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Portulaca oleracea Reduces Lipemia, Glycemia, and Oxidative Stress in Streptozotocin-induced Diabetic Rats Fed Cholesterol-enriched Diet

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Authors' contributions

This work was carried out in collaboration between all authors. Author FD collected and prepared the samples, participated in the laboratory procedures and wrote the first draft of the manuscript. Authors DK and MALD managed the analyses and the literature searches and performed the statistical analysis. Author MB designed the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, the effects of *Portulaca oleracea* (*Po*) in the treatment of hypercholesterolemia combined with diabetes in rats were investigated.
Place and Duration of Study: Laboratory of Clinical and Metabolic Nutrition, Department of Biology, Nature and Life Sciences Faculty, University of Oran1, Algeria, between September and July 2017.
Study Design: The effects of an aqueous extract from the leaves of *Po* were studied on glucose homeostasis, lipid parameters, and oxidative stress markers, in streptozotocin (STZ) induced diabetic rats on a cholesterol-enriched diet.
Methodology: Experimental hypercholesterolemia was induced by feeding rats with 1% cholesterol-enriched diet for 15 days. Hypercholesterolemic male Wistar rats (n=28) were rendered diabetic by

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a single intraperitoneal injection dose (55 mg/kg BW) of STZ. Hypercholesterolemic diabetic rats were divided into two groups (n=10): Hypercholesterolemic diabetic group (HC-D), and hypercholesterolemic-diabetic group supplemented with 1% of *Po* aqueous extract (HC-D*Po*) during 28 days. Assessed parameters were blood glucose, insulinemia, glycosylated haemoglobin (HbA1c), liver and plasma lipids, lipid peroxidation, and antioxidant enzymes in plasma, red blood cells (RBCs), and tissues.

Results: A significant increase in final body weight was found between both groups. In HC-D*Po vs* HC-D group, glycemic parameters were improved by decreasing glucose, HbA_1C levels, and by enhancing insulinemia. Low plasma values of total cholesterol (TC) (-42%), triacylglycerols (TG) (-63%), very low and low density lipoprotein cholesterol (VLDL-C, LDL-C) (-66% and -80%), and increase of high density lipoprotein cholesterol (HDL-C) (-26%), leading to decreased atherogenic indices TC/HDL-C (-57%) and LDL-C/HDL-C (-85%) were noted in rats treated by *Po* compared to untreated group. In the liver, heart and aorta, lipid peroxidation was lowered, and inversely, antioxidant enzymes activities were increased after treatment with *Po*.

Conclusion: On the basis of these finding, it can be concluded that the *Po* aqueous extract is an interesting natural product to prevent hypercholesterolemia in diabetic rats. Protocols in hypercholesterolemia diabetic patients are needed to verify and validate these results.

Keywords: Portulaca oleracea; streptozotocin; hypercholesterolemia; diabetes; oxidative stress antioxidant enzyme.

1. INTRODUCTION

Vascular homeostasis can be broken in numerous physiopathological circumstances, at the origin of noxious effects of oxidative stress for the concerned cells and their vascular functions. The causes of this imbalance are multiple and recruit at least partially the classic cardiovascular risk factors such as hypercholesterolemia and diabetes [1,2,3]. Hypercholesterolemia is one of the main factors of the atherogenic risk, directly bound to the increased serum level of small-dense low density lipoprotein cholesterol (LDL-C)and conversely linked to the lower rate of high density lipoprotein cholesterol (HDL-C) [4,5]. Previous studies have shown that a hypercholesterolemic diet-induced in animal models and man results in numerous metabolic disorders concomitant with antioxidant defence deficiency and altered inflammatory response [1,6], which could induce a cardiovascular metabolic disorder [7]. Diabetes is related to a glycemic biological regulation mechanisms deficiency, leading to hyperglycemia [8]. In addition, it has been reported that the lipid profile during diabetes is impaired, characterised by high concentrations of triacylglycerols (TG), total cholesterol (TC), LDL-C, and reduced HDL-C [9]. These abnormalities in lipid metabolism are intensified by the over-consumption of cholesterol [10]. Moreover, elevation levels of apolipoproteins glycation, oxidised LDL and oxidative stress are also observed [11,9]. In the presence of diabetes and Hypercholesterolemia, two cardiovascular risk factors, namely, the balance between pro-oxidant and antioxidant species is broken in favour of pro-oxidants, favouring an overproduction of oxygen-reactive species (ROS) [12,13].

Traditional medicinal plants play an important role in the research and development of new medicines. Recent studies revealed promising results for the prevention and treatment of diseases, such as atherosclerosis [14,15], diabetes [16,17] and cardiovascular diseases [18,19].

Portulaca oleracea L. (Portulaceae), known as purslane “Rigla” in Arabic is a succulent annual herbaceous plant found in the temperate and tropical regions of the world. *Po* can be consumed fresh as salad and pickle or can be cooked as a vegetable similar to spinach [20, 21,22]. In Algeria, it is used for the preparation of traditional couscous sauce. *Po* is a great source of beneficial health compounds including alkaloids, polysaccharides, ω3 fatty acids, proteins, carbohydrates, flavonoids, tannins, vitamins (vitamin A, vitamin C, vitamin B and carotenoids), and minerals (magnesium, calcium, potassium and iron) [23,24,25,26,27,28,20]. Besides of its culinary uses, *Po* is widely prescribed in folk medicine. Experimental research has shown an important pharmacological potential of *Portulaca* in the treatment of hyperglycemia [29], hyperlipidemia [23] and oxidation [30].

There were no reports of *Po* supplementation in Wistar rats with hypercholesterolemia (HC) and

diabetes (D). Hence, the present study was designed to evaluate the protective effect of *Po* extract on glycemia, lipid profile and oxidative stress in a rat model with HC-D.

2. MATERIALS AND METHODS

2.1 Sources of Animals, Plant Material and Diets

Twenty-eight adult male Wistar rats were obtained from the Animal Research Center, Pasteur Institute, Algiers, Algeria. *Portulaca oleracea* L. was collected on July 2016 in Ouargla southeastern city (Algeria). This plant was identified taxonomically and authenticated by the Botanical Research Laboratory of Oran 1 University. A voucher specimen has been deposited in the herbariumof the Laboratory ofClinical and Metabolic Nutrition, Faculty of Natural and Life Sciences, under the number *P0* 1965.

The casein, 20%, was obtained from Prolabo, Paris, France. Cellulose, 5%, cornstarch, 59%, minerals, 4%, and vitamins, 2%, were collected from Merck, Darmstadt, Germany. Sunflower oil, 5%, and sucrose, 4%, were obtained at a local market in Oran city, Algeria.

2.2 Extraction of Plant Material

Fresh aerial parts (leaves) were air dried at an ambient temperature (24°C) for 7 days, and then grounded. The *Po* extract was prepared as follows: a fine powder of *Po* (50 g) was suspended in 500 ml of distilled water, heated under reflux for 45 min and then filtered. The filtrate was frozen at -70°C and lyophilised (CHRIST, ALPHA 1-2 LD, Germany). The crude extraction yield of the *Po* lyophilised extract was approximately 30% (wt/wt). It was stored in the dark at ambient temperature until further use.

2.3 Chemical Separation

A previous phytochemical analysis of the aqueous extract of *Po* Zidan et al. [23] was achieved leading to the conclusion of the presence of phenolic compounds including flavonoids, tannins and other phenolic compounds, carbohydrates and terpenoids. Furthermore, some pure secondary metabolites were isolated by successive column chromatography techniques [column chromatography on Sephadex LH 20, Medium Pressure Liquid Chromatography (MPLC) on normal and reversed-phase silica gel] and identified by 1D and 2D NMR and mass spectroscopic techniques as adenosine, allantoin, and adenine by comparing with NMR and mass spectroscopic data with literature values.

2.4 Evaluation of the Antioxidant Activity of *Po* Extract

In vitro test for the antioxidant activity of the *Po* extract was measured by using KRL test [31]. This evaluates the resistance to free radical aggression by measuring the capability of total blood to resist free radical-induced hemolysis. Trolox, a water-soluble synthetic analogue of vitamin E was used as a standard, and the antioxidant activity of the extract was compared with those of trolox (mmol/l).

2.5 Treatment of Animals

Adult male Wistar rats (Animal Research Center, Pasteur Institute, Algiers, Algeria) (n = 28) weighing 138 ± 12 g were housed in wire bottom cages at a temperature of 23±1°C, relative humidity of 60% and light were automatically turned on from 07:00 to 19:00 h. Diets and tap water were freely available. Food consumption and weight gain were measured in every three days. This study was approved by the Institutional Animal Research Committee (*International Research grants 04 MDU 629*). The General Guidelines on the Use of Living Animals in Scientific Investigations Council of European Communities (1987) were followed.

Hypercholesterolemia was experimentally induced by feeding rats a cholesterol-enriched diet (1%) and cholic acid (0.5%) (Merck, Darmstadt, Germany), for 15 days. After this phase, cholesterolemia value was 5.76±0.80 mmol/l *vs* value in normal rat <3.90mmol/l [32]. Diabetes was induced by the administration to hypercholesterolemic rats of an intraperitoneal injection of STZ diluted in citrate buffer (0.1mol/l, pH 4.5) at a single dose of 55 mg/kg body weight (BW). After 48 h, the response to the injection of STZ was checked. The diabetic state was assessed by measuring glycemia using a glucometer (One-touch glucometer Accu-Chek Active, Germany). Only rats in which fasting blood glucose levels were greater than 16 mmol/l within 3 days after STZ injection were considered diabetic. Hypercholesterolemic-diabetic (n = 20) rats were divided into two groups (n = 10) and fed for 28 days with the same diet supplemented (HC-D*Po*) or not (HC-*D*) with *Po* lyophilised aqueous extract (1 g/100 g diet).

2.6 Collection of Samples

At d 28, the animals were food deprived for 12 h, weighed, anaesthetized with sodium pentobarbital (60 mg/kg BW) (Sigma, St Louis, MO, USA), and euthanised with an overdose. Blood was obtained from the abdominal aorta and collected into tubes containing Na_2-EDTA (0.1%) (Sigma, St Louis, MO, USA). Plasma was achieved by low-speed centrifugation at 1000 × g for 20 min at 4°C. Red blood cell sediment was washed twice with ice-cold distilled water (1/4, v/v) and centrifuged (1000 × g for 10 min, 4°C). Liver, heart, aorta and adipose tissue were quickly excised, rinsed with a cold saline solution (0.9%), blotted on filter paper and weighed. Aliquots of plasma, red blood cells and tissues were stored at -70°C until analysis.

2.7 Chemicals

All the chemicals and reagents used were of analytical grade and purchased from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany). Kits used in this study for HbA1c, cholesterol, triacylglycerol, urea and creatinine were from Biocon, Germany and for insulin release, chemicals were obtained from Spi-Bio, Bertin groupe, Montigny Le Bretonneux, France. Other EIA kits for the determination of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSH-Red) were supplied by the Sigma Chemical Company.

2.8 Methodology

Fasting glycemia was determined by the glucose oxidase method using a one-touch glucometer (Accu-Chek® Active, Germany). HbA1c was measured by ion exchange chromatography method (kit Biocon, Germany). Plasma urea, creatinine, TC and TG were determined with the enzymatic methods (Kits Biocon, Germany).

Insulin was measured at d28 using an enzyme immunoassay (EIA), based on the competition between unlabeled rat insulin and acethylcholinesterase (AChE) linked to rat insulin (tracer) for limited specific Guinea-Pig anti-rat insulin antiserum sites (EIA kit, SpiBio, Montigny-le-Bretonneux, France). The colouration intensity was determined spectrophotometrically at 405 nm.

Plasma VLDL, LDL and HDL lipoproteins were isolated by differential dextran sulphate magnesium chloride precipitation using $MgCl_2$ (Merck, Germany) and phosphotungstate (Prolabo, Paris, France) by the method of Burstein et al. [33,34]. Total cholesterol of each lipoprotein was estimated by enzymatic colourimetric method (kit Biocon Germany).

RBCs lipid peroxidation was measured by the determination of thiobarbituric acid reactive substances (TBARS) Quintanilha et al. [35], using malondialdehyde as a standard. Liver, heart and aorta were homogenised in four volumes of 0.1 M phosphate buffered saline (PBS) and centrifuged at 2000 × g, at 4°C, for 10 min. Tissue homogenates were used for determination of tissues susceptibility to lipid peroxidation according to the method of Ohkawa et al. [36]. GSH concentration was determined by the method of Sedlak and Lindsay [37]. GSH reacts with DTNB [5.5'-dithio-bis (2-nitrobenzoic acid)] to form TNB (5-thio-2-nitrobenzoic acid) which can be quantified at 412 nm. One ml of RBCs was mixed with 800 µl of ice-cold distilled water and 200 µL of 50% trichloroacetic acid (TCA), and incubated for 15 min. After centrifugation at 1200 × g for 15 min, 400 µl of the supernatant wasmixed with 800 µl of Tris buffer (0.4 mol/l, pH = 8.9) and 20 µl of DTNB reagent (0.01 mol/l). After 5 min of incubation, the absorbance of the reaction mixture was measured at 412 nm and reduced glutathione was used as a standard.

SOD (EC. 1.15.1.1) is a metalloenzyme that catalyses the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. Superoxide dismutase 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. SOD activity in RBCs and tissues was measured at 440 to 460 nm using a plate reader. Glutathione peroxidase assay (kit Cayman, Ann Arbor USA) measures GSH-Px activity indirectly by a coupled reaction with glutathione reductase. Oxidised glutathione, produced upon reduction of hydroperoxide by GSH-Px, was recycled to its reduced state by glutathione reductase and NADPH (nicotinamide adenine dinucleotide phosphate, reduced form). The oxidation of NADPH to $NADP^+$ was accompanied by a decrease in absorbance at 340 nm. One unit of GSH-Px is defined as the amount of enzyme that catalyses the oxidation of 1 nmoL of NADPH per min at 25°C. GSH-Px activity in RBCs and

tissues was measured at 340 nm using a plate reader.

GSSH-Red (EC 1.6.4.2) is a flavoprotein that catalyses the NADPH-dependent reduction of GSSG to GSH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm and is directly proportional to the GSSH-Red activity in the sample. The procedure for assays was according to the instructions provided by the manufacturer (kit; Cayman). CAT (EC. 1.11.1.6) catalyses the decomposition of hydrogen peroxide to water and oxygen. Catalase activity was assayed in RBCs and tissues by measuring the rate of hydrogen peroxide (H_2O_2) decomposition according to the method described by Aebi [38]. The absorbance was measured at 240 nm.

2.9 Statistical Analysis

Data are expressed as means ± SEM or 10 rats per group. Statistical analysis was performed using Student's- *t* test using a statistical package program STATISTICA 6.1 (StatSoftInc. software, Tulsa, USA). The limit of statistical significance was set at: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ between the hypercholesterolemic-diabetic group treated with *Po* extract (HC-D*Po*) and untreated group (HC-D).

3. RESULTS

At the dose of 1 g/l, the *Po* aqueous extract developed an important antioxidant activity similar to those of 422 mmol/l of trolox. This antioxidant activity increased with the concentration of the *Po* extract (Fig. 1).

At d28, in treated group *vs* untreated group, FBW was increased by 10% and liver relative weight was decreased by 16%. However, there was no significant difference in food intake, relative weight of the heart, aorta and adipose tissue (Table 1).

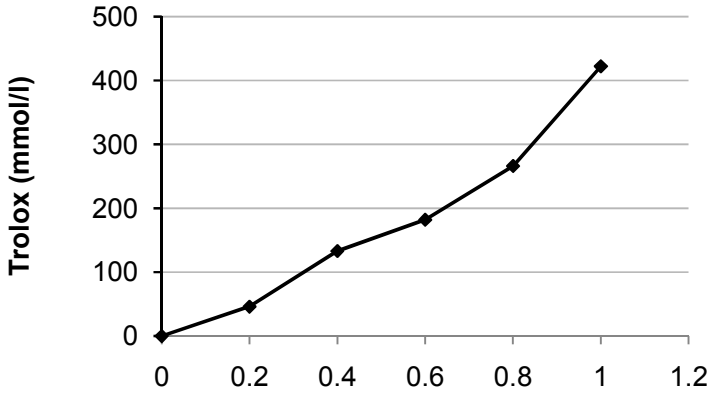

Fig. 1. *Po* (g/l) antioxidant activity compared to trolox

Table 1. Body weight (BW), food intake and relative organ weight of hypercholesterolemic-diabetic rats treated or not with *Portulaca oleracea*

	HC-D	HC-D*Po*
BW (g)	176.34 ± 5.56	195.59 ± 6.67*
Food intake ($g.d^{-1}.rat^{-1}$)	21.38 ± 1.57	21.15 ± 0.85
Relative organ weights		
Liver	4.06 ± 0.25	3.39 ± 0.30*
Heart	0.42 ± 0.03	0.39 ± 0.04
Aorta	0.03 ± 0.01	0.03 ± 0.01
Adipose tissue	0.39 ± 0.02	0.36 ± 0.02

Values are means ± SEM of 10 rats per group. HC-D: Untreated hypercholesterolemic-diabetic rats, HC-DPo: Portulaca oleracea treated hypercholesterolemic-diabetic rats.
*Relative weight = [organ weight/BW] × 100. Statistical analysis was performed using the student's -t test. *p < .05; HC-DPo vs. HC-D group*

As shown in Table 2, daily administration of *Po* to hypercholesterolemic-diabetic rats induced a significant reduction in glycemia (-67%), and HbA1c (-42%) ($p < .05$). Inversely, insulinemia was increased by 11%. The renal function markers showed a reduction of plasma urea and creatinine concentrations (-31% and -28%, respectively).

Table 3 displays the treatment effect on the liver and plasma lipids concentrations and atherogenic index. It was observed that the plasma TC and TG were reduced by 42 and 63%, respectively, in hypercholesterolemic-diabetic rats fed with *Po* aqueous extract supplemented diet. Furthermore, in liver, CT and TG values were lowered by 39% and 36%, respectively. Moreover, VLDL-C and LDL-C concentrations were found to be 3-and 5-fold lower, inversely; HDL-C amounts were 1.4-fold higher in HC-D*Po* than HC-D group. Treatment with *Po* lyophilised aqueous extract induced reduced atherogenic ratios LDL-C/HDL-C (85%) and TC/HDL-C (57%).

In hypercholesterolemic-diabetic rats RBCs, purslane caused a significant reduction of TBARS concentrations (28%), whereas, GSH amounts were increased by 18% (Table 4).

In RBCs of hypercholesterolemic-diabetic rats treated with *Po* extract, the antioxidant defence mechanism was ameliorated *via* enhancement of the enzymatic activities of SOD, CAT, GSH-Px and GSSH-Red. Indeed, these activities were increased by 15%, 26%, 20% and 39%, respectively.

Table 5 presents the data on the tissues antioxidant enzymes activities. In HC-D*Po*, liver, heart and aorta SOD activities were 1.3-, 1.2- and 1.2-fold higher than those of HC-D group (Table 5). In addition, an increase of CAT activity was observed in the heart (11%) and aorta (16%), whereas, there was no significant difference in the liver. GSH-Px enzyme activity was 1.6-, 1.3- and 1.3-fold higher, in liver, heart and aorta, respectively. In addition, GSSH-Red activity was increased in liver (14%), heart (16%) and aorta (24%).

Table 2. Glycemia, glycosylated haemoglobin percentage, plasma insulin, urea and creatinine concentrations

	HC-D	HC-D*Po*
Glycemia	20.60 ± 1.22	6.73 ± 0.46***
HbA1c	11.24 ± 1.48	6.57 ± 0.92**
Insulin	61.70 ± 1.74	69.41 ± 1.85*
Urea	8.77 ± 0.64	6.05 ± 0.53**
Creatinine	61.92 ± 2.57	44.76 ± 1.60*

Values are means ± SEM of 10 rats per group. Statistical analysis was performed using the Student-t test. HC-D: Untreated hypercholesterolemic-diabetic rats, HC-DPo: Portulaca oleracea treated hypercholesterolemic-diabetic rats.

****p < .001; HC-DPo vs. HC-D group; **p < .01; HC-DPo vs. HC-D group; *p < .05; HC-DPo vs. HC-D group*

Table 3. Liver and plasma lipids concentrations and atherogenic index

	HC-D	HC-D*Po*
Liver (µmol/g)		
Total cholesterol	79.86 ± 2.60	48.67 ± 3.21**
Triacylglycerols	35.39 ± 2.56	22.66 ± 1.01**
Plasma (mmol/l)		
Total cholesterol	5.52 ± 0.40	3.22 ± 0.12**
Triacylglycerols	1.78 ± 0.16	0.65 ± 0.09***
VLDL-C	1.18 ± 0.02	0.40 ± 0.03***
LDL-C	2.44 ± 0.04	0.49 ± 0.03***
HDL-C	1.70 ± 0.03	2.30 ± 0.03*
Atherogenic index		
LDL-HDL_1-C/HDL-C	1.44 ± 0.03	0.21 ± 0.01***
TC/HDL-C	3.25 ± 0.06	1.40 ± 0.06***

Values are means ± SEM of 10 rats per group. Statistical analysis was performed using the Student's- t test. HC-D: Untreated hypercholesterolemic-diabetic rats, HC-DPo: Portulaca oleracea treated hypercholesterolemic-diabetic rats.

****p < .001; HC-DPo vs. HC-D group; **p < .01; HC-DPo vs.HC-D group; *p < .05; HC-DPo vs. HC-D group*

Table 4. Lipid peroxidation and antioxidant defense in red blood cells

	HC-D	HC-D*Po*
Lipid peroxidation		
TBARS (nmol/ml)	11.09 ± 0.68	8.01 ± 0.90*
Antioxidant defense		
GSH (nmol/ml)	21.77 ± 1.12	26.68 ± 1.43*
SOD (U/ml)	183.09 ± 10.79	218.41 ± 13.87*
CAT (pmol/min/ml)	1.15 ± 0.07	1.56 ± 0.12*
GSH-Px (nmol/min/ml)	22.89 ± 1.07	28.50 ± 1.87*
GSSH-Red (nmol/min/ml)	33.14 ± 2.69	54.36 ± 4.11**

Values are means ± SEM of 10 rats per group. Statistical analysis was performed using the Student's- t test. HC-D: Untreated hypercholesterolemic-diabetic rats, HC-DPo: Portulaca oleracea treated hypercholesterolemic-diabetic rats.

***p < .01; HC-D vs. HC-DPo group; *p < .05; HC-D vs. HC-DPo group*

Table 5. Tissues antioxidant enzymes activities

		HC-D	HC-D*Po*
SOD (U/g)	Liver	11.29 ± 0.77	14.28 ± 0.66*
	Heart	34.94 ± 0.57	42.09 ± 0.68*
	Aorta	33.02 ± 1.04	40.14 ± 1.62*
CAT (nmol/min/g)	Liver	293.04 ± 31.88	313.50 ± 28.21
	Heart	116.79 ± 6.15	130.60 ± 5.56*
	Aorta	121.65 ± 5.79	144.71 ± 4.34*
GSH-Px (nmol/min/g)	Liver	64.68 ± 5.61	103.63 ± 6.20**
	Heart	19.89 ± 1.02	26.91 ± 1.93*
	Aorta	21.29 ± 1.73	28.74 ± 2.20*
GSSH-Red (nmol/min/g)	Liver	31.91 ± 1.17	37.14 ± 1.50*
	Heart	23.04 ± 0.77	27.58 ± 0.98*
	Aorta	24.14 ± 0.61	31.75 ± 0.82*

Values are means ± SEM of 10 rats per group. Statistical analysis was performed using the Student's- t test. HC-D: Untreated hypercholesterolemic-diabetic rats, HC-DPo: Portulaca oleracea treated hypercholesterolemic-diabetic rats.

***p < .01; HC-D vs. HC-DPo group;*p < .05; HC-D vs. HC-DPo group*

4. DISCUSSION

The present study aims to evaluate the effects of *Po* aqueous extract on certain plasma lipids, and glycemic markers, oxidative stress, and antioxidant defence, in streptozotocin-induced-diabetic rats fed with a cholesterol-enriched diet.

In rats treated by *Po* extract compared to the untreated group, low levels in plasma amounts of TC, TG, VLDL, LDL, were associated with significant increase of HDL-C, leading to decreased atherogenicity indices TC/HDL-C, LDL/HDL, and TC-HDL-C/HDL-C. Similar results have been obtained in hypercholesterolemic rats treated with a 0.5 g/100 g diet of *Po* aqueous lyophilised extract for 28 d [23]. As compared to this study, there is no aggravation, due to the association of hypercholesterolemia and diabetes. In fact, in our study, plasma and liver TC, TG, and CT/HDL-C ratio is respectively decreased by 42%, 63%, 39%, 36% and 57%. These values are sensitively similar to those observed by Zidan et al. [23].

Hypocholesterolemic potential of other medicinal plants is reported in many studies, using aqueous extract in hypercholesterolemic rats. For example, Aslam and Najam reported the result of consumption of *Lagenaria siceraria* at the doses of 200 and 400 mg/kg BW for 8 weeks [39]. Ntchapda et al. reported the effect of*Zanthoxylum heitzii* (225, 300 and 375 mg/kg BW) after four months of administration [40].

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to impaired insulin secretion, or altered glucose utilization in

the vital organs or both [41,42,8]. The STZ is generally used to induce diabetes in Wistar rat, leading to a severe loss of body weight [43], inspite of increased food intake, and relative liver weight [44]. This body weight loss in diabetes is due to high lipolysis, and muscle wasting, and reduced tissue proteins caused by insulin deficiency [45].

In the present study, the FBW of *Po* treated group increased significantly, as compared to the untreated rats. However, no significant difference was noted in food intake in both groups. In a study done by Ashraf et al., the aqueous extract of *Berberis integerrima* root (250 and 500 mg/kg of BW) improved FBW in STZ-induced diabetic male Wistar rats when administered daily by gavage, for six weeks, [46]. Similar findings were reported by Solanki and Bhavsar, after four weeks treatment with *Ficus racemosa* aqueous extract (250 and 500 mg/kg of BW), in STZ-induced diabetic rats [47]. In the present study, the decrease in relative liver weight in the treated group indicated the ability of *Po* aqueous extract to reduce the diabetic liver hypertrophy. These results are in accordance with those of Eleazu et al. [44], where, STZ diabetic rats were fed pelletised *Colocasia esculent,* and *Musa paradisiacal* for 21 days. Furthermore, *Po* ethanolic extracts (1 g/kg BW), administered by gavage for 8 weeks to hyperlipidemic rats, and had significant beneficial effects on the liver [48]. This is probably due to improved liver lipoprotein excretion, and increased insulinemia, lowering AG influx, and TG accumulation in the liver [49,44].

In the present study, glycemic parameters (Glycemia and HbA1c) are significantly reduced. These results are in accordance with those of Guenzet et al. [29] in STZ-diabetic rats (60 mg/kg BW) treated with *Po* (1 g/kg BW), for 28 days. In both studies, *Po* treatment induced a decrease of glycemia (-67% and -65%, respectively), and HbA1c (-42% and -41%, respectively). The relating data between these two studies revealed that enriched cholesterol diet (or induced hypercholesterolemia) did not aggravate the diabetic state in treated and untreated groups.

Several experimental studies revealed a hypoglycemic effect of aqueous extract of some plants, in STZ-induced diabetic rats. Indeed, The consumption of *Kalanchoe pinnata* (140 mg/kg BW), for one month, had a significant decrease in blood glucose [8]. Also, the administration of *Evolvulus alsinoides* (150 mg/kg BW) involved high insulinemia [50]. These results are similar to the current results, i.e., oral administration of *Po* induced hypoglycemia; reduced the levels of HbA1c, and increased the insulin secretion. This improvement in glycemic parameters can result in increased insulin-like action [51], stimulation of insulin secretion from partial cells of pancreas which might involve the closure of K^+-ATP and the intensification of calcium influx through voltage-sensitive Ca^{2+} channels [52], protection against degeneration of β cells, promoting glucose utilisation in muscle, and inhibiting the glucose output from the liver [53,54].

Also, natural products classified into terpenoids, alkaloids, flavonoids, phenolics, and some other categories have shown antidiabetic potential through the insulinomimetic activity of the plant extract [55]. Elevated levels of serum urea and creatinine, which are considered as significant markers of renal dysfunction [56], were significantly decreased in HC-D*Po* group *vs* HC-D. This could be explained by the reduction of plasma glucose concentration, and subsequent glycosylation of glomerular renal basement membranes [29]. Such a nephroprotector potential was noted by Nabi et al. [57], in STZ-induced diabetic rats fed with an enriched diet with *Piper longum* aqueous extract (200 mg/kg BW), for one month.

Hypercholesterolemia and diabetes can be at the origin of a noxious oxidative stress for concerned cells, and their vascular functions [1, 2,3]. In the present study, the decreased levels of TBARS in RBCs showed the antioxidant capacity of *Po*. These results are corroborated by the study of Ntchapada et al. [40] on hypercholesterolemic rats treated with *Zanthoxylum heitzii* (225, 300 and 375 g/kg BW). Under pathological conditions, the imbalance between prooxidants and antioxidants encourages the overproduction of reactive oxygen species (ROS). This elevated rate is counterbalanced and eliminated from the enzymatic and/or non-enzymatic antioxidants in regulated cellular redox status [12,13]. Phenolic compounds contribute largely to the plant's antioxidant activities [58]. Our data showed that *Po* extract treatment enhances antioxidant enzymes activities (CAT, SOD, GSH-Px and GSSH-Red). Indeed, Dkhil et al., (2011) noted similar results with *Po* juice (1.5 ml/kg BW) administered in rats for 12 days [30]. At the moment, there is no scientific evidence to link the constituents which have been detected in the preliminary phytochemical screening to the pharmacological actions revealed in this study.

However many secondary metabolites isolated from plants such as terpenoids, alkaloids, flavonoids, phenolics have shown antidiabetic and hypocholesterolemic potential. The antioxidant properties of *Po* are attributed to their compounds, as gallotannins, fatty acid omega-3, ascorbic acid, α-tocopherol, kaempferol, quercetine, apigenine [59,20].

5. CONCLUSION

The current experimental evidence demonstrates that metabolic disorders induced by combined hypercholesterolemia and diabetes in rats, such as high levels of lipids, glycemic and oxidative stress parameters can significantly be improved by *Po* supplementation in the diet. The richness of *Portulaca* in different nutrients such as alkaloids, polysaccharides, ω3 fatty acids, proteins, carbohydrates, flavonoids, tannins, vitamins, minerals and other healthful compounds improves its ability to control and to reduce side effects of associated hypercholesterolemia and diabetes in rats. These cumulated beneficial effects encourage the experimentation of this potent safety plant on a human to make *Po* as a useful plant in the prevention and treatment of patients with combined hypercholesterolemia and diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per the international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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