Portulaca oleracea aqueous extract reduces oxidative stress in erythrocytes and tissues, in rats fed enriched-cholesterol diet

Yahiaoui Zidan¹, Sherazede Bouterbala¹, Anne-Claire Mitaine-Offer², Marie-Aleth Lacaille-Dubois², Malika Bouchenak¹

ABSTRACT

Objective: The aim of this study was to determine the effect of aqueous extract of Portulaca oleracea L. (Po) on lipid peroxidation as well as antioxidant enzymes activities in erythrocytes and tissues, in hypercholesterolemic rat.

Methods: Male Wistar rats were fed on 1% cholesterol-enriched diet for 10 days, and were divided into two groups fed the same diet supplemented (Po-HC) or not (HC) with Po (0.5%) for four weeks. Results: Thiobarbituric acid reactive substances were significantly decreased in erythrocytes (-48%), adipose tissue (-37%) and heart (-37%) in Po-HC. Superoxide dismutase activity in erythrocytes, liver, muscle, heart and kidney was respectively, 1.2-, 1.3-, 1.2-, 1.5- and 1.2-fold higher in Po-HC group. Catalase activity in erythrocytes, liver and heart was respectively, 1.6-, 1.7- and 1.5-fold higher in Po-HC. Glutathione peroxidase activity was 1.2-fold increased in adipose tissue, whereas that of glutathione reductase was respectively 1.4 and 1.6-fold increased in adipose tissue and liver. Conclusion: Po is able to reduce oxidative stress in hypercholesterolemic rats by decreasing the lipid peroxidation and increasing the antioxidant enzymes activities of erythrocytes and tissues. The high content of phytoconstituents present in Po as flavonoids, alkaloids, omega-3 fatty acids, β-carotene, vitamin C, saponins and tannins are considered to be responsible for this effect.

KEY WORDS: Hypercholesterolemia, Portulaca oleracea, Erythrocytes, Tissues, Oxidative stress

ABBREVIATIONS

CAT, Catalase;
CHD, Coronary heard diseases;
GSH, Glutathione;
GSH-Px, Glutathione peroxidase;
GRed, Glutathion reductase;
GSSG, Oxidized glutathione;
HC, Hypercholesterolemic group;
LDL-C, Low-density lipoprotein cholesterol;
MDA, Malondialdehyde;
Po, Portulaca oleracea L.
RBC, Red blood cells;
SOD, Superoxide dismutase;
TBARS, Thiobarbituric acid reactive substances;
TC, Serum total cholesterol.

INTRODUCTION

Hyperlipidemia or a high level of serum triacylglycerols and cholesterol is a risk factor for premature atherosclerosis and coronary heard diseases (CHD). Strong evidences have been put forward by various investigators for the involvement of free radicals production and lipid peroxidation in the onset of atherosclerosis [1].

Oxidative stress is currently suggested as a mechanism underlying hypercholesterolemia. Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Enzymatic antioxidant defenses include SOD, GSH-Px, and CAT. Nonenzymatic antioxidants are represented by ascorbic acid (vitamin C), α-tocopherol (vitamin E), GSH, carotenoids, flavonoids, and other antioxidants. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health [2]. Oxidative stress results from imbalance between radical-generating and radical scavenging systems, that is increased free radical production, or reduced activity of antioxidant defenses, or the both [3]. Hypercholesterolemia, high-cholesterol diet, and oxidative stress increased serum TC and LDL-C, resulting in increased risk for atherosclerosis development [4].

Recent researchers showed that many medicinal plants have the potential to reduce oxidative stress. Oral administration of Astragalus mongholicus (0.4 to 0.8% for 5 weeks), in rats fed high cholesterol diets has caused a significant reduction
in oxidative stress of liver by decreasing lipid peroxidation and increasing antioxidant enzymes activities [5].

The presence of bioactive compounds in some medicinal plants involved reduction of oxidative stress. The increase of the antioxidant enzymes activities and the reduction of TBARS concentrations in plasma and tissues, in hypercholesterolemic rats treated with aqueous extract of Spermacoce hispida may be due to bioactive molecules like flavonoids [6].

Portulaca oleracea L. (Po) (family Portulacaceae) is known as purslane in English, El raja in arabic and Khorfeh in persian. Po leaves have been used in foods like soups, salads and in treatment of several disorders such as hyperlipidemia, inflammatory disorders and some other urinary and topical diseases [7-9].

Po contains many nutrients; some of the active compounds include omega-3 fatty acids, flavonoids, coumarins, vitamins A, C and E, β-carotene, melatonin, dopamine, noradrenaline, oxalates and minerals [10-12]. In pharmacological tests and trials, different extracts of Po are reported to have analgesic, anti-inflammatory, smooth and skeletal muscle relaxant, anti-convulsant, antioxidant, and antifungal effects [13-14]. Therefore, this study was undertaken to determine the effects of lyophilized aqueous extract of Po on lipid peroxidation, as well as antioxidant enzymes activities, in erythrocytes and tissues, in rat fed a cholesterol-rich diet (1%).

MATERIALS AND METHODS

Plant material and preparation of Po aqueous extract

Po plant was collected on April 2011 from west Algeria (Sahara) and identified by A. Marouf, in Botanical Laboratory (Faculty of Nature and Life Sciences, University of Oran). A voucher specimen has been deposited in the herbarium of the Laboratory of Clinical and Metabolic Nutrition, Faculty of Nature and Life Sciences, University of Oran, under the number 2 011 0 6 11 [15].

The leaves of Po were dried at ambient temperature. Then, 500 mL of distilled water was added to 50 g of finely powered leaves and the mixture heated under reflux for 60 min at 75°C, and the decoction was filtered. The filtrate was frozen at -20°C and then lyophilized (Po). The crude yield of the lyophilized material was approximately 30% (w/w).

As previously described by Zidan et al., (2014) [15], the preliminary phytochemical analysis of the aqueous extract revealed the presence of phenolic compounds including flavonoids, tannins and other phenolic compounds, carbohydrates, terpenoids and alkaloids. In addition, some pure secondary metabolites were isolation and identified as adenosine, allantoin and adenine.

Animals and dietary treatment

Male Wistar rats (Pasteur Institute, Algiers, Algeria) (n = 12) weighing 120±5g were kept in wire bottom cages at temperature of 24°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. The General Guidelines on the Use of Living Animals in Scientific Investigations Council of European Communities, (1987) [16] were followed. The protocol and use of rats were approved by our Institutional Committee on Animal Care and Use. Hypercholesterolemia was induced by feeding rats cholesterol-enriched diet (1%) and cholic acid (0.5%) (Merck, Darmstadt, Germany) for 10 days. The composition of the diet (expressed in g/kg) was: casein, 200 (95% purity; Prolabo, Paris, France); sunflower oil, 50; sucrose, 40; cellulose, 50; cornstarch, 590; minerals, 40 (CaHPO4; KCl; NaCl; MgO; MgSO4; FeSO4; Fe3O4; 7H2O; MnSO4; H2O; CuSO4; 5H2O; ZnSO4; 7H2O; CuSO4; 7H2O; KI); vitamins, 20 (Vit A; Vit D3; Vit B1; Vit B2; Vit B2; Vit B3; Vit B6; Vit B7; Vit B12; Vit C; Vit E; Vit K; Vit PP (Villemonois, 91360, Epinay, S'Orge, France). After this phase, serum total cholesterol (TC) concentration was 5.66±1.60 mmol/L.

Then, hypercholesterolemic rats were divided into two groups fed for 28 days with the same diet treated or not with Po. Lyophilized aqueous extract (0.5g/100 g diet) (Po-HC treated and untreated control HC group).

Blood and tissue samples

After four weeks, rats were food deprived for 12 hours, anaesthetised with sodium pentobarbital (60 mg/kg body weight) and euthanized with an overdose. Blood was collected from abdominal aorta into dried tubes and centrifuged at 4°C, 1000g for 15 min. Serum was taken, and separated RBC was then washed 3-times by resuspending in 0.9% NaCl solution and repeating the centrifugation. The washed cells were lysed in an equal volume of water and mixed thoroughly. Liver, adipose tissue, muscle, aorta, heart and kidney were quickly excised in ice-cold saline, blotted on filter paper and weighed. Serum and tissues samples were stored at -70°C until use.

Lipid peroxidation products and endogenous antioxidant enzymes analysis

As a marker of the lipid peroxidation, serum TBARS concentrations were measured according to the method of Quintaniilha et al., (1982) [17] and those of tissues by the method of Ohkawa et al., (1979) [18], as previously described by Bouderbala et al., (2008) [19]. 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’s Chemical, Ann Arbor, MI, USA). Glutathione peroxidase (EC. 1.11.1.9) catalyzes the reduction of hydrogen peroxides, including H2O2, by reduced GSH and functions to protect the cell from oxidative damage (kit Cayman’s Chemical, Ann Arbor, MI, USA). Glutathion reductase (EC 1.6.4.2) is a flavoprotein that catalyzes 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 GRed activity in the sample (kit Cayman’s Chemical, Ann Arbor, MI, USA). Catalase (EC 1.11.1.6) catalyses the decomposition of H₂O₂ to water and oxygen. CAT activity was assayed in tissues by measuring the rate of H₂O₂ decomposition according to the method described by Aebi, (1974) [20].

Protein concentrations were measured using bovine serum albumin (Sigma, Chemical Company, St Louis, MO, USA) as a standard, according to the method of Lowry et al., (1951) [21].

Statistical analysis

Results are expressed as means ± SEM for six rats per group. Statistical analysis was carried out by Student’s t-test. The calculations were performed using STATISTICA 6.0 (for Windows; StatSoft Inc. software, Tulsa, USA). The limit of statistical significance was set at *P<0.05, **P<0.01, ***P<0.001 between the hypercholesterolemic group treated with Portulaca oleracea L. extract (Po-HC) and the untreated hypercholesterolemic group (HC).

RESULTS

Erythrocytes and tissues lipid peroxidation

After four weeks of Po treatment, in hypercholesterolemic group, lipid peroxidation was significantly decreased in erythrocytes, adipose tissue and heart, whereas there was no significant difference in liver, muscle and kidney, compared with untreated HC group (Table 1). Indeed, in Po treated HC group compared to untreated group, TBARS concentrations were decreased by 48% in erythrocytes (p<0.001), and by 37% in both adipose tissue and heart (p<0.01).

Table 1. The levels of TBARS in erythrocytes and different tissues of HC- group against Po-HC group

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>Po-HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>34.60±0.95</td>
<td>17.90±1.34**</td>
</tr>
<tr>
<td>Liver</td>
<td>33.57±5.69</td>
<td>33.76±5.71</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>96.14±6.84</td>
<td>60.12±6.53**</td>
</tr>
<tr>
<td>Muscle</td>
<td>52.30±2.61</td>
<td>51.27±2.34</td>
</tr>
<tr>
<td>Heart</td>
<td>106.27±10.00</td>
<td>66.46±2.99**</td>
</tr>
<tr>
<td>Kidney</td>
<td>182.04±1.09</td>
<td>177.23±2.45</td>
</tr>
</tbody>
</table>

HC, hypercholesterolemic rats; Po-HC, hypercholesterolemic rats treated with Portulaca oleracea L. extract. Values are means± SEM of 6 rats per group. **P<0.01, ***P<0.001, Po-HC treated vs untreated HC group.

Antioxidant enzymes activities in erythrocytes

In erythrocytes, SOD and CAT activities were 1.2- and 1.6-fold higher in Po-HC treated than untreated group, whereas no significant difference was observed in GRed and GSH-Px (Table 2).

Table 2. The activities of antioxidant enzymes in erythrocytes of HC- group against Po-HC group

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>Po-HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/ml)</td>
<td>104.50±63.64</td>
<td>126.05±7.72**</td>
</tr>
<tr>
<td>GSH-Px (nmol/min/ml)</td>
<td>283.14±10.00</td>
<td>311.53±2.86</td>
</tr>
<tr>
<td>GRed (nmol/min/ml)</td>
<td>64.31±05.27</td>
<td>69.20±3.15</td>
</tr>
<tr>
<td>CAT (nmol/min/ml)</td>
<td>53.26±05.80</td>
<td>87.10±4.20**</td>
</tr>
</tbody>
</table>

HC, hypercholesterolemic rats; Po-HC, hypercholesterolemic rats treated with Portulaca oleracea L. extract. Values are means± SEM of 6 rats per group. **P<0.01, Po-HC treated vs untreated HC group.

Antioxidant enzymes activities in different tissues

SOD activity was respectively, 1.2-, 1.3-, 1.5- and 1.2-fold higher in muscle, liver, heart and kidney (Table 3). CAT activities in liver and heart were respectively, 1.7- and 1.5-fold higher in treated Po-HC than untreated HC group. Compared to untreated HC group, GSH-Px activity was 1.2-fold higher in adipose tissue and GRed activity was 1.6- and 1.4-fold higher in liver and adipose tissue, in treated Po-HC (Table 3). However, there was no significant difference in GSH-Px of liver, muscle, heart or kidney, in GRed of muscle, heart and kidney, and in CAT of adipose tissue, muscle, heart and kidney.

Table 3. The activities of antioxidant enzymes in different tissues of HC- group against Po-HC group

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>Po-HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>107.68±33.70</td>
<td>147.67±14.00</td>
</tr>
<tr>
<td>GSH-Px (nmol/min/g)</td>
<td>09.96±01.27</td>
<td>11.32±01.60</td>
</tr>
<tr>
<td>GRed (nmol/min/g)</td>
<td>49.61±07.40</td>
<td>81.16±11.30**</td>
</tr>
<tr>
<td>CAT (nmol/min/g)</td>
<td>09.28±02.09</td>
<td>16.36±06.60*</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>153.01±13.00</td>
<td>153.70±13.00</td>
</tr>
<tr>
<td>GSH-Px (nmol/min/g)</td>
<td>254.65±4.50</td>
<td>311.66±8.60**</td>
</tr>
<tr>
<td>GRed (nmol/min/g)</td>
<td>57.90±7.00</td>
<td>86.10±2.86*</td>
</tr>
<tr>
<td>CAT (nmol/min/g)</td>
<td>122.00±0.01</td>
<td>121.80±0.01</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>99.20±14.10</td>
<td>121.00±1.34**</td>
</tr>
<tr>
<td>GSH-Px (nmol/min/g)</td>
<td>106.30±3.20</td>
<td>108.10±3.43</td>
</tr>
<tr>
<td>GRed (nmol/min/g)</td>
<td>173.81±7.66</td>
<td>171.50±8.30</td>
</tr>
<tr>
<td>CAT (nmol/min/g)</td>
<td>54.45±4.70</td>
<td>59.21±5.90</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>68.40±12.00</td>
<td>108.00±12.00</td>
</tr>
<tr>
<td>GSH-Px (nmol/min/g)</td>
<td>123.76±2.10</td>
<td>125.80±2.00</td>
</tr>
<tr>
<td>GRed (nmol/min/g)</td>
<td>55.40±4.40</td>
<td>56.76±4.70</td>
</tr>
<tr>
<td>CAT (nmol/min/g)</td>
<td>27.00±8.67</td>
<td>42.86±16.00**</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>159.54±7.69</td>
<td>189.20±11.00**</td>
</tr>
<tr>
<td>GSH-Px (nmol/min/g)</td>
<td>75.54±9.24</td>
<td>75.30±9.50</td>
</tr>
<tr>
<td>GRed (nmol/min/g)</td>
<td>98.67±5.67</td>
<td>99.00±5.60</td>
</tr>
<tr>
<td>CAT (nmol/min/g)</td>
<td>43.00±6.31</td>
<td>43.54±5.08</td>
</tr>
</tbody>
</table>

HC, hypercholesterolemic rats; Po-HC, hypercholesterolemic rats treated with Portulaca oleracea L. extract. Values are means± SEM of 6 rats per group. * P<0.05; ** P<0.05; ***P<0.001, Po-HC treated vs untreated HC group.

Zidan et al: Portulaca oleracea and reduced oxidative stress
DISCUSSION

In this study, the effect of lyophilized aqueous extract from Po on lipid peroxidation as well as its influence on the antioxidant enzymes activities in erythrocytes and tissues, were reported in rats fed a cholesterol-enriched diet.

Hypercholesterolemia is a dominant risk factor of atherosclerosis [22]. In this study, cholesterol-enriched diet (1%) for 10 days induced hypercholesterolemia and oxidative stress in rats. In addition, feeding of atherogenic diet cholesterol (1%) for 2 weeks resulted in high total cholesterol in rat [23].

Oxygen free radicals have been implicated in the pathogenesis of hypercholesterolemic atherosclerosis and antioxidants suppress its development [24]. Hypercholesterolemia can increase the cholesterol content of platelets, polymorphonuclear leukocytes and endothelial cells, so that endothelial and smooth muscle cells, neutrophils and platelets may be sources of oxygen free radicals [25].

High-cholesterol diet provides a relevant example of endogenous chronic oxidative stress due to the resulting hypercholesterolemia. Indeed, Otunola et al., (2010) [26] confirmed the efficiency of cholesterol-enriched diet to produce a state of oxidative stress with biochemical and biological characteristics of hypercholesterolemia in rat Wistar. It has been reported that hypercholesterolemia increased oxygen-free radical production. Feeding high cholesterol diet by animals showed a depressed antioxidant system and the formation of free radicals. Thus, it has been suggested that the antioxidant effects may potentially improve hypercholesterolemic status [27].

Oxidative stress and lipid peroxidation have been implicated in atherogenesis. One of the hyoprodcts of lipid peroxidation is the MDA. Modified proteins by MDA changes antigenicity, function, and turnover kinetics of various proteins and has been implicated as a pathogenetic mechanism of atherosclerosis [28].

In this study, Po aqueous extract treatment reduced TBARS concentrations in erythrocytes, adipose tissue and heart, in hypercholesterolemic rats. These decreases, observed with Po treatment, could be good indicators of lowered lipid peroxidation in hypercholesterolemic rats. The same result was observed in rat fed high-cholesterol diet (1%) and treated with Curcuma longa L., and Ruta Chalepensis extracts L. [29].

Bouderbala et al., (2008) [19] observed no changes in TBARS of liver, muscle and adipose tissue in hypercholesterolemic rats treated with Ajuga iva L. aqueous extract, compared to untreated hypercholesterolemic rat, but Alagumanivasagam et al., (2012) [30] were noted increased TBARS concentrations and lowered lipid peroxidation in liver, heart and aorta, in hypercholesterolemic rat treated with methanolic extract of Teramnus labialis (L), compared to untreated rats.

In this study, hypercholesterolemic rats treated with Po presented a significant decrease in lipid peroxidation in adipose tissue and heart, compared to untreated rats. This increasing lipid peroxidation would lead to the generation of harmful free radicals which impair membrane function and ultimately result in microvascular and macrovascular complications [31]. This could be suggesting that the aqueous extract of Po may possess antioxidant effects.

It has been demonstrated in this investigation that the consumption of cholesterol-enriched diet (1%) for 10 days induced oxidative stress by decreasing the antioxidant enzymes activities in tissue rat. The results of the above study clearly demonstrated that Po aqueous extract had an antioxidant effect. In erythrocytes, liver and heart, SOD and catalase activities were higher in Po treated group than untreated hypercholesterolemic group. The higher SOD activity was observed in muscle and adipose tissue. This increase might be due to enzyme activation by Po treatment. The same effect was observed in the hypercholesterolemic rats treated with aqueous extract of Asparagus racemosus Willd, compared with untreated rats [32].

In this study, high SOD activity in erythrocytes and all tissues was believed to be due to increased dismutation of superoxide anions due to their production. Hao et al., (2009) [33] reported that Po can be used as a medicinal plant, where it was used for anti-aging, thereby increasing level of SOD and decreasing that of MDA in brain of mice treated with D-galactosamine. CAT and SOD activities in heart were higher in HC-Po compared with HC group; this treatment of Po (0.5%) prevented TBARS accumulation in this tissue and inhibited lipid peroxidation. The same effect was observed in the hypercholesterolemic rats treated with aqueous extract of Astragalus mongholicus compared with untreated group suggesting that Astragalus mongholicus extract consumption can improve lipid profile, inhibit peroxidation, and increase the antioxidant enzymes activity, and is thereby likely to reduce the risk of coronary heart disease associated with hyperlipidemia and oxidative stress in hypercholesterolemic rats [5].

Po is also reported as an excellent source of antioxidant vitamins α-tocopherol, ascorbic acid and β-carotene, as well as glutathione. Moreover, Po is considered as a rich source of many amino acids like isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine and valine. It has been described as a “power food of the future” because of its high nutritive and antioxidant properties [34].

The decreased enzyme activities in the current study were possibly due to the antioxidants and bioactive compounds present in Po which acted against oxidative stress, such as flavonoids, alkaloids, omega-3 fatty acids, β-carotene, and vitamins.

CONCLUSION

Po, lyophilized aqueous extract treatment, in hypercholesterolemic rats, can reduce oxidative stress by
preventing lipid peroxidation and increasing antioxidant enzymes activities in erythrocytes and tissues. Such effect may be due to the presence of several secondary metabolites belonging principally to the phytochemical class of phenolic compounds, nitrogen compounds, vitamins and terpenoids.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest to disclose.

ACKNOWLEDGMENTS
This work was supported by the Algerian Ministry of Higher Education and Scientific Research and by the French Foreign Office, with International Research Extension Grant 11 MDU $23.

REFERENCES

Zidan et al: Portulaca oleracea and reduced oxidative stress


© TEMKODER. This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, noncommercial use, distribution and reproduction in any medium, provided that the work is properly cited.

Conflict of Interest: None declared