



POSITIVE INFLUENCE OF *PORTULACA OLERACEA* L. IN RATS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Chronic hyperglycemia is associated with oxidation of cellular machinery and hyperlipidemia which could end with high risk of complications. *Portulaca oleracea* (PO) is confirmed to excellent source of antioxidant components and hypolipidemic agent. The basic idea behind this study was to check the influence of PO in Diabetic rats. Thirty male rats were classified into three groups, Group1: control group, Group2: diabetic rats (*Diabetes mellitus*) and Group 3; diabetic rats with PO. Dietary supplement of 200 mg.kg⁻¹ of PO was daily through oral gavage for 21 days. The results revealed that the extracted contains several species in addition to the trace elements. The sugar level and lipid profile in Group 3 was significant lower compared to Group2, point to it has protective effects against DM. Contrariwise, a significant higher in antioxidant enzymes in Group3 than Group2. While a significant lower in protein carbonyl and malondialdehyde (MDA) in Group3 than Group2. PO may exert preventive influences by acting as an antioxidant and antihyperlipidemic agent to diabetic rats.

Keywords : *Portulaca oleracea*, type 2 diabetes, antioxidant enzymes, lipid profile

Introduction

Diabetes organization continue to recommend that diet and lifestyle change are the fundamental control of diabetes mellitus and prevention diabetes mellitus complication (Beckman *et al.*, 2013; American Diabetes, 2017). Until recently, many anti-diabetes medication does not prevent diabetes mellitus complications (DMC) (Tzoulaki *et al.*, 2009). As a result, leads to serious complications affecting certain organs such as nephropathy, neuropathy and retinal vascular disease (Inzucchi *et al.*, 2010). Finally, the coronary artery diseases are major cause of death in diabetic patients (Dokken, 2008).

Oxidative stress is increased in patients with *Diabetes mellitus* (DM), in which imbalance between free radicals and antioxidant system leads to crushed antioxidant by excessive free radicals (ROS/RNS) production (Valko *et al.*, 2007). Chronic hyperglycemia associated with increased in free radicals production and ultimately ending with microvascular and macrovascular complications. Sidewise, to oxidative stress there is another factor; hyperlipidemia plays a pivotal role in pathogenesis of DM leading to high risk of DMC (Ayepola *et al.*, 2014; Kangarkar *et al.*, 2010). Antioxidant are substances that may protect cells from the damage involve endogenous antioxidant and exogenous antioxidants from diet, such as superoxide dismutase (SOD), caeruloplasmin, catalase(CAT), vitamins (C, A , E) and cofactors like uric acids and folic acid. It's neutralized free radicals and keeps the homeostasis of body (Asmat *et al.*, 2016).

Portulaca oleracea L. (common purslane) is a tropical to warm climates; annual herbaceous with succulent leaves plant with an extensive distribution, a member of the portulacaceae family (Elkhayat *et al.*, 2008). *Soft, purslane leaves* provides many health benefits its rich in omega-3 fatty acids than in some of the fish oils and antioxidant (vitamins A and E) (Palaniswamy *et al.*, 2001). Traditionally, it's used in several countries as an antiseptic, vermifuge, antipyretic,

and so on (Lee *et al.*, 2012). Purslane exhibits a large spectrum of pharmacological influences, such as antibacterial (Zhang *et al.*, 2002), antiulcerogenic (Karimi *et al.*, 2004), anti-inflammatory (Chan *et al.*, 2000), antioxidant (Chen *et al.*, 2012), and cut-healing (Rashed *et al.*, 2003) properties. World Health Organization listed it as one of the most important widely used medicinal plants (Xu *et al.*, 2006). Purslane has been used in traditional Chinese Medicine since ancient times and described it as vegetable for long life (Jin *et al.*, 2013). It has a slightly sour taste and cold in nature and is used to aid in clearing heat, stanch bleeding, cooling blood and expelling toxins. The dried purslane is particularly effective in the treatment of pyrexia, diarrhea, dysentery, carbuncle, hematochezia and dermatitis, with 9–15 grams dose (Ramalingam *et al.*, 2016).

Materials and Methods

Preparation of extracts and Chemical detection of the plant components

PO was purchased from Baghdad. The plant identified by Faculty of Pharmacy, Baghdad University. Dry Purslane was cleaned and ground into coarse powder. The aqueous extract contains a number of chemical components as shown in table 1. They included: phenolic compounds, saponins, alkaloids, glycosides, resins, tannins, proteins and flavonoids (Mohammed, 2012).

Determination of Trace elements

The following trace elements concentrations of Purslane (Nutritive value per 100 g): Magnesium (Mg), Potassium (K), Iron (Fe), Sodium (Na), Phosphorus (P), Manganese (Mn), Copper (Cu) and Selenium (Se) were estimated by standard reference methods followed by the Nutrition Research Institute.

Experimental design

Thirty male Wistar albino rats weighing 200-230 grams were used in the experiments. All rats were maintained under

standard conditions. The rats were categorized into three groups ($n = 10$ / group), Group 1: control group (NDM), Group 2: diabetic rats (DM) got no diet therapy and Group 3: diabetic rats supplemented with diet therapy. Group 1 and group 2 received 0.9% NaCl, while group 3 was administered through oral gavage with PO extract daily (at 200mg .kg⁻¹) period for three weeks consecutive days (Ramalingam *et al.*, 2016). *P. oleracea* was re-dissolved in deionized water before used. Blood sugar level and body weight were determined weekly throughout the study.

Induction of diabetes

Intraperitoneal injection of (60 mg.kg⁻¹) dose of freshly prepared streptozotocin (STZ) in overnight fasted adult wistar to induced diabetes (Bagri *et al.*, 2009). After three days, using glucometer to measured fasting blood glucose level. Rats were considered to be diabetic with glucose levels above 15 mmol.L⁻¹ and selected to be used in this study.

Blood sugar and lipid profile

Fasting blood sugar was measured by kit (BioSystems SA, Spain), whereas total cholesterol, triglyceride were determined by commercial kit (Teco Diagnostics, Anaheim). The results of calculations are expressed as mmol.L⁻¹.

Oxidative stress assessment

Thiobarbituric acid method was using to determine lipid peroxidation (Stocks and Dormandy, 1971). In this method malondialdehyde (MDA), formed from the collapse of polyunsaturated fatty acids was identified as a marker to lipid peroxidation, malondialdehyde (MDA) react with thiobarbituric acids (TBA) to give a red chromophore absorbed at 532 nm. Results of calculations are expressed in unit of nmol.g⁻¹ of protein. While, protein carbonyl groups as biomarkers of protein oxidation (Levine *et al.*, 1990), in this method, measurement of protein carbonyl after reaction with 2, 4-dinitrophenylhydrazine (DNPH) (Sigma-Aldrich St. Louis, USA) by spectrophotometrically (UV 160A, shidmazu, Japan) at 360 nm. Results of calculations are expressed as nmol.mg⁻¹ of protein.

Antioxidant enzymes status

Superoxide dismutase (SOD) activity was done due to the method reported by (Beyer and Fridovich, 1987). Nitroblue tetrazolium (NBT) reduction by superoxide anion to purple formazan absorbed at 560 nm, in which one unit of SOD inhibition 50% of purple formazan formation. Results of calculations are expressed as E (mg.min⁻¹) the activity of catalase (CAT) was estimated due to the method reported by (Aebi, 1984). Decomposition of H₂O₂ by CAT was estimated using spectrophotometer at 240 nm every 30 seconds interval for 3 minutes. Results of calculations are expressed as mmol.min⁻¹.mg⁻¹ of protein. The spectrophotometric reader assay method for glutathione (GSH), involves oxidation of GSH by Ellman's reagent (5, 5-dithiobis-2-nitrobenzoic (DTNB)) to form the yellow-coloured complex 5'-thio-2-

nitrobenzoic (TNB), measured at 412 nm (Ellman, 1959). The results of calculations are expressed as mmol/mg of protein.

Statistical analysis

The statistical analyses were carried out by the aid of SPSS version 23. ANOVA was used to analyses the data, while a test of post-hoc Tukey used to estimate the difference between the groups. The differences were about p<0.01.

Results and Discussion

The starting point of this work is to analyze the aqueous extracted of the PO. The phytochemical composition of the aqueous extracts illustrated in Table 1. It can be clearly seen from this table that the leaves contents: proteins, glycosides, saponins, tannins, resins, various phenolic compounds, flavonoids and alkaloids. Our data are in agreements with previous studies (Yan *et al.*, 2015).

Table 1 : List of chemical contents of aqueous extracted species of *Portulaca oleracea* L. Leaves.

Phytochemical	Result
Saponins	+
Glycosides	+
Flavonoids	+
Protein	+
Alkaloids	+
Resins	+
Phenolic compounds	+
Tannins	+

Furthermore, several trace elements such as K, Mg, Ca, Na, P with high concentration 43, 57, 51, 39, 37 mg, low concentrations of Fe, Mn, Cu with 1.99, 0.303, 0.113 ppm respectively and very low concentrations of Sn with 0.7 µg in purslane leaves were also indicated as shown in Table 2.

Table 2: The concentration of several trace elements in purslane leaves (Nutritive value per 100 g).

Elements	Per 100 g PO
Potassium	432mg
Magnesium	57mg
Calcium	51mg
Sodium	39mg
Phosphorus	37 mg
Iron	1.99mg
Manganese	0.303 mg
Copper	0.113mg
Selenium	0.7 µg

Figure 1 shown significant lower in sugar level in Group3 compared to Group2, indicating it's protective effects against DM ,also a significant decrease in both dangerous fats in the blood total cholesterol and triglyceride in Group3 compared to Group2.

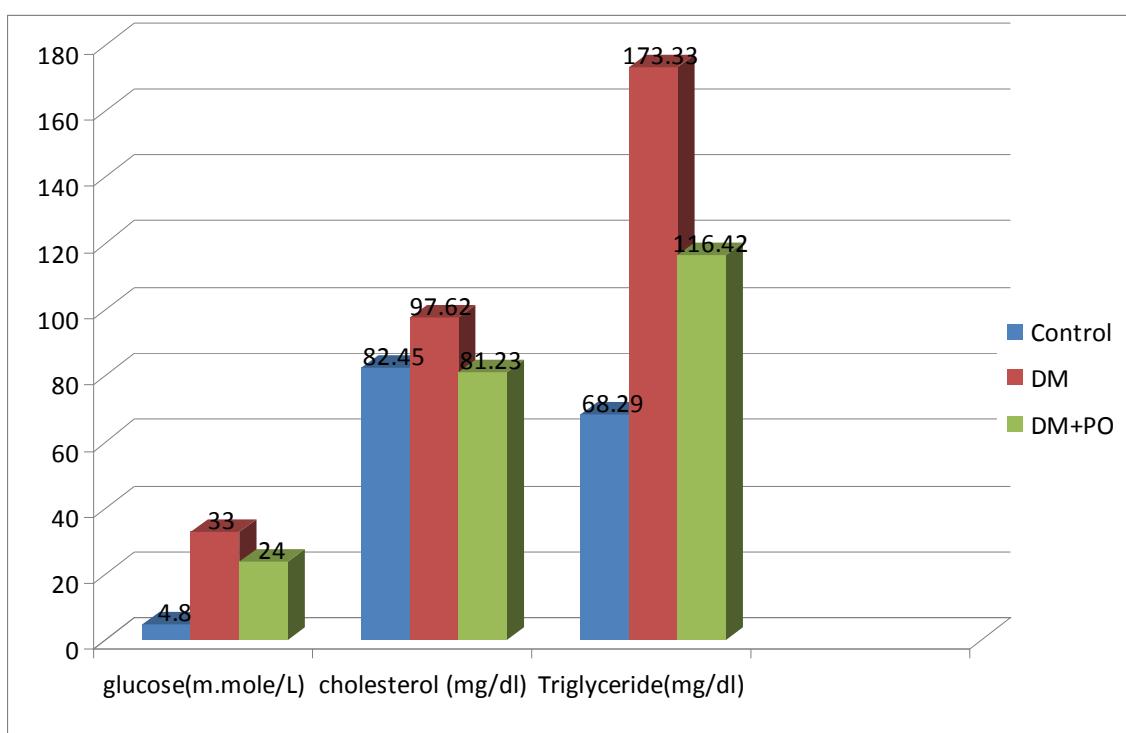


Fig. 1 : Effect of 200 mg/kg dose of aqueous extract of *Purslane* on glucose and lipid profile of rats

Purslane alleviates body weight, levels of dangerous fats in the blood, and hyperinsulinemia. It also makes the cells of the body more sensitive to insulin and improved lipid metabolism and glucose tolerance in rats were injected with a diabetes type 2-causing streptozotocin (60 mg.kg^{-1}) and high calorie forage diet, suggesting that that *purslane* attenuates insulin resistance (Shen and Lu, 2003). The aqueous extract of *Purslane* lowers blood sugar concentration and also inhibit a complication involving inflammation of the vascular and endothelial dysfunction in diabetic rat type 2,

suggesting it's have antidiabetic properties and prevents vascular complications (Lee et al., 2012). Extract crude polysaccharide from *Portulaca oleracea* also prevents hyperglycemia, regulates the lipids metabolism in blood and glucose after toxic glucose analogue by alloxan in diabetic mice (Gong et al., 2009), whilst seeds of this plant attenuates triglycerides, the levels of total cholesterol in the blood that have been linked to heart disease and fasting blood sugar in diabetes mellitus type 2 (El-Sayed, 2011).

Table 3: Oxidative stress markers and antioxidant status in Group1, Group2and Group3 groups after 21 days of study

Parameter	Control	DM	DM + PO	P
SOD (E/mg/min)	945.84 ± 46.3	$289.91 \pm 11.38a$	$561.31 \pm 27.51a, b$	0.01
CAT(mmol/min/mg)	135.36 ± 5.16	$64.47 \pm 6.47a$	$71.92 \pm 3.38a, b$	0.01
GSH (mmol/mg)	0.12 ± 0.01	$0.07 \pm 0.004a$	$0.07 \pm 0.006a,b$	0.01

Portulaca oleracea, actually, is excellent source of antioxidant due to characteristic of its constituents, such as ascorbic acids, α -tocopherols, gallotannins, omega-3 fatty acids, quercetin, kaempferol, and apigenin (Zhu et al., 2010). The comet assay is a rapid, simple, and cheap manner for measuring DNA strand breaks, the aqueous extract of *Portulaca oleracea* L. significantly alleviated H_2O_2 -induced oxidative DNA deleterious in human lymphocytes, whereas there was no influence of the ethanolic extract, probably as a result of their antioxidants content in the aqueous extract (Behravan et al., 2011). The aqueous extract lowers diet high in fat-elicted oxidative injury by modifying liver and blood antioxidant enzyme activities, elevating liver PPAR α/β -actin and leptin/ β -actin and preventing the protein expression of FAS mRNA and p-PERK expression of spleen and liver in male mice (Chen et al., 2012). Another study in this field, aqueous and ethanolic extracts exerted cytoprotective effects against hemolytic damages of RBCs induced by 2, 2'-azobis hydrochloride in a concentration-dependent method (Karimi et al., 2011).

Conclusion

The current study was to evaluate the effect of *Portulaca oleracea* in experimental rats as antidiabetic and diabetic complication. *Portulaca oleracea* efficiently trims glucose level, triglyceride and total cholesterol. The results support the *Portulaca oleracea* has a antidiabetic agent and it's efficiently increase the levels of endogenous antioxidant enzymes this finding, indicate the *Portulaca oleracea* as an antioxidant agent that lead to its prevent diabetic complication this can be attributed to the synergistic effects of the diverse chemical constituents of this plant. These finding strengthen, *Portulaca oleracea* could be more effective in controlling diabetic and diabetic complication than synthetic antidiabetes medication.

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Conflict of Interest

Authors declare that, there is no conflict of interest regarding the publication of this paper.

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