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# Protective Effects of Simvastatin, an HMG-CoA Reductase Inhibitor, Against Oxidative Damage in Experimental Diabetic Rats

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**Abstract**: The role of oxidative stress has been reported in various diabetic complications. The present study was undertaken to evaluate the possible protective effects of simvastatin (SMV), a HMG-CoA reductase inhibitor against oxidative stress in streptozotocin (STZ) induced diabetic rats. Diabetes induced in rats by a single intraperitoneal injection of STZ (6o mg/Kg). A dose of 10 mg/kg of either SMV or glibenclamide (0.6 mg/kg) was orally administered 3 days prior to STZ administration and these supplementations were continued to the end of the study (5 weeks). The effects of SMV on blood glucose, hemoglobin (Hb), glycosylated hemoglobin (HbA1c), urea, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipid profiles, glutathione (GSH) and vitamin C were measured. Reduced GSH, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were measured in the liver and renal tissues. At the end of experiment, fasting blood glucose, HbA1c, BUN, creatinine, AST, ALT and lipid profiles were significantly increased, whereas Hb, GSH and vitamin C were significantly decreased in diabetic rats. Moreover, liver and renal tissue TBARS was markedly increased while GSH, SOD, CAT and GSH-Px were significantly decreased. *In vitro* studies on the effect of SMV on scavenging 1,1-diphenyl-2-picrylhydrazyl

(DPPH$^{\bullet}$) and total antioxidant capacity (TAC) also confirmed the free radical scavenging and antioxidant activity of SMV. Oral administration of SMV improved the lipid profile, liver and renal function tests. Tissue GSH, SOD, CAT and GSH-Px were significantly increased while TBARS was markedly reduced. Therefore, the present results revealed that SMV has a protective effect against STZ-induced oxidative damage by scavenging the free radicals generation and enhancement of the antioxidant defense mechanisms.
**Key words:** Simvastatin, diabetes, oxidative stress, streptozotocin.

## INTRODUCTION

Diabetes is a major threat to global public health, and the numbers of diabetic patients are rapidly increasing world-wide. More than 220 million people worldwide have diabetes and this number is likely to more than double by the year of 2030 [1]. Apart from this, more than 60% of the world population with diabetes will come from Asia [2]. It has already been established that chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and eventually failure of organs, especially the kidneys, nerves, heart, eyes and blood vessels [3]. About 50% of individuals with diabetes are affected with one or more of the above complications.

Oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation [4,5]. Streptozotocin (STZ) is frequently used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells [6]. The cytotoxic action of STZ is associated with the generation of reactive oxygen species (ROS) causing oxidative damage [7]. Oxidative stress increases due to several factors: enhancement of glucose auto-oxidation, stimulation of the polyol pathway, production of advanced glycation products and reduction in antioxidant defenses, such as depletion of cellular antioxidant levels and decreased antioxidative enzyme activity [3,8]. In diabetes there are significant changes such as increased lipid peroxidation, dyslipidemia and irregularities in the metabolism of proteins, lipids and carbohydrates [9]. Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cells and protect pancreatic islets against cytotoxic effects of STZ [6,10].

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statins) including simvastatin (SMV) have been previously demonstrated in diabetic patients the ability to reduce albuminuria [11-13]. Furthermore, administration of statins in experimental diabetes has previously been reported to be associated with a reduction in the renal expression of the prosclerotic cytokine, transforming growth factor- β1 (TGF-β1) [14,15], improve wound healing [16] and improve endothelial function in experimental diabetic rats [17]. Furthermore, a meta-analysis provided evidence that statins reduce the progression of retinopathy [18] and nephropathy [19].

One of the pleiotropic mechanisms receiving much attention is the antioxidant effect of statins [20,21]. Mechanisms for this may be the inhibition of oxidant formation by affecting NADPH-oxidase, blocking of the effects of ROS by up regulation of antioxidant enzymes, or an increase in nitric oxide bioavailability which neutralizes radicals [22]. ROS, including free radicals such as $HO^{\bullet}$ and $O_2^{\bullet-}$, and molecules such as hydrogen peroxide, are involved and contribute to the development of atherosclerosis [23]. Important sources of ROS are NADPH oxidases from endothelial cells, smooth muscle cells, fibroblasts, and infiltrated monocytes/macrophages [24]. Therefore, the present study was designed to investigate the effects of simvastatin on oxidative stress markers as well as on the antioxidative defense system in STZ-induced diabetic rats.

## MATERIALS & METHODS

### *Chemicals*

Simvastatin (SMV), Streptozotocin (STZ), hydrogen peroxide, glutathione, 5,5′-dithio-*bis*-(2-nitrobenzoic acid), thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane, glutathione reduced form, glutathione reductase, glutathione oxidized, NADPH-tetra salt and ethylenediamine tetra acetic acid (EDTA) disodium salt were purchased from Sigma-Aldrich Chemical (St Louis, MO, USA). Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) reagent assay kits were purchased from Cayman chemical (MI, USA). Glucotest (glucose urine strips) was purchased from Roche Diagnostics (Mannheim, Germany). All other chemicals were of the highest grade available commercially

### *Total Antioxidant Activity Determination*

The antioxidant activity of SMV was determined according to the thiocyanate method [25]. Ten milligrams of SMV was dissolved in 10 ml water. SMV at a various concentrations (25, 50 and 75 μg/ml) or standard sample (α-tocopherol) in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) was

added to linoleic acid emulsion. Five milliliters linoleic acid emulsion consists of 17.5 µg tween-20, 15.5 µl linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). On the other hand, 5.0 ml control consists of 2.5 ml linoleic acid emulsion and 2.5 ml potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37 ◦C in a glass flask and in the dark. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer, after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides formed. These compounds oxidize $Fe^{2+}$ to $Fe^{3+}$. The latter $Fe^{3+}$ ions form complex with $SCN^-$, which had maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without SMV or standards were used as blank samples. All data about total antioxidant activity are the average of duplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by following equation:

Inhibition (%) = $[A_0 - A_1/ A_0]$ x *100*

Where $A_0$ was the absorbance of the control reaction and $A_1$ was the absorbance in the presence of the sample of SMV.

***DPPH scavenging activity***

The free radical scavenging activity of SMV, buthylated hydroxyanisole (BHA) and α-tocopherol were measured using the method of Shimada et al. [26] with a slight modification. A 0.1 mM solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH.) in ethanol was prepared and 1 ml of this was added to 3 ml of SMV solution in ethanol at different concentrations (20–80 µg). After 30 minutes, a decrease in absorbance was measured at 517 nm and the actual decrease in absorption induced by the test compound was calculated by subtracting that of the control.

***Experimental Animals***

Forty male Sprague-Dawley rats, weighing 200–220 g, were obtained from King Fahd Medical Research Center, King Abdulaziz University (Jeddah, Saudi Arabia). Guides for the care and use of laboratory animals were approved by the local ethics committee at the King Abdulaziz University. Rats were housed in wire-floored cages under a 12 h light–dark cycle for at least 7 days prior to treatment and were fed standard laboratory chow and tap water *ad libitum*. The room temperature was kept at 22 ± 2°C. All stressful conditions were avoided. Rats were fasted overnight prior to the study and housed in mesh-bottomed cages to minimize coprophagia. Except for the last hour, water was supplied *ad libitum*.

***Experimental Induction of Diabetes***

Animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (60 mg/kg) in 0.1 mol/L citrate buffer (pH 4.5) [27]. The dosing volume was 1 ml/kg. To prevent fatal hypoglycemia, rats were kept on 5% glucose solution for 24 h after STZ injection. Successful induction of diabetes was confirmed by measuring the fasting blood glucose concentration in rats 6 h after injection of STZ. Rats with a fasting blood glucose level > 250 mg/dl were considered diabetic and included in the present study.

***Experimental Design***

One week after the administration of STZ and citrate buffer, control and diabetic rats were randomly assigned to treatments with SMV or glibenclamide. Rats were divided into five groups, with eight rats in each group, as follows: (i) Group I, control rats receiving vehicle solution (citrate buffer; 1 ml/kg/day); (ii) Group II, control rats receiving SMV (10 mg/kg/day) only; (iii) Group III, diabetic control rats injected with STZ (60 mg/kg bodyweight, i.p.) only; (iv) Group IV, diabetic rats treated with SMV (10 mg/kg per day) in aqueous solution via an intragastric tube 3 days after STZ treatment and continued for 5 weeks [28]; and (v) Group V, diabetic rats treated with glibenclamide (0.60 mg/kg per day) in aqueous solution daily via an intragastric tube for 5 weeks.

The following parameters were assessed in each of the study groups during the treatment period: daily fluid and food consumption, weekly body weight and blood glucose concentration (Glucostix strips tested in a glucometer; Abbott Laboratories, Medisense Products, Bedford, MA, USA). Food consumption was determined by subtracting leftovers from the diet provided to rats at 2 day intervals

After the last treatment (5 weeks), rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected in two separate tubes, with and without anticoagulant, for the estimation of glucose, hemoglobin, $HbA_{1c}$, lipid profiles, liver and kidney function tests. Liver and kidney tissues were excised immediately from the rats and stored in ice-cold containers.

***Preparation of liver and kidney homogenates***

After blood sample collection, the animals were killed then the liver and kidneys were removed immediately and placed in ice-cold 0.1 M phosphate buffer saline (PBS) with pH 7.5. The tissues were then

blotted dry and weighted. A 10% tissue homogenates (w/v) of the liver and kidney were prepared in PBS. Homogenates were then centrifuged at 1000 rpm for 10 min in a cooling centrifuge to remove the cell debris. Then the supernatants were placed in -80 $^{o}$C until further use to determine antioxidant enzymes activities and lipid peroxidation. Protein was estimated by the method of Lowry et al. [29].

## Biochemical Analysis

***Plasma glucose assay***

Plasma glucose levels were estimated using a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by the method of Trinder [30].

***Total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) assays***

Total Hb and HbA1c were estimated by Diagnostic kit-Bio Systems (Costa Brava, Spain).

***Plasma lipid profile***

Total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-C) were determined spectrophotometrically, using commercial kits. Low density lipoprotein (LDL) was calculated by using Friedewald formula [31].

***Liver Function Tests***

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed in serum using standard kits from Merck using colorimetric method [32]. The results were expressed as U/L.

***Kidney Function Tests***

Serum creatinine and urea were determined at 37$^{o}$C colorimetrically by the modified Jaffe method, and the modified Berthelot-Searcy enzymatic method respectively. They were assayed using reagents obtained from assay kits (Quimica Clinica Applicada, Spain). Urinary protein was quantified by the Biuret method using bovine serum albumin as the standard.

***Determination of plasma antioxidants***

Reduced glutathione (GSH) was estimated by the method of Ellman [33]. A 0.1 ml of plasma was precipitated with 5% TCA. The contents were mixed well for complete precipitation of proteins and centrifuged. To an aliquot of clear supernatant, 2.0 ml of 0.6 mM 5,50-dithiobis-2-nitrobenzoic acid (DTNB) reagent and 0.2 M phosphate buffer (pH 8.6) were added to obtain a final volume of 4.0 ml. The absorbance was read at 412 nm against a blank containing 5% TCA instead of sample. A series of standards treated in a similar way were also run to determine the glutathione content. The amount of glutathione was expressed as mg/dl in plasma.

Ascorbic acid (Vitamin C) concentration was measured by the method of Omaye et al. [34]. To 0.25 ml of plasma, 0.75 ml of 6% TCA was added and centrifuged (3500g, 20 min). To 0.25 ml of supernatant, 0.25 ml of dinitrophenylhydrazone (DNPH) reagent (2% DNPH and 4% thiourea in 4.5 M sulphuric acid) was added and incubated for 3 h at room temperature. After incubation, 1.25 ml of 85% sulphuric acid was added and color developed was read at 530 nm after 30 min.

***Lipid Peroxidation (LPO) Assay***

LPO was determined by measuring thiobarbituric acid reactive substances (TBARS) content in tissue homogenates according to the method of Uchiyama and Mihara [35], with some modifications. Briefly, 0.01 g liver or kidney tissue was homogenized with 0.9 ml of 1.15% KCl solution and the TBARS content was measured spectrophotometrically at 532 nm. The TBARS content was calculated based on a standard curve using 1,1,3,3-tetraethoxypropane as a standard.

***Glutathione (GSH) Assay***

GSH was measured in liver and kidney tissue homogenates by the reaction of the sulphydryl groups (SH) in the non-protein fractions with 5,5-dithiobis-(2-nitrobenzoic acid; DTNB or Ellman's reagent). The GSH levels were compared with a standard curve prepared using different known concentrations of GSH. The product was measured spectrophotometrically at 412 nm [33].

***Enzymatic Antioxidants Assays***

Superoxide dismutase (SOD) activity was determined in homogenates and erythrocytes according to the methods of Marklund and Marklund [36]. A colorimetric assay was performed that involved generation of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) to formazan by SOD, measured at 570 nm. The amount of MTT formazan was calculated by using a molar extinction coefficient *E*570 of 17 000 L/mol per cm. One unit of SOD was defined as the amount of protein required to inhibit MTT reduction by 50%.

Catalase (CAT) activity was measured according to the method described by Aebi [37].

One unit of CAT activity was defined as the amount of enzyme required to decompose 1 mmol $H_2O_2$ in 1 min. A 50 ml aliquot of tissue supernatant was added to a cuvette containing 1.95 ml of 50 mmol/L phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml freshly prepared 30 mmol/L $H_2O_2$. The rate of decomposition of $H_2O_2$ was measured spectrophotometrically at 240 nm for 1 min.

The activity of glutathione peroxidase (GSH-Px) was determined according to the method of Lawrence and Burk [38]. The assay mixture consisted of 2.0 ml of 75 mmol/L phosphate buffer (pH 7.0), 50 ml glutathione, 0.1 ml of 30 units/ml glutathione reductase, 0.1 ml of 15 mmol/L EDTA, 0.1 ml of 3 mmol/L NADPH and the appropriate amount of tissue supernatant to reach a final volume of 3.0 ml. The reaction was started by the addition of 0.1 ml of 7.5 mmol/L $H_2O_2$. The rate of change of absorbance during the conversion of NADPH to $NADP^+$ was recorded spectrophotometrically at 340 nm for 3 min. The GSH-Px activity for tissues was expressed as mmol GSH oxidized/min per mg protein.

***Determination of protein content***

The protein content of tissue homogenates was determined by the Lowry protein assay using bovine serum albumin as the standard [29].

## Statistical analysis

The Graph Pad (ISI Software, Philadelphia, PA, USA) computer program was used to conduct regression analysis and to plot collected data. Data are expressed as the mean ± SEM. Results were assessed using one-way ANOVA followed by Tukey–Kramer multiple comparisons tests using Graph Pad Instat (Version 3.06; Graph Pad Software La Jolla, CA, USA). $P < 0.05$ was used as the criterion for significance.

**Table 1 In vitro total antioxidant and free radical scavenging activities of simvastatin (SMV), buthylated hydroxyanisole (BHA), and α-tocopherol (α-Toc).**

| Groups | Total antioxidant activity (Inhibition of lipid peroxidation %)[a] | Free radical scavenging activity ($DPPH^{\cdot}$ μM) |
|---|---|---|
| α-Toc (80 μg) | 46.2 | 76 |
| BHA (80 μg) | 93.7 | 83 |
| SMV (20 μg) | 41.2 | 38 |
| SMV (40 μg) | 60.2 | 44 |
| SMV (80 μg) | 98.3 | 72 |

Values are the mean ± SEM for eight animals in each group.

[a]The antioxidant activity of different doses of simvastatin (20-80 μg) was determined by the thiocyanate method. The peroxide values were determined by reading the absorbance at 500 nm after reaction with $FeCl_2$ and thiocyanate. [b]Free radical scavenging activity of different doses of SMV, BHA, and α-tocopherol by 1,1-diphenyl-2-picrylhydrazyl radicals.

**Table 2 Effect of simvastatin(SMV) and glibenclamide supplementation on fluid and food intake and body weight of rats in the different experimental groups.**

| Groups | Fluid intake (ml/day) | Food intake (g/day) | Weight gain (g/day) |
|---|---|---|---|
| Control | 28 ± 3 | 15.3 ± 0.2 | 4.90 ± 0.03 |
| SMV | 33 ± 2 | 18.2 ± 0.42 | 4.41 ± 0.06 |
| Diabetic | 96 ± 5* | 25.7 ± 0.3* | 2.96 ± 0.04* |
| Diabetic + SMV | 46 ± 4*# | 22.1 ± 0.5* | 3.30 ± 0.05*# |
| Diabetic + Glibenclamide | 36 ± 2*# | 23.0 ± 0.2* | 3.71 ± 0.03*# |

Values are the mean ± SEM for eight animals in each group.

*$P < 0.001$ compared with the control group.

#$P < 0.05$ compared with the untreated diabetic group.

**Table 3 Effect of simvastatin (SMV) and glibenclamide supplementation on blood glucose, haemoglobin (Hb) and glycosylated haemoglobin ($HbA_{1c}$) of rats in different experimental groups**

| Groups | Blood glucose (mg/dl) | Hb (g/dl) | $HbA_{1c}$ (% Hb) |
|---|---|---|---|
| Control | 84.3 ± 4.2 | 12.8 ± 0.41 | 5.8 ± 0.32 |
| SMV | 87.5 ± 3.6 | 13.0 ± 0.36 | 6.1 ± 0.21 |
| Diabetic | 336 ± 9.8* | 9.8 ± 0.43* | 12.7 ± 0.5* |
| Diabetic + SMV | 260 ± 7.3*# | 10.6 ± 0.52 | 10.2 ± 0.33*# |
| Diabetic + Glibenclamide | 127.5 ± 6.4*# | 12.3 ± 0.48# | 6.7 ± 0.03# |

Values are the mean ± SEM for eight animals in each group.
*$P < 0.001$ compared with the control group.
#$P < 0.05$ compared with the untreated diabetic group.

**Table 4 Effect of simvastatin (SMV) and glibenclamide supplementation on serum total cholesterol, triglycerides, high-density lipoprotein–cholesterol (HDL-C) and low-density lipoprotein–cholesterol (LDL-C) triglycerides for rats in different experimental groups**

| Groups | Total cholesterol (mg/dl) | Triglycerides (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) |
|---|---|---|---|---|
| Control | 77.4 ± 4.4 | 57.3 ± 1.8 | 42.5 ± 1.3 | 23.6 ± 1.1 |
| SMV | 70.8 ± 3.1 | 50.1 ± 2.1 | 44.6 ± 1.6 | 16.6 ± 1.3 |
| Diabetic | 110.3 ± 5.1* | 128.6 ± 3.6* | 31.2 ± 2.2* | 53.3 ± 3.4* |
| Diabetic + SMV | 84.2 ± 2.9*# | 64.2 ± 2.4# | 50.7 ± 2.8*# | 20.6 ± 1.5*# |
| Diabetic + Glibenclamide | 89.3 ± 3.6*# | 68.7 ± 3.6*# | 45.3 ± 1.9# | 30.6 ± 1.7*# |

Values are the mean ± SEM for eight animals in each group.
*$P < 0.001$ compared with the control group.
#$P < 0.05$ compared with the untreated diabetic group.
SMV, simvastatin

## RESULTS

### In vitro antioxidant of SMV

The effects of various amounts of SMV on the peroxidation of linoleic acid emulsion are shown in table 1. The antioxidant activity of SMV in the concentration of 40 µg and 80 µg were greater than that of α-tocopherol 80 µg and showed 60.2% and 98.3% inhibition on peroxidation of linoleic acid, respectively, greater than that of α-tocopherol (46.2%). The antioxidant activity of SMV in the concentration of 20 µg was close to that of α-tocopherol and showed 41.5% inhibition on peroxidation of linoleic acid. Also, table 1 illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of standards and SMV in a concentration dependant manner. The scavenging effect of SMV and standards on the DPPH radical decreased in the order of BHA> α-tocopherol> SMV. These results indicated that SMV and standards have a noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration of SMV in a concentration dependant manner.

### Fluid and food intake, bodyweight and organ weight

**Table 2** shows significant differences in fluid and food intakes and bodyweight gain between control and diabetic rats. Increased fluid and food intakes and decreased bodyweight were observed in diabetic rats compared with control rats. Administration of SMV or glibenclamide tended to increase bodyweight to that seen in untreated control rats and the effect was more pronounced in the group of rats treated with glibenclamide. There was no significant change in control rats treated only with SMV.

### Blood glucose, total hemoglobin and $HbA_{1c}$

Levels of blood glucose, total hemoglobin (Hb) and glycosylated ($HbA_{1c}$) in control and diabetic rats are given in Table 3. The fasting blood glucose levels and $HbA_{1c}$ were significantly higher in diabetic animals when compared to control rat values, whereas

Hb levels were decreased significantly in diabetic rats compared to control rat values. Treatment of diabetic rats with SMV non-significantly increased total Hb and significantly (P<0.05) decreased blood glucose level and $HbA_{1c}$ compared to untreated diabetic rat values. On the other hand, glibenclamide significantly reduced fasting blood glucose level and $HbA_{1c}$ when compared with untreated diabetic animals (P<0.001).

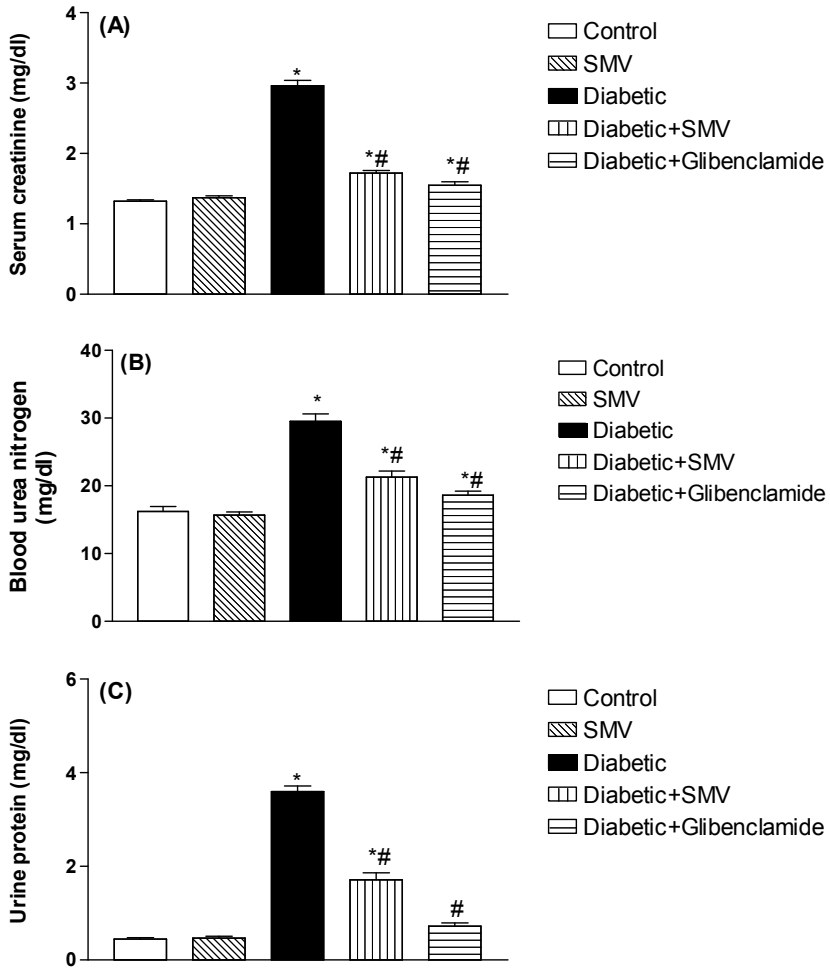
**Lipid profiles**

In diabetic rats, there was a significant increase ($P$<0.001) in serum cholesterol, triglyceride and LDL-C levels and concomitant decrease in the level of HDL-C compared to untreated diabetic rats. Oral administration of SMV significantly decreased the levels of serum cholesterol, triglycerides and LDL-C with concomitant increase in the level of HDL-C in diabetic rats compared to untreated diabetic rats. Furthermore, results obtained following treatment with SMV was comparable to those obtained following glibenclamide treatment (Table 4).

**Serum creatinine, BUN and urine protein**

Figure 1 shows a significant increase (P<0.05) in the serum creatinine, BUN and urine protein in untreated diabetic rats when compared with control group. STZ-induced almost a two-fold increase in the creatinine and urea levels and an eight-fold increase in the urine protein levels over the controls rats. All the indices were reduced to near control levels when the SMV was administered to the untreated diabetic rats. In the case of control and SMV only treated rats, the levels of the abovementioned parameters remained unaltered.

**Figure 1. Effect of simvastatin (SMV) and glibenclamide treatment on serum creatinine (A), blood urea (B) and urinary protein (C) in normal and streptozotocin-induced diabetic rats.**




Data are expressed as means ± SEM ($n$ = 8).
*Significantly different from control group ($P$ < 0.01).
#Significantly different from diabetic-untreated group ($P$ < 0.01).

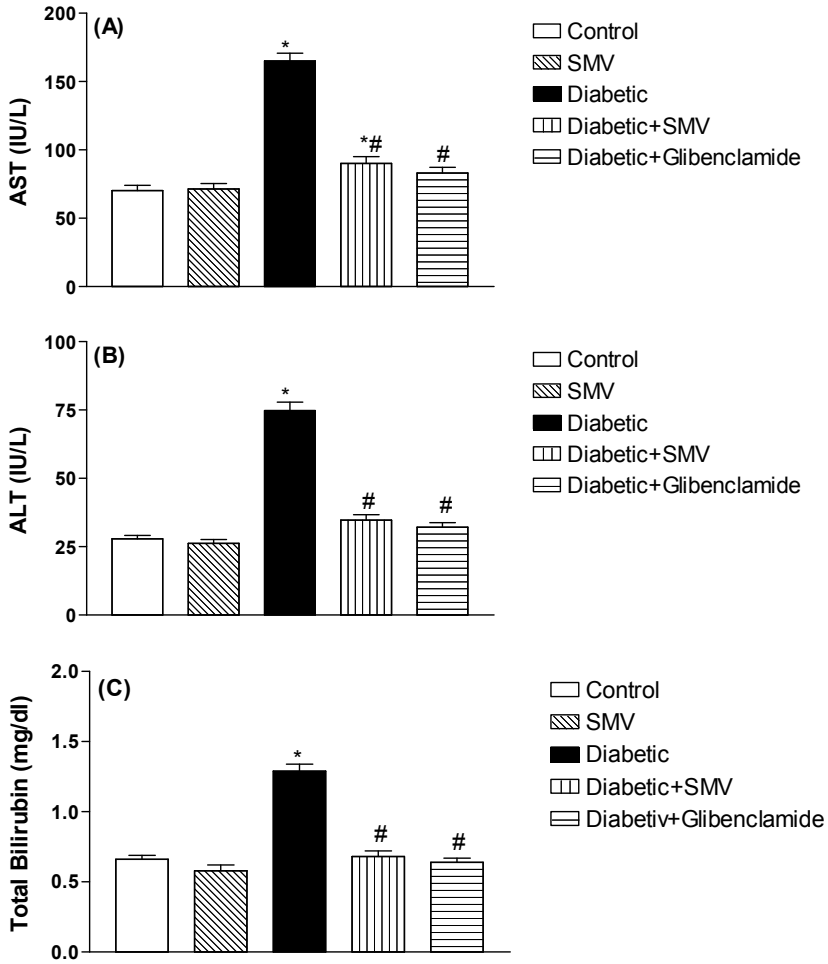
**Serum ALT, AST and total bilirubin**

The effect of SMV and glibenclamide on STZ induced liver damage in rats with reference to the changes in the level of AST, ALT and total bilirubin is shown in Figure 2. Diabetic rats showed significant increase in the levels of AST, ALT and total bilirubin as compared to the normal control group. Whereas blood samples analysis from the animals treated with SMV or glibenclamide showed significant decrease in the levels of serum marker enzymes and total bilirubin to the near normal value.

**Plasma Non-enzymatic antioxidants**

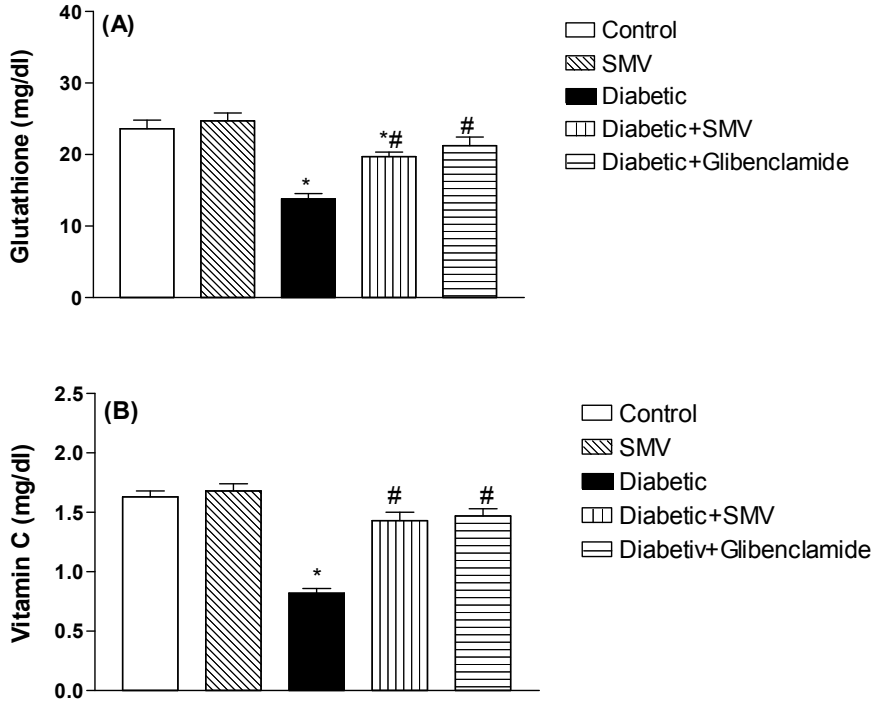
The levels of non-enzymatic antioxidants in normal and diabetic rats are given in Figure 3. There was a significant ($p < 0.05$) decrease in the levels of GSH (13.8 ± 0.78 vs. 23.6 ± 1.72 mg/dl) and vitamin C (0.82 ± 0.06 vs. 1.63 ± 0.11 mg/dl) in diabetic control rats than normal rats. Oral administration of SMV and glibenclamide to diabetic rats lead to a significant ($p < 0.05$) increase in the plasma levels of GSH and vitamin C.

**Figure 2.** Effect of simvastatin (SMV) and glibenclamide treatment on (A) serum aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT) and (C) total bilirubin in normal and streptozotocin-induced diabetic rats.




Data are expressed as means ± SEM ($n = 8$).
*Significantly different from control group ($P < 0.01$).
#Significantly different from diabetic-untreated group ($P < 0.01$).

**Figure 3. Effect of simvastatin (SMV) and glibenclamide treatment on (A) plasma glutathione and (B) vitamin C in normal and streptozotocin-induced diabetic rats.**




Data are expressed as means ± SEM ($n$ = 8).
*Significantly different from control group ($P < 0.01$).
#Significantly different from diabetic-untreated group ($P < 0.01$).

**Reduced GSH in liver and kidney homogenates**

**Figure 4** (A and B) shows the GSH content in liver and kidney homogenates of control and diabetic rats. There was a significant decrease in the concentration of GSH in the liver and kidney homogenates (50 and 36%, respectively) in diabetic rats compared with control rats. Administration of SMV and glibenclamide increased the GSH content in the liver (74 and 44 %, respectively) and kidney homogenates (35 and 18%, respectively) of the diabetic group of rats. The effect was more pronounced in the group of rats treated with SMV and not significant in the groups treated with glibenclamide ($P > 0.05$).

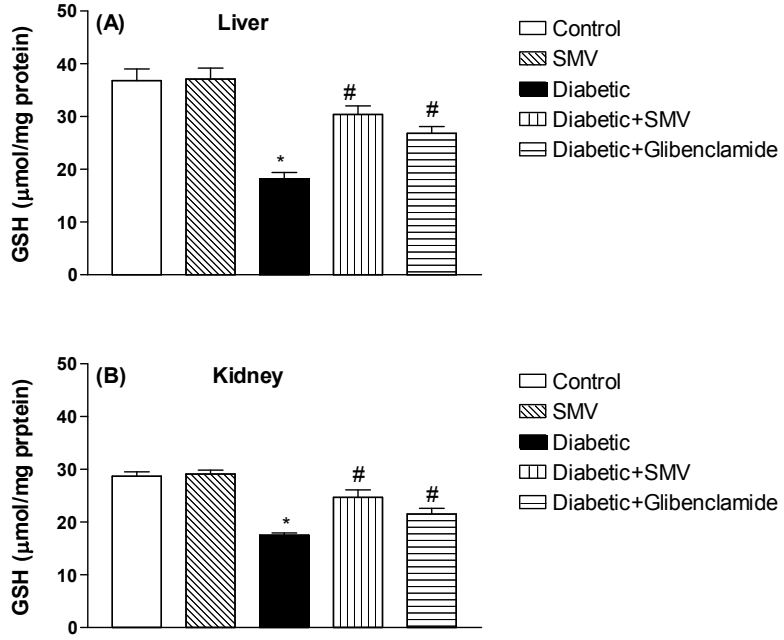
**Lipid peroxidation**

Liver and kidney TBARS levels, an index of lipid peroxidation, were higher in diabetic rats (3.96 ± 0.36 and 5.14 ± 0.12 nmol/mg protein, respectively) compared with control rats (2.03 ± 0.14 and 1.96 ± 0.11 nmol/mg protein, respectively) and markedly decreased ($P<0.001$) by SMV. Level of TBARS in liver and kidney had no significant change in healthy rats treated with SMV (**Figure 5 A and B**).

**Antioxidant enzymes**

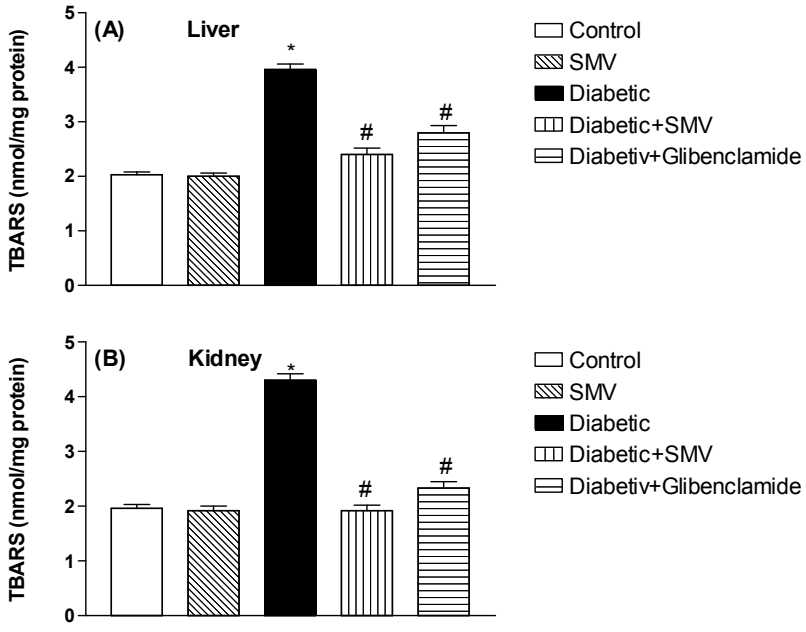
**Figures 6 and 7** shows SOD, CAT and GSH-Px activities in liver and kidney homogenates of control and diabetic rats. In the diabetic group, there was a significant reduction in SOD, CAT and GSH-Px activities in the liver (64, 49 and 40%, respectively) and kidney (43, 34 and 39%, respectively) homogenates compared with the control group. Treatment with SMV and glibenclamide increased SOD, CAT and GSH-Px activity in diabetic rats and the effect was more pronounced in the liver than in the kidney.

**Figure 4. Effect of simvastatin (SMV) and glibenclamide treatment on (A) liver glutathione and (B) kidney glutathione in normal and streptozotocin-induced diabetic rats.**



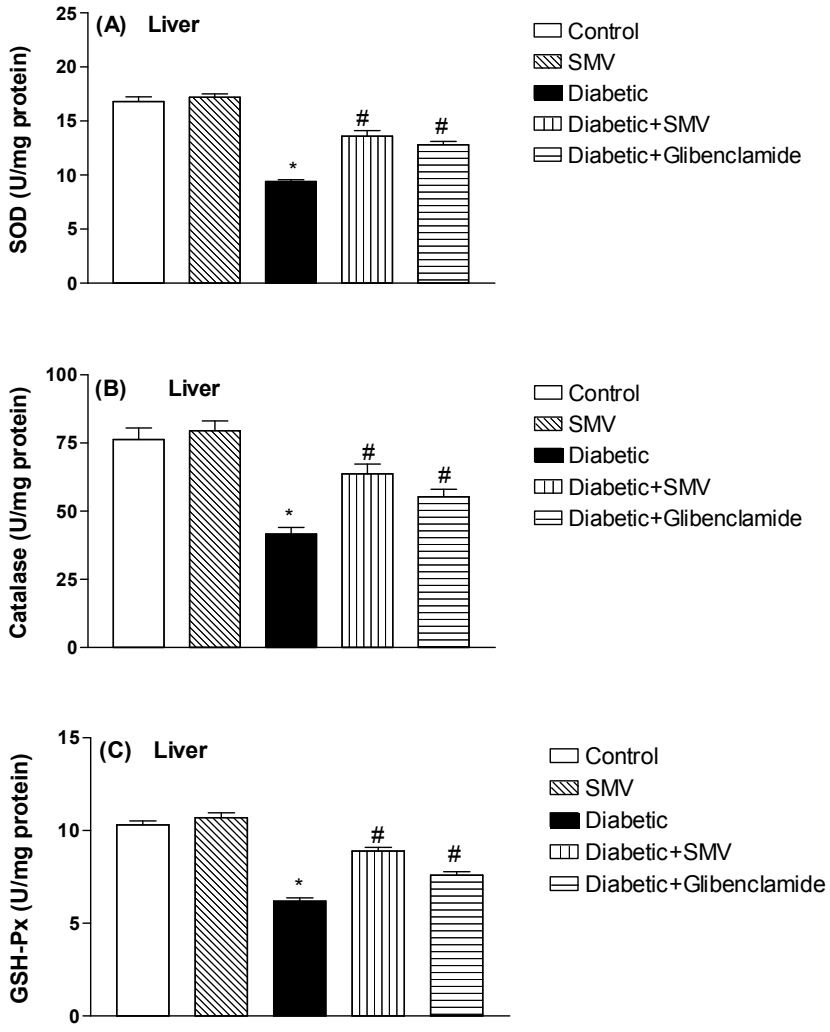

Data are expressed as means ± SEM ($n$ = 8).
*Significantly different from control group ($P < 0.01$).
#Significantly different from diabetic-untreated group ($P < 0.01$).

**Figure 5. Effect of simvastatin (SMV) and glibenclamide treatment on liver thiobarbituric acid reactive substances (TBARS) in liver (A) and kidney (B) in normal and streptozotocin-induced diabetic rats.**



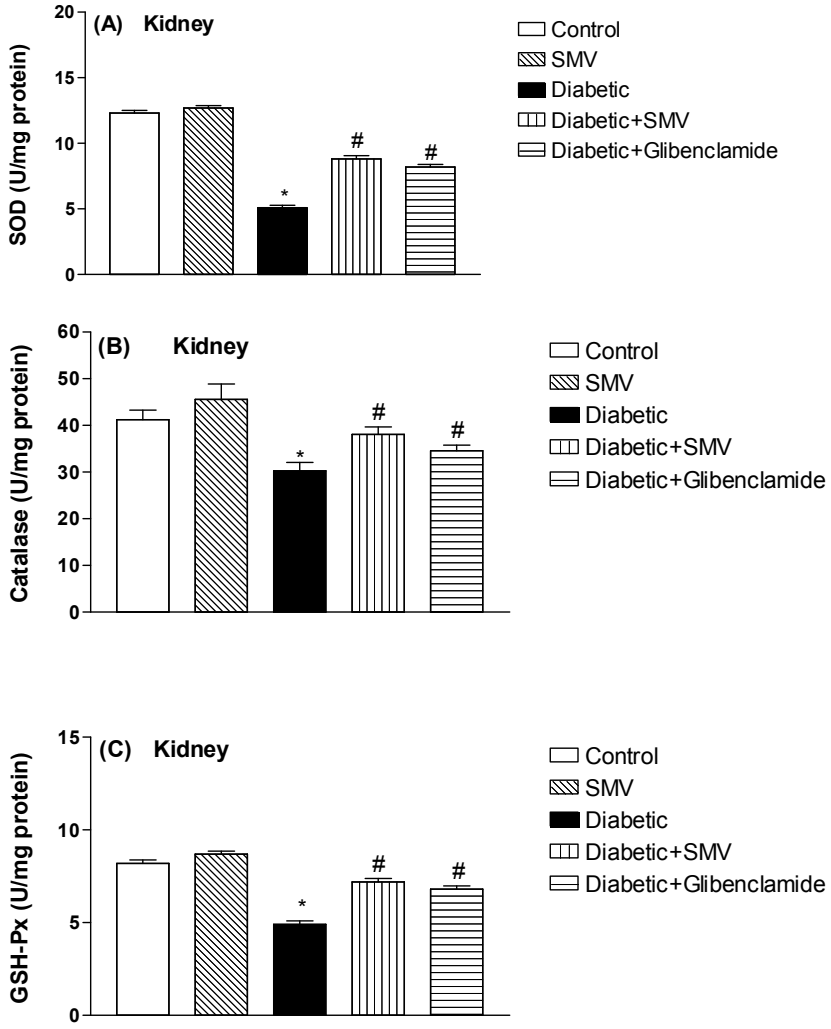

Data are expressed as means ± SEM ($n$ = 8).
*Significantly different from control group ($P < 0.01$).
#Significantly different from diabetic-untreated group ($P < 0.01$).

**Figure 6. Effect of simvastatin (SMV) and glibenclamide treatment on liver enzymatic antioxidants; (A) SOD, (B) CAT and (C) GSH-Px in normal and streptozotocin-induced diabetic rats.**




Data are expressed as means ± SEM ($n$ = 8).
SOD, superoxide dismutase ; CAT, catalase; GSH-Px, glutathione peroxidase.
*Significantly different from control group ($P < 0.01$).
#Significantly different from diabetic-untreated group ($P < 0.01$).

**Figure 7. Effect of simvastatin (SMV) and glibenclamide treatment on kidney enzymatic antioxidants; (A) SOD, (B) CAT and (C) GSH-Px in normal and streptozotocin-induced diabetic rats.**




Data are expressed as means ± SEM ($n$ = 8).
SOD, superoxide dismutase ; CAT, catalase; GSH-Px, glutathione peroxidase.
*Significantly different from control group ($P < 0.01$).
#Significantly different from diabetic-untreated group ($P < 0.01$).

## DISCUSSION

Diabetes mellitus is a highly prevalent chronic illness. More than 220 million people worldwide have diabetes and this number is likely to more than double by the year of 2030 [1]. It has been reported in various studies that increased oxidative stress may play a role in the pathogenesis and progression of diabetic tissue damage [39,40]. Chronic hyperglycemia in diabetic patients or animals can cause oxidative stress, depleting the activity of the antioxidative defense system and resulting in elevated levels of oxygen free radicals [6]. Consequences of oxidative stress induces the production of highly reactive oxygen radicals that are toxic to cells, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides and lead to organs oxidative damage [41]. Reduced oxidative stress in the diabetic condition had been observed in experimental animals following the administration of certain antioxidants [42].

In the current study, administration of STZ resulted in a significant increase in the blood glucose level and reduction in plasma insulin level. Persistent hyperglycemia results in glycation of Hb that lead to the formation of HbA1c [43]. The observed increase in the levels of HbA1c with a concomitant decrease in Hb in the experimental diabetic rats implies the oxidation of sugars, extensive damage to both sugars and proteins in the circulation and reinforcing the cycle of oxidative stress and damage. Agents with antioxidant or free radical scavenging power have been shown to inhibit oxidative reactions associated with glycation [44]. In this regard treatment with SMV significantly reversed the imbalance in the oxidative stress status.

In the present study, lipid profile markers, such as total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride in blood, significantly increases from their normal level in diabetic rats. These results further confirm that there is a strong correlation between oxidative stress and diabetes occurrence. Antioxidants such as resveratrol, vitamin C and vitamin E have been reported to reduce STZ-induced oxidative damage [5,45]. Similarly, we observed that administration of SMV significantly restored abnormal levels of lipid profile markers in blood in diabetic rats. This suggests that SMV may improve lipid dysfunction of diabetic rats and retard development of diabetic complications. Our results are in agreement with previous reports [19,46].

Elevated activities of serum AST, ALT and total bilirubin are a common sign of liver disease and are observed more frequently among people with diabetes than in the general population (Arkkila et al., 2001). SMV treatment prevented the increase in these enzymatic activities in serum that was caused by STZ administration. Our results are agreement to those of Imaeda [47] who also found that, antioxidants inhibited the increase in serum levels of AST and ALT in STZ-treated mice. Increased serum levels of urea and creatinine, indicators of impaired renal function [19], were observed in the diabetic rats might indicate renal damage. Treatment with SMV significantly decreased serum creatinine and urea. These data suggest that SMV may help in the repair of renal damage.

During diabetes, an increased oxidative stress in certain tissues may lead to a rise in the rate of lipid peroxidation [8]. The formation of the lipid peroxide product, TBARS, was measured in tissue as an index of increased lipid peroxidation in diabetic liver and kidneys [48]. Our present study showed a significant increase of tissue thiobarbituric acid reactive substances (TBARS) level in diabetic rats. The increased TBARS content of diabetic rats suggests that peroxidative injury may be involved in the development of diabetic complications. TBARS levels in liver and kidneys were significantly lower in the SMV and glibenclamide treated groups compared to the diabetic control rats. Recently, agents with antioxidant or free radical scavenging power have been shown to inhibit oxidative reactions associated with lipid peroxidation [5,45]. The above result suggests that the SMV may exert antioxidant effects and protect the tissues from lipid peroxidation.

Non-enzymatic antioxidants such as GSH and vitamin C play an excellent role in preventing the cells from oxidative damage. GSH is an intra cellular thiol rich tripeptide, which plays a major role in the protection of cells and tissue structures [49]. GSH is required for the recycling of vitamin C [50] and acts as a substrate for GSH-Px that is involved in preventing the deleterious effect of free radicals [51]. In our study, diabetic rats exhibited decreased level of GSH, which might be due to increased utilization of GSH for scavenging free radicals by GSH-Px. Administration of SMV reversed GSH level in plasma of diabetic rats, which could be due to the low peroxidisability and thus its low utilization. The present findings of decreased GSH in liver and kidneys are consistent with the studies of Panda et al. [48].

Vitamin C is a well known physiological hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species [52]. The observed significant decrease in the level of plasma vitamin C could be caused by increased utilization of vitamin C as an antioxidant defense against ROS or by a decrease in GSH, which is required for the recycling of vitamin C. Treatment with SMV brought vitamin C to near normal levels which could be as a result of decreased membrane damage as evidenced by the antioxidant nature.

In the present study, antioxidant enzyme activities were significantly decreased and MDA levels were significantly increased in diabetic rats. Reactive oxygen species-induced oxidative damage has been implicated in the pathogenesis of several disorders, including diabetes mellitus [53]. Oxidative stress is the imbalance between production and removal of reactive oxygen species (ROS). Increased oxidative stress, which contributes substantially to the pathogenesis of diabetic complications, is the consequence of either enhanced ROS production or attenuated ROS-scavenging capacity. Several studies have demonstrated both lower non-enzymatic antioxidant levels and enzymatic antioxidant activities in streptozotocin-induced diabetic rats [48,9]. Recently, Sefi [54] have reported an elevated lipid peroxidation

and lowered antioxidants in streptozotocin-induced diabetes mellitus.

Enzymatic antioxidants (SOD, CAT and GSH-Px) form the first line of the antioxidant defense mechanism to protect the organism from ROS-mediated oxidative damage [50]. In the current study, SOD, CAT and GSH-Px showed lower activities in liver and kidney during diabetes and the results agree well with the earlier published data [55,56]. The decreased activities of SOD, CAT and GSH-Px may be a response to increased production of $H_2O_2$ and $O_2^{\cdot -}$ by the auto-oxidation of the excess of glucose and non-enzymatic glycation of proteins [57]. Pigeolet et al. [58] have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The decreased activity of SOD and CAT could also be due to their decreased protein expression levels in the diabetic condition, as recently reported in liver [59]. The decreased GSH-Px activity represents a compensatory mechanism to degrade $H_2O_2$. Treatment of the diabetic rats with SMV restored the altered antioxidant enzyme activities significantly ($p < 0.001$).

In conclusion, the present investigation showed that SMV possesses an antioxidant activity and also protects lipid peroxidation and enhances its effect on enzymatic antioxidant (SOD, CAT and GSH-Px) and non-enzymatic antioxidant (GSH and vitamin C) defense. This activity contributes to the protection against oxidative damage in STZ induced diabetes.

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