

# ***Portulaca oleracea* Leaf Aqueous Lyophilized Extract Reduces Hyperglycemia and Improves Antioxidant Status of Red Blood Cells and Liver in Streptozotocin-Induced Diabetic Wistar Rats**

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**Abstract:** The aim of this study was to investigate the antihyperglycemic and antioxidant effects of *Po* (*Portulaca oleracea*) lyophilised aqueous extract in diabetic male Wistar rats. Diabetes was induced intraperitoneally by a single injection of STZ (streptozotocin) (60 mg/kg bw (body weight)). Twenty diabetic rats, weighing  $263 \pm 5$  g, were divided into two groups fed a casein diet supplemented or not with *Po* extract (1 g/kg bw), for four weeks. Control group ( $n = 6$ ) received 0.23–0.25 mL of citrate buffer and was fed a standard diet during the experiment. The study was carried out at Oran University, Algeria and the entire experiments lasted from September 2011 to July 2012. Blood was obtained from the abdominal aorta of rats after fasting overnight and standard methods were used for the extraction of spices, determination of glycemia, insulinemia, lipid peroxidation and antioxidant enzymes activities. *Portulaca oleracea* treated compared to untreated rats, glycemia and HbA1c values were respectively 2.8- and 1.7-fold lower. TBARS (thiobarbituric acid reactive substances) concentrations were reduced in RBC (red blood cells) (–54%) and plasma (–65%). Moreover, in liver and kidney, TBARS values were respectively 1.8- and 2-fold lower. SOD (superoxide dismutase) and GSH-Px (glutathione peroxidase) activities were increased respectively by +38% and +85%, in liver. GSSG-Red (glutathione reductase) activity was 1.9-fold higher in kidney, while CAT (catalase) was improved in kidney (+48%). In RBCs, SOD, GSH-Px, GSSH-Red and CAT activities were increased by 31%, 42%, 56% and +50%, respectively. These data have cast a new light on the actions of *Portulaca oleracea* and its antioxidant potential benefits in preventing diabetes and its complications.

**Key words:** *Portulaca oleracea*, rats, diabetes, streptozotocin, antioxidative enzymes, TBARS.

## **1. Introduction**

DM (diabetes mellitus) is a major health problem worldwide [1]. Moreover, it is known that DM is a metabolic disorder characterized by hyperglycemia, abnormal lipids and proteins metabolism resulting from either an absolute or relative deficiency of insulin secretion or action [2]. Such disorders lead to various profound secondary complications, like as atherosclerosis, hypertension, hypercholesterolemia, myocardial infarction, ischemic attacks, retinopathy and nephropathy [3]. Several previous investigations

have confirmed the role of oxidative stress in developmental diabetic-mediated damages [4], possibly by oxygen free-radical formation, nonenzymatic protein glycosylation, auto-oxidation of glucose [5, 6], alteration of antioxidant enzymes [6] and lipid peroxides production [7]. A reverse correlation was observed between the intake medicinal and dietary plants and the incidence of diabetes [8], this health benefit is partly owed to plant bioactive components called “polyphenols”, which exhibit antioxidant properties [9]. *Portulaca oleracea* L., locally named “Redjila”, is widely used as an ingredient in a green salad. Tender stems and leaves are usually eaten raw, alone or with other greens.

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They are also cooked or pickled for consumption. Recent research demonstrated that this plant is a good source of compounds with a positive impact in human health. Those compounds include omega-3 fatty acids, phenolics, coumarins and alkaloids. The aqueous extracts of *Po* (*Portulaca oleracea*) show no cytotoxicity or genotoxicity, and have been certified safe for daily consumption as a vegetable [10]. Thus, the purpose of the present study was to evaluate in streptozotocin-induced diabetic rats, the effects of the lyophilized aqueous extract of *Po* leaves on hyperglycemia and oxidative stress, in red blood cells and several tissues.

## 2. Experimental Section

### 2.1 Plant Material

*Portulaca oleracea* was collected in Southern of Algeria (Touggourt), between March and April 2012, identified taxonomically and authenticated by the Botanical Research Institute of Oran University (voucher specimen number *Po*1965). The plant material was stored at room temperature in a dry place before use. Fresh aerial parts (leaves) of the plant were dried at ambient temperature (24 °C) for seven days and ground to a powder. The *Po* extract was prepared as follows: 50 g of the powdered aerial parts was refluxed at 60~70 °C in 500 mL distilled water for 30 min and the decoction was filtered with cotton wool. The filtrate was concentrated at 65 °C by a rotavapor (Buchi Labortechnik AG, Postfach, Switzerland) under a reduced pressure and frozen at -70 °C before lyophilization (Christ, alpha 1-2 LD). The crude yield of the lyophilized extract was approximately 25% (wt/wt (weight/weight)). It was stored at ambient temperature until further use.

### 2.2 Experimental Animals and Treatment

Male Wistar rats (Iffa Credo, l'Arbresle, Lyon, France), weighing  $263 \pm 5$  g were housed under standard environmental conditions ( $23 \pm 1$  °C,  $55 \pm 5\%$  humidity and a 12 h light/dark cycle) and

maintained with free access to water and a standard diet *ad libitum*. The *General Guidelines on the Use of Living Animals in Scientific Investigations* [11] were followed, while the protocol and use of rats were approved by our institutional committee on animal care and use. Diabetes was induced by intraperitoneal injection of STZ (streptozotocin) (Sigma, St. Louis, Mo, USA) at a dose of 60 mg/kg bw (body weight) STZ was dissolved in 0.05 mol/L cold sodium citrate buffer, pH 4.5 immediately before use. After 48 h, hyperglycemia was confirmed using a Glucometer (Accu-Chek® active, Germany). Only animals with fasting blood glucose levels greater than 16 mmol/L were considered diabetic and then included in this study. Diabetic rats ( $n = 12$ ) were randomly divided into two groups. The untreated group received a casein diet and the treated group received the same diet supplemented with the *Po* extract (1%), for four weeks. Control group ( $n = 6$ ) received 0.23~0.25 mL of citrate buffer and was fed a standard diet during the experiment. The ingredient composition of the diets is shown in Table 1.

### 2.3 Blood and Tissue Samples

After the four weeks of the experiment, the rats were fasted overnight and anesthetized with chloral hydrate 10% (3 mL/kg bw) and euthanized with an overdose. Blood was obtained from the abdominal aorta of rats and collected into tubes containing ethylenediaminetetraacetic acid- $\text{Na}_2$  (Sigma, St. Louis, Mo, USA). Blood plasma was prepared by lowspeed centrifugation at 1,000 g for 20 min at 4 °C. The RBC (red blood cells) sediment was washed twice with ice-cold distilled water (1/4, v/4v (one volume for four volume)) and centrifuged (1,000 g for 10 min, 4 °C). Liver and kidney were removed immediately, rinsed with cold saline, and weighed. Aliquots of plasma, red blood cells and 50~100 mg of each tissue were stored at -70°C until analyzed.

### 2.4 Biochemical Analysis

Blood glucose levels were determined as described

**Table 1** Ingredient composition of the diets fed to rats<sup>a</sup>.

Ingredients	Control (g/kg diet)	Diabetic	
		Untreated (g/kg diet)	Po-treated (g/kg diet)
Casein <sup>b</sup>	200	200	200
Corn starch <sup>c</sup>	590	590	573.4
Sunflower oil <sup>d</sup>	50	50	50
Sucrose <sup>e</sup>	50	50	50
Cellulose <sup>b</sup>	50	50	50
Vitamin mix <sup>f</sup>	20	20	20
Mineral mix <sup>g</sup>	40	40	40
Portulaca oleracea extract <sup>h</sup>	0	0	1

<sup>a</sup>Diets were isoenergetic (16.28 MJ/kg) and given in powdered form;

<sup>b</sup>PROLABO (Paris, France);

<sup>c</sup>ONAB (Sidi Bel Abbès, Algeria);

<sup>d</sup>CEVITAL (Béjaïa, Algeria);

<sup>e</sup>ENASUCRE (Sfifef, Algeria);

<sup>f</sup>Commercial source UAR 200 (Villemoisson, 91360, Epinay/S/Orge, France). Vitamin mixture provided the following amounts (mg/kg diet): Vitamin A, 39,600 UI; Vitamin D<sub>3</sub>, 5,000 UI; Vitamin B<sub>1</sub>, 40; Vitamin B<sub>2</sub>, 30; Vitamin B<sub>3</sub>, 140; Vitamin B<sub>6</sub>, 20; Vitamin B<sub>7</sub>, 300; Vitamin B<sub>12</sub>, 0.1; Vitamin C, 1,600; Vitamin E, 340; Vitamin K, 3.80; Vitamin PP, 200; choline, 2,720; folic acid, 10; paraaminobenzoic acid, 180; biotin, 0.6; cellulose, qsp, 20 g;

<sup>g</sup>Commercial source UAR 205B (Villemoisson, 1360, Epinay/S/Orge, France). Mineral mixture provided the following amounts (mg/kg diet): CaHPO<sub>4</sub>, 17,200; KCl, 4,000; NaCl, 4,000; MgO<sub>2</sub>, 420; MgSO<sub>4</sub>, 2,000; Fe<sub>2</sub>O<sub>3</sub>, 120; FeSO<sub>4</sub>·7H<sub>2</sub>O, 200; MnSO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O, 98; CuSO<sub>4</sub>·5H<sub>2</sub>O, 20; ZnSO<sub>4</sub>, 80; CuSO<sub>4</sub>, 80; CuSO<sub>4</sub>·7H<sub>2</sub>O; KI, 0.32;

<sup>h</sup>Prepared in our laboratory as previously described.

above. Insulin was measured using an enzyme immunoassay kit based on the competition between unlabeled rat insulin and acetyl cholinesterase linked to rat insulin (tracer) for limited specific Guinea-pig anti-rat insulin antiserum sites (enzyme immunoassay kit; Spi-Bio, Le Bretonneux, France). The color intensity was determined by spectrophotometer at 405 nm. HbA1c (glycosylated haemoglobin) was estimated by ion exchange chromatography method (Kit Biocon, Germany). Uric acid and albumin levels were measured by enzymatic colorimetric methods (Kits Spinreact, Girona Spain). ALT (alanin aminotransferase; EC (Enzyme Commission) Number 2.6.1.1) and AST (aspartate aminotransferase; EC Number 2.6.1.2) were determined by kinetic colorimetric assay (Kits Biocon, Germany).

### 2.5 Plasma and Tissues Lipid Peroxidation

TBARS (thiobarbituric acid-reactive substances) were determined in plasma according to the method of

Quantanilha et al. [12]. One milliliter of diluted plasma was added to 2 mL of TBA (thiobarbituric acid) (final concentration, 0.017 mmol/L), plus butylated hydroxytoluene (concentration, 3.36 μmol/L) and incubated for 15 min at 100 °C. After cooling and centrifugation, the absorbance of supernatant was measured at 535 nm.

The lipid peroxidation in tissues was assessed by the complex formed between MDA (malondialdehyde) and TBA (thiobarbituric acid) [13]. Briefly, the liver, heart, kidney and brain (0.5 g) was homogenized with 4.5 mL of KCl (1.15%). The homogenate (100 μL) was mixed with 0.1 mL of sodium dodecylsulfate (8.1%), 750 μL of acetic acid (20%), and 750 μL of TBA reagent (0.8%). The reaction mixture was heated at 95 °C for 60 min. After heating, the tubes were cooled, and 2.5 mL of n-butanol-pyridine (15:1) was added. After mixing and centrifugation at 4,000 g for 10 min, the upper phase was taken for measurement at 532 nm.

### 2.6 RBCs (Red Blood Cells) Lipid Peroxidation

The lipid peroxidation in RBCs was measured as described by Brown et al. [14]. Briefly, 100  $\mu$ L of RBCs was diluted to 900  $\mu$ L PBS buffer, then added to 100  $\mu$ L of H<sub>2</sub>O<sub>2</sub> (1.15%) and incubated for 60 min at 37 °C. The reaction was inhibited with 1 mL of trichloroacetic acid (20%). After centrifugation at 2,000 g for 10 min, 100  $\mu$ L of butylated hydroxytoluen (2%) was mixed with supernatant sample. The lipid peroxidation in RBCs was estimated as previously described [12].

### 2.7 Enzymatic Antioxidant Defense

SOD (superoxide dismutase; EC 1.15.1.1) is a metalloenzyme that catalyzes the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. SOD assay uses a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (kit; Cayman). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. One gram of tissue was homogenized in 5~10 mL of cold 20 mmol/L N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, pH 7.2, containing 1 mmol/L ethyleneglycol-bis (2-aminoethoxy)-tetraacetic acid, 210 mmol/L mannitol, and 70 mmol/L sucrose. The homogenate was centrifuged at 1,500 g for 5 min at 4 °C, the supernatant was removed, and SOD activity was measured at 440~460 nm using a plate reader.

GSH-Px (glutathione peroxidase; EC 1.11.1.9) catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. Glutathione peroxidase assay measures GSH-Px activity indirectly by a coupled reaction with glutathione reductase (kit; Cayman). The oxidation of  $\beta$ -Nicotinamide adenine dinucleotide-2'-phosphate, reduced (NADPH) to  $\beta$ -Nicotinamide adenine dinucleotide-2'-phosphate (NADP<sup>+</sup>) was accompanied by a decrease in absorbance at 340 nm. One unit of

GSH-Px was defined as the amount of enzyme that catalyzed the oxidation of 1nmol of NADPH per minute at 25 °C. One gram of tissue was homogenized in cold buffer (50 mmol/L Tris-HCl, pH 7.5, 5 mmol/L ethylenediaminetetraacetic acid, and 1 mmol/L 1,4-dithiothreitol). The homogenate was centrifuged at 10,000g for 15 min at 4 °C. The supernatant containing GSH-Px was removed and measured at 340 nm using a plate reader.

GSSH-Red (glutathione reductase; EC 1.6.4.2) activity was evaluated at 340 nm by measuring the decrease in NADPH absorbance in the presence of oxidized glutathione [15]. One unit of enzyme reduces 1  $\mu$ mol oxidized glutathione per min at pH 7 at 25 °C.

CAT (catalase; EC 1.11.1.6) catalyses the decomposition of hydrogen peroxide to water and oxygen. Catalase activity was assayed in tissues by measuring the rate of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposition according to the method described by Aebi [16]. Briefly, 250  $\mu$ L of homogenate (100 mg of tissue in 0.9 mL KCl), 250  $\mu$ L H<sub>2</sub>O<sub>2</sub> (30 mmol in phosphate-buffered saline 50 mmol/L), and 250  $\mu$ L of phosphate-buffered saline were added. The contents were shaken and incubated for 5 min, and then titanium sulfate (TiOSO<sub>4</sub>) was added. The absorbance was measured at 240 nm.

### 2.8 RBCs and Tissues Reduced GSH (Glutathione) Levels

Reduced GSH (glutathione) concentration was measured according to Sedlak and Lindsay [17]. GSH reacts with DTNB (5,5'-dithio-bis (2-nitrobenzoic acid)) to form TNB (5-thio-2-nitrobenzoic acid) can be quantified at 412 nm. 1 mL of sample was mixed with 800  $\mu$ L distilled water icy, 200  $\mu$ L TCA (50%) and incubated for 15 min. It was centrifuged at 1,200 g for 15 min and 400  $\mu$ L of the filtrate was added to 800  $\mu$ L of tris buffer (0.4 M, pH = 8.9), followed by the addition of 20  $\mu$ L of DTNB reagent (0.01 M). After 5 min for incubation, the absorbance of the reaction mixture was measured at 412 nm and the

reduced glutathione was used as a standard.

### 2.9 Statistical Analysis

All data are presented as means  $\pm$  SEM of six rats per group. Statistical analysis was carried out by STATISTICA (Version 4.1; Statsoft, Tulsa, Okla). The significance of differences was performed with one-way analysis of variance at a significance level of  $p < 0.05$ . Further specific group differences were determined with Tukey honestly test.

## 3. Results and Discussion

In the present study, we have demonstrated the favorable effects of *Po* lyophilized aqueous extract in STZ-induced diabetic rats after 4-week treatment on hyperglycemia and oxidative stress, in red blood cells and several tissues. Also, the *Po* extract prevented hyperglycemia could act as a protective agent against potential of STZ-induced changes in rats, by reducing lipid peroxidation and enhancing the antioxidant enzymes activity, especially in red blood cells, liver and kidney.

### 3.1 Levels of Glycemia, Insulinemia and Glycosylated Hemoglobin

STZ is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic beta cells [18]. Induction of diabetes in the experimental rats was confirmed by higher fasting blood glucose level. A significant ( $p < 0.05$ ) increase in the level of blood glucose and glycosylated

hemoglobin was observed in diabetic rat (untreated group) when compared to control group (Table 2). Our results indicated that the treatment with *Po* extract during 28 days reduced glycemia in STZ-induced diabetic rats ( $-62\%$ ), but had no effect on plasma insulin concentrations. It could be suggested that the observed hypoglycemic with *Po* treatment may be due to an extrapancreatic mechanism by decrease in glucose absorption from the small intestines by various mechanisms [19].

Glycosylated haemoglobin is one of those glycosides compounds in particular that reflects average blood glucose in patients 2~3 months before blood collection [20]. The results obtained from the measurement of HbA1c show a clear deference between untreated diabetic (10%) and *Po*-diabetic groups (6%). It has been shown that the treatment with antioxidants could prevent hemoglobin glycosylation caused by hyperglycemia.

### 3.2 Plasma Albumin, Uric Acid and Transaminases Activities

One factor that has been associated with renal and cardiovascular disease is serum uric acid. It has been found that level of uric acid circulating in the upper end of normal range concentration is an independent predictor for development of diabetic nephropathy, which supports the concept that uric acid, may be involved in the pathogenesis of diabetic microvascular complications [21]. In our study, increased acid uric level was noted in the diabetic group (Table 3).

**Table 2 Glycemia, plasma insulin levels and glycosylated hemoglobin percentage.**

Control		Diabetic	
		Untreated	<i>Po</i> -treated
Glycemia (mmol/L)	6.16 $\pm$ 0.35	27.09 $\pm$ 2.29 <sup>‡</sup>	9.54 $\pm$ 2.18 <sup>***</sup>
HbA1c (%)	2.17 $\pm$ 1.72	10.11 $\pm$ 0.38 <sup>‡</sup>	69.85 $\pm$ 6.22
Insulin (pmol/L)	82.41 $\pm$ 1.28	64.13 $\pm$ 4.10	5.94 $\pm$ 0.58 <sup>**</sup>

Values are mean  $\pm$  SEM of six rats per group;

<sup>‡</sup> $p < 0.05$  vs. control rats;

<sup>\*</sup> $p < 0.05$  vs. diabetic rats;

<sup>\*\*</sup> $p = 0.01$ ;

<sup>\*\*\*</sup> $p = 0.001$ .

**Table 3** Plasma albumin, uric acid levels and transaminase activities.

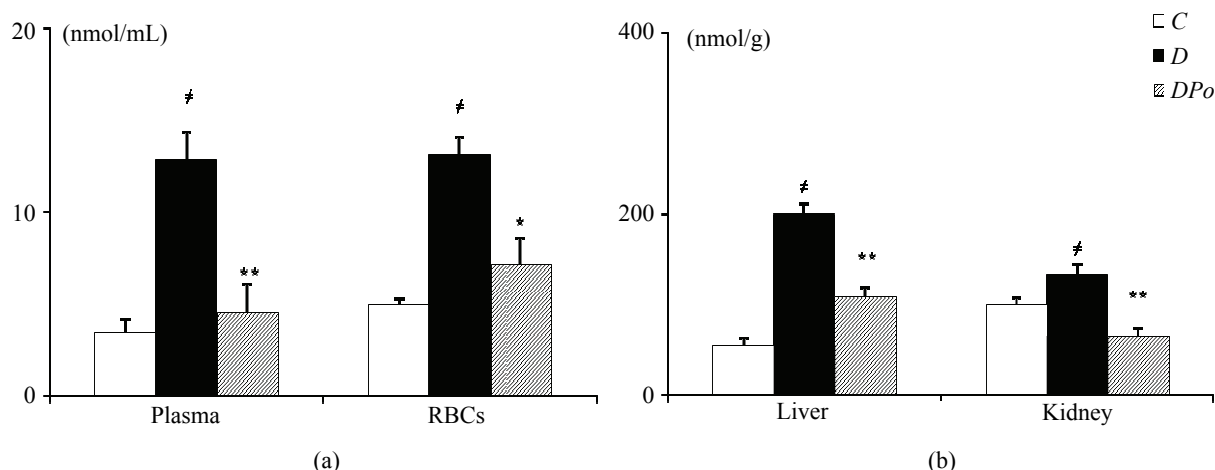
Control		Diabetic	
		Untreated	<i>Po</i> -treated
Albumin (g/L)	37.22 ± 2.94	20.77 ± 1.06	24.22 ± 1.76*
Uric acid (mmol/L)	295.51 ± 1.06	376.12 ± 0.59	290.06 ± 0.35**
AST (IU/L)	51.23 ± 7.27	91.43 ± 3.09	52.93 ± 0.61**
ALT (IU/L)	50.82 ± 3.24	60.01 ± 2.47	56.58 ± 4.12

Values are mean ± SEM of six rats per group;

<sup>#</sup>*p* < 0.05 vs. control rats;

\**p* < 0.05 vs. diabetic rats;

\*\**p* = 0.01.



**Fig. 1** TBARS levels in: (a) plasma and RBCs; (b) liver and kidney.

Values are mean ± SEM of six rats per group;

<sup>#</sup>*p* < 0.05 vs. control rats;

\**p* < 0.05 vs. diabetic rats;

\*\**p* = 0.01.

Interestingly, the higher levels of uric acid in diabetic rats are diminished significantly (−23%) after the administration of *Po*, which could be related to a delay in the onset of the complications of diabetes, as has been previously suggested [21]. In the diabetic rats, daily administration of *Po* extract induced a significant increase of plasma albumin levels (+14%). ALT and AST are common intracellular enzymes that increase the liver damage induced by diabetes [22]. Our data showed that plasma ALT (alanin aminotransferase) and AST (aminotransferase) activities were significantly increased in diabetic rats. In contrast, they were diminished in *Po*-treated group (−42%), most likely due to its antioxidant properties. Elkhayat et al. [23] showed that treatment with alcoholic extract of *Po* restored the hepatic marker enzymes, which demonstrated hepatoprotective

activity in hepatic injured rats.

### 3.3 Plasma, RBCs (Red Blood Cells) and Tissues Lipid Peroxidation

The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species and decreased level of antioxidant defenses, such as GSH and ascorbic acid. Recently, it has been reported that auto oxidation of glucose in diabetes mellitus is major cause for generating oxidative stress [24]. Hyperglycemia is a well-known cause for elevated free radical concentration and this can lead to increased lipid peroxidation (TBARS), in tissues and blood of STZ-induced diabetic rats [25]. Our study showed that in the diabetic group in comparison with

the control, TBARS values were increased significantly ( $p < 0.05$ ). Inversely, *Po*-treatment normalized TBARS levels and induced a significant decrease in plasma (-65%), RBCs (-54%), liver (-46%) and kidney (-51%) (Fig. 1). These results indicated that *Po* extract might protect the plasma and tissues against the cytotoxic action and oxidative stress of streptozotocin. Moreover, the reduction in the lipid peroxidation could be due to the improvement of the glycemic control and the increased of antioxidant status, since *Po* aqueous extract was had hypoglycaemic activity in STZ-diabetic rats. The contribution of polyphenolic antioxidants to the overall therapeutic properties of medicinal plants used for prevention of oxidative stress related disorders is still disputed [26].

### 3.4 Antioxidant Enzymes Activities

Elevated free radical concentration and lipid peroxidation decreases the antioxidant defense in the biological systems. In untreated diabetic compared with the control rats, the antioxidant enzyme activities in liver and kidney declined significantly ( $p < 0.05$ ) (Table 4). In liver, SOD and GSSH-Red activities levels were decreased by -45% and -47%, respectively. Inversely, CAT activity was increased (+44%).

However, SOD, GSH-Px and CAT activities were lower (-54%, -76% and -49%), respectively. Treatment with *Po*, increased significantly antioxidant defense in RBCs ( $p < 0.05$ ), indicating the efficacy of the *Po* extract to reduce oxidative stress in vivo. The constituents of *Po*, such as flavonoids, omega-3 and ascorbic acid have antioxidant activity [27], so the observed antioxidant and antidiabetic activities of this plant may be attributed to the presence of these bioactive principles and their synergistic properties.

The present data indicates that *Po* increased SOD and GSH-Px activities in liver by +38% and +85%, respectively, but these activities remained unchanged in kidney. These increases might constitute a protection against superoxide anion elevation, because SOD catalyses the decomposition of superoxide radicals to hydrogen peroxides ( $H_2O_2$ ) [28]. The  $H_2O_2$  produced by SOD is excreted as  $H_2O$  based on the activity of GSH-Px and catalase. These result explained the low TBARS levels in liver since GSH-Px has been known to inactivate lipid peroxidation reactions. Additionally, glutathione reductase activity was 1.9-fold higher, only in kidney. Indeed, catalase activity is increased in kidney (+48%), but no significant difference was observed in liver.

GSSH-Red regenerates reduced GSH (glutathione)

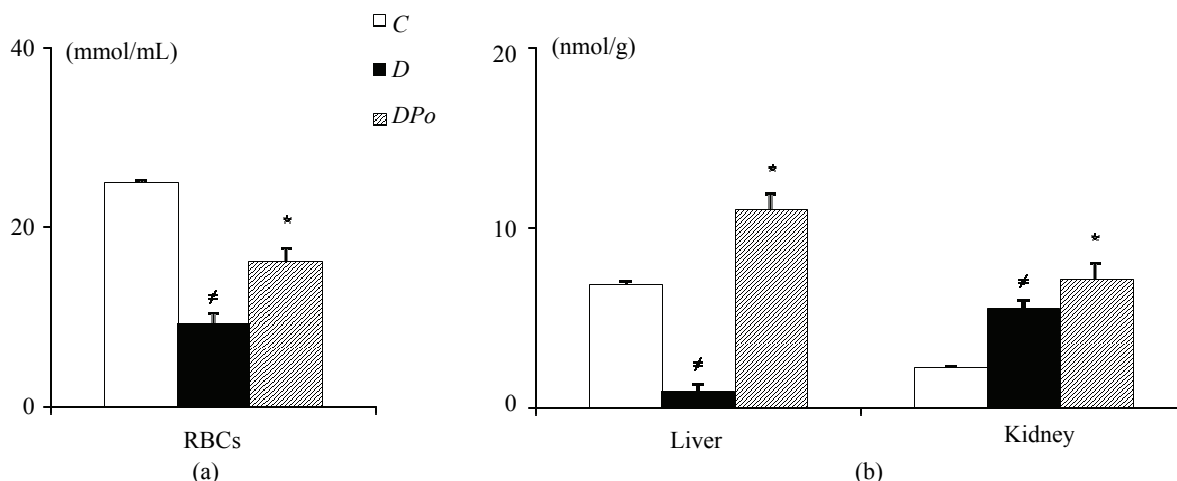
**Table 4** Antioxidant enzymes activities in RBCs and various tissues in rats.

Control			Diabetic	
			Untreated	<i>Po</i> -treated
SOD	RBCs (U/mL)	188.76 ± 9.22	154.50 ± 18.11	224.20 ± 9.68*
	Liver (U/g)	14.31 ± 1.02	7.95 ± 1.07 <sup>#</sup>	12.80 ± 0.61*
	Kidney (U/g)	11.64 ± 0.34	5.34 ± 1.14 <sup>#</sup>	8.75 ± 1.92
GSH-Px	RBCs (nmol/min/mL)	38.86 ± 3.17	20.00 ± 1.88	34.17 ± 4.62*
	Liver (nmol/min/g)	31.23 ± 2.88	25.57 ± 3.42	176.51 ± 09.07**
	Kidney (nmol/min/g)	98.59 ± 7.94	24.48 ± 1.19 <sup>#</sup>	25.63 ± 14.99
GSSH-Red	RBCs (nmol/min/mL)	57.28 ± 13.41	20.60 ± 11.13 <sup>#</sup>	46.77 ± 5.82*
	Liver (nmol/min/g)	61.05 ± 3.7	32.42 ± 3.68 <sup>#</sup>	32.66 ± 2.95
	Kidney (nmol/min/g)	40.14 ± 12.42	20.54 ± 2.82 <sup>#</sup>	39.08 ± 7.96*
CAT	RBCs (pmol/min/mL)	2.31 ± 1.47	1.04 ± 0.56	1.56 ± 0.43*
	Liver (nmol/min/g)	189.85 ± 16.89	338.20 ± 08.71 <sup>#</sup>	307.00 ± 07.81
	Kidney (nmol/min/g)	145.83 ± 20.19	126.80 ± 04.80	247.70 ± 08.11*

Values are mean ± SEM of six rats per group;

<sup>#</sup> $p < 0.05$  vs. control rats;

\* $p < 0.05$  vs. diabetic rats.



**Fig. 2** Reduced glutathione levels in: (a) RBCs; (b) tissues.

Values are mean  $\pm$  SEM of six rats per group;

<sup>#</sup> $p < 0.05$  vs. control rats;

<sup>\*</sup> $p < 0.05$  vs. diabetic rats.

from oxidized glutathione, which has been formed by oxidation while CAT is responsible for the scavenging or detoxification of  $H_2O_2$ . These antioxidant enzymes have a complementary catalytic activity leading to reduced TBARS concentrations in these tissues. These findings are in agreement with those of Sharma et al. [29], who noted, in the liver and kidney, increased SOD and CAT activities. Mineral elements form part of some enzymes for example, catalase contains iron, superoxide dismutase contains copper and zinc while glutathione peroxidase is a selenium containing enzyme. *Portulaca oleracea* belongs to the plant family *Portulacaceae*, found to be rich in minerals [30]. The results obtained for diabetic rats treated with extract of *Po* may be an indication of the availability of these metals for the formation of the enzymes.

### 3.5 Reduced Glutathione Content of Red Blood Cells and Tissues

In our study, lower level of GSH in red blood cells, liver and kidneys of STZ induced diabetic rats is in accordance with those reported earlier studies [31]. Treatment with aqueous extract of *Po* significantly increased GSH levels in RBCs (+43%), in liver (+40%) and kidney (+65%) (Fig. 2). GSH plays an important

role in the detoxification and metabolism as a cofactor or a substrate for enzymes GSH-Px and GST (glutathione-S-transferase).

## 4. Conclusions

The effect produced by *Portulaca oleracea* lyophilized aqueous extract on hyperglycemia, lipid peroxidation and antioxidant status in diabetic animals has been studied. The result of our study showed that *Po* possesses antidiabetic activity and exhibits potent antioxidant potential in diabetic rats, these effects may be due to the presence of tannins and flavanoids which makes this plant very important to be exploited by clinicians and scientists for developing value-added foods and nutraceuticals. Further clinical investigations are necessary to validate the chemotherapeutic activities and potential effects of this plant in the treatment of diabetes and diabetic complications.

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## Conflict of Interest

All authors of this research have no conflict of



interest related with employment, consultancies, stock ownership, grants or other funding.

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148 ***Portulaca oleracea* Leaf Aqueous Lyophilized Extract Reduces Hyperglycemia and Improves Antioxidant Status of Red Blood Cells and Liver in Streptozotocin-Induced Diabetic Wistar Rats**

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