</sup>
Vitamin C+Rutin (Diab.) (100 and 50)	348.91±9.39 <sup>b***</sup>	32.28±1.33 <sup>b***</sup>	58.17±3.86 <sup>b***</sup>	394.56±18.12 <sup>b***</sup>

<sup>a</sup>Non-diabetic treated and diabetic groups were compared to control group. <sup>b</sup>Drugs treated diabetic groups were compared to diabetic vehicle group. \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Six rats were used in each group

**Effect on serum glucose, insulin and lipids:** Blood glucose levels mg dL<sup>-1</sup> were found 481.49±31.64 (mg dL<sup>-1</sup>) in untreated diabetic rats and that significantly reduced by rutin (p<0.05), vitamin C (p<0.05) and their combination (p<0.001) treatments, respectively. Here, also the protection level showed higher in combine treatment group compared to the individual treatments. Significant decrease in insulin (p<0.001) levels of diabetic rats were corrected only in co-treated group. Serum total cholesterol and triglyceride levels were significantly (p<0.01) increased in untreated diabetic rats compared to controls. Treatments groups showed decrease in the increase caused by streptozotocin injection although the protection was higher in co-treated group (Table 3).

**Effect on hepatic GSH levels:** Diabetic-induced oxidative stress significantly (p<0.001) reduced the GSH levels in hepatic cells. Rutin (p<0.05), vitamin C (p<0.01) and their

combination (p<0.001) increased the decrease values of GSH in diabetic rats. The protective effect was greater in combined treatment group (Fig. 1).

**Effect on hepatic MDA levels:** One of the lipidperoxidation marker MDA significantly (p<0.001) elevated in liver of diabetic rats and that increase was significantly reduced after treatments with rutin (p<0.05), vitamin C (p<0.001) and their combination (p<0.001) for five consecutive weeks. Protection against the oxidation-induced by streptozotocin injection found higher in combined treatment group (Fig. 2).

**Effect on hepatic SOD activities:** Superoxide dismutase activity was significantly (p<0.001) reduced in liver of untreated diabetic rats compared to normal rats. The treatments with rutin, vitamin C and their combination was significantly (p<0.05, p<0.05 and p<0.01) enhanced

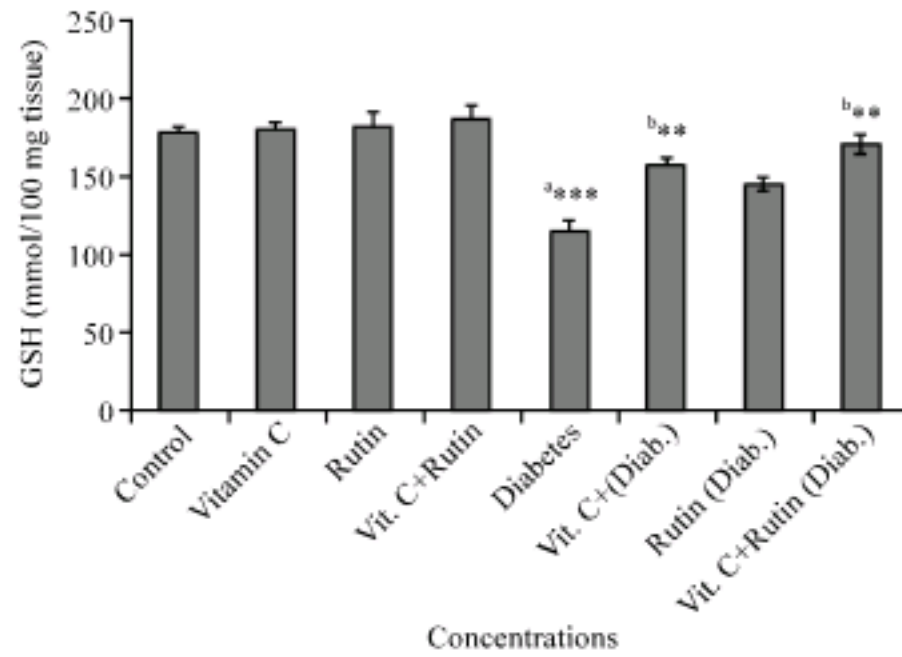


Fig. 1: Effect on rutin and vitamin C on liver glutathione concentrations of normal and diabetic rats. <sup>a</sup>Non-diabetic treated and diabetic groups were compared to control group. <sup>b</sup>Drugs treated diabetic groups were compared to diabetic vehicle group. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . Values were expressed as Mean $\pm$ SEM and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Six rats were used in each group

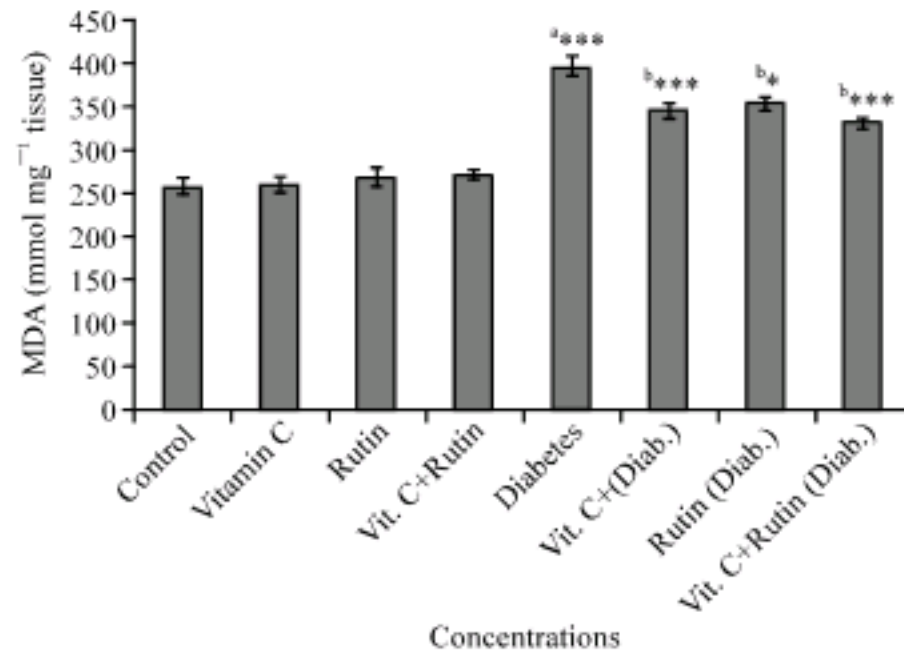


Fig. 2: Effect on rutin and vitamin C on liver malondialdehyde concentrations of normal and diabetic rats. <sup>a</sup>Non-diabetic treated and diabetic groups were compared to control group. <sup>b</sup>Drugs treated diabetic groups were compared to diabetic vehicle group. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . Values were expressed as Mean $\pm$ SEM and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Six rats were used in each group

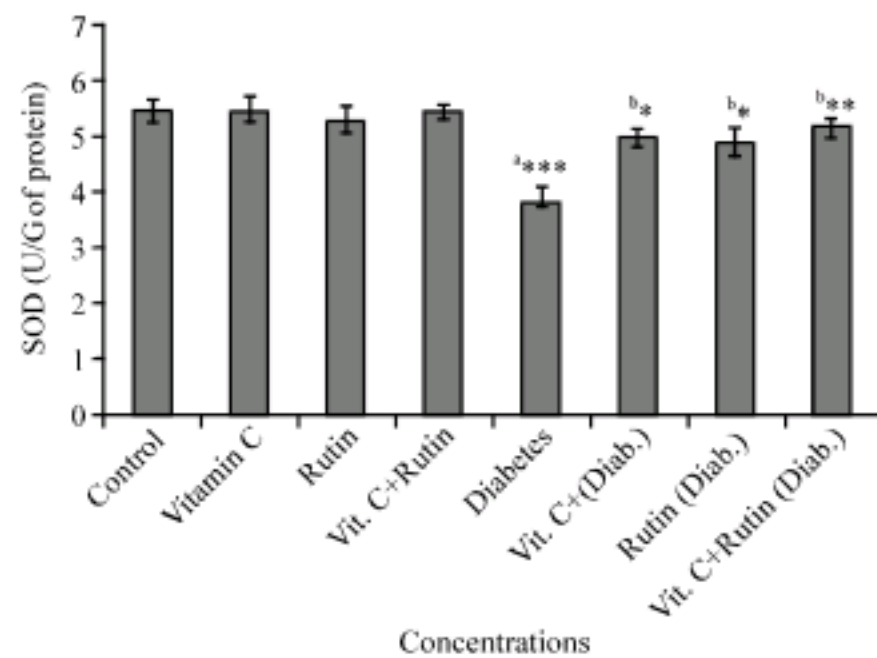


Fig. 3: Effect on rutin and vitamin C on liver superoxide dismutase activity in normal and diabetic rats. <sup>a</sup>Non-diabetic treated and diabetic groups were compared to control group. <sup>b</sup>Drugs treated diabetic groups were compared to diabetic vehicle group. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . Values were expressed as Mean $\pm$ SEM and analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Six rats were used in each group

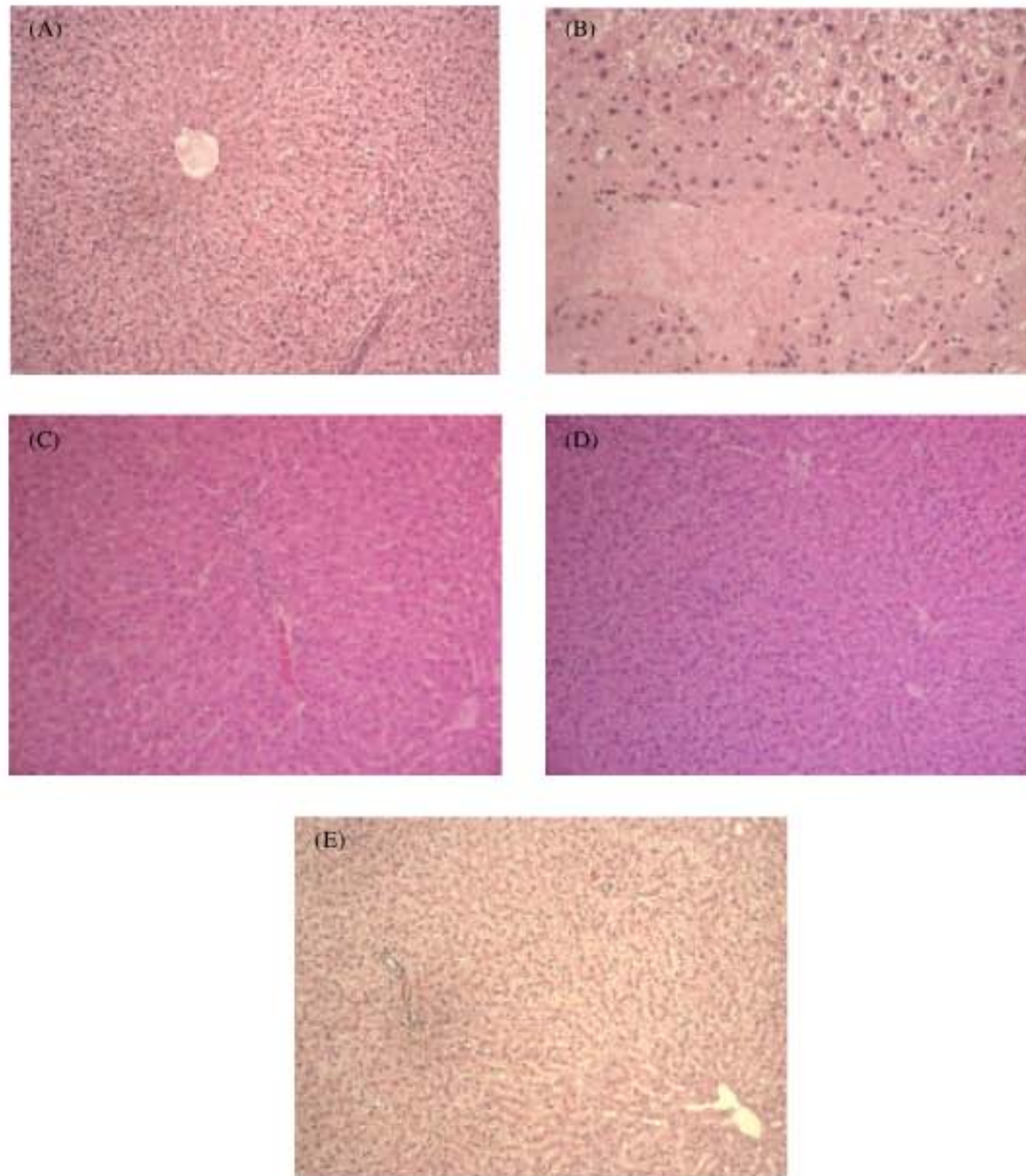


Fig. 4: (A) Normal liver showing portal triad and normal hepatocytes, (B) STZ-induced diabetic caused inflammation, hemorrhage and congestion in liver, (C) Rutin treated to the diabetic rats showing mild inflammation and congestion in liver, (D) Vitamin C treated diabetic rats showing mild inflammation in hepatocytes and (E) Combined (rutin+vitamin C) treatment to the diabetic rats showing normal hepatocyte

respectively the reduced activity (Fig. 3). However, here also the combined treatment group showed higher protection against diabetic-induced changes in liver.

**Effect on histopathology:** Histopathological examination of control and treated non-diabetic groups showed normal appearance of hepatic cells (Fig. 4A). STZ-induced diabetic liver (Fig. 4B) showed moderate inflammation, mild hemorrhage and mild congestion. Rutin treated diabetic liver showed (Fig. 4C) mild inflammation and sinusoidal dilatation whereas vitamin C treated diabetic liver (Fig. 4D) showed only mild inflammation. However, combined treated diabetic liver showed (Fig. 4E) normal hepatocytes.

## DISCUSSION

Streptozotocin is 1-methyl-1-nitrosourea attached to the carbon-2 position of glucose that causes  $\beta$ -cell animal models (Thulesen *et al.*, 1997). The glucose moiety of STZ allows preferential uptake of STZ in to  $\beta$ -cells, probably via glucose transporter-2 (GLUT-2), which are abundantly expressed in rodent  $\beta$ -cells of pancreas. The intracellular metabolism of STZ aggravates the situation by yielding potential free radicals such as nitric oxide which also precipitates the additional DNA strand breaks (Yang and Wright, 2002).

The destruction of  $\beta$ -cells during diabetes ultimately causes physic-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 (Brodsky, 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.

Aminotransferases, such as ALT and AST measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatases act as markers of biliary function and cholestasis. It is hypothesized that elevation in ALT, AST and ALP are considered as predictors of diabetes (Harris, 2005). Further, the elevation in the levels of these gluconeogenic enzymes whose gene transcription is suppressed by insulin could indicate impairment in insulin signaling rather than purely hepatocyte injury (O'Brien and Granner, 1991). Other potential explanations for elevated aminotransferase in insulin-resistant states include oxidative stress from reactive lipid peroxidation, peroxisomal  $\beta$ -oxidation and recruited inflammatory cells. In present study, serum elevated intracellular hepatic enzymes and cytokines in diabetic rats were significantly decreased by the treatments. TNF- $\alpha$  and IL-6 are highly involved with microphage activation and increased levels of these cytokines have been observed in insulin resistance stages and diabetic mellitus development (Pickup *et al.*, 2000). However, combined treatment (rutin+vitamin C) showed higher protection than the individual supplemented groups.

In agreement with the present results, the individual hypoglycemic effect on 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 combined treatments for 5 weeks to hyperglycemic rats found decrease and increase the glucose and insulin levels, respectively but the effect was more pronounced in the group of rats treated with combined vitamins (C and P). Earlier studies, recommends the combined treatments with antioxidant vitamin against diabetes mellitus (Hamden *et al.*, 2008; Aksoy *et al.*, 2005).

The levels of serum lipids are usually elevated in diabetes and such increase might lead to a higher risk for cardiovascular diseases (CVD) in some cases. Lowering of serum lipids level through dietary or drug therapy is associated with a decrease in the risk of CVD (Deedwania and Fonseca, 2005; Weiss and Sumpio, 2006). The results of this study revealed that daily treatment of rutin and vitamin C to the diabetic rats for 5 consecutive weeks brings the elevated serum lipids levels to the normal.

An imbalance between the production of ROS and free radicals and antioxidant capacity leads to a state of oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, protein and DNA (Kasai *et al.*, 1986; Dreher and Junod, 1996). Potential causes of increased oxidative stress in diabetes mellitus include increased production of ROS by NADPH oxidase, decreased antioxidant enzyme activity and reduced levels of GSH,  $\alpha$ -tocopherol and ascorbate (Aliciguzel *et al.*, 2003). Glutathione provides a first line of defense against ROS, as it can scavenge free radicals and reduce  $H_2O_2$ . The decreased concentration of GSH in liver might be due to consumption of GSH in the removal of peroxides (Yadav *et al.*, 1997). The impact that free radicals make on lipids is known as lipid peroxidation. Several animals' studies revealed that, STZ-induced diabetic increased the lipid peroxidation and they observed that of hyperglycemia can be taken under control by antioxidant vitamins (Kamalakkannan and Prince, 2006a, b). Similar results also been seen in the present study but the combined treatment showed higher effect. The decreased liver tissue levels of SOD activities in diabetes were restored with treatments found in the present study, indicating that the depression of SOD activity is an early and persistent effect on diabetes in some organs, which can be a response to increased production of  $H_2O_2$  and  $O_2$  by the outoxidation of glucose and nonenzymatic glycation. However the results showed that, combine treatment protected more than the individual vitamin against the oxidation-induced by diabetes.

In conclusion, from present study, diabetes in rats decreased body weights, increased intracellular enzymes, cytokines and lipids in serum and oxidative stress in liver. These results indicate that consumption of rutin and vitamin C in combination exerted beneficial effects of antioxidative defense systems against that imposed by diabetes mellitus.

#### ACKNOWLEDGMENT

The author is thankful to King Saud University, Riyadh, Kingdom of Saudi Arabia, for sanction of grant (2816025677) to conduct the present research.



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