Efficacy of Portulaca Oleracea Leaves Extract on Lipid Profile in Induced Hyperlipidemic Rats

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Abstract:

The increased incidence of cardiovascular diseases and hyperlipidemia was observed among the Egyptians in the last decades. The present study aims to explore the role of Purslane on some biological activities of hyperlipidemic rats. Therefore, different concentrations ranged 200 to 400 mg/kg body weight (BW) of Purslane extract were consumed by hyperlipidemic rats and the plasma total cholesterol, triglycerides, HDL-c, LDL-c, lipid peroxide as well as total antioxidants capacity levels were investigated. Results revealed that significant differences (P<0.01) among all experimental groups regarding total cholesterol, triglycerides, HDL-c, LDL-c, lipid peroxide and total antioxidants capacity levels were observed. Concentration of 400 mg/kg BW of purslane extract recorded the most decrease in all previous plasma parameters except the HDL-c and total antioxidant capacity levels which recorded the opposite direction.

Key words: Portulaca oleracea, triglycerides- HDL-c, LDL-c, antioxidant capacity- lipid peroxide

Introduction:

Purslane (Portulaca oleracea, L.) family (Portulacaceae) was a nutritious vegetable used for human consumption, and it was mentioned in Egyptian texts a long time ago (Mohamed, 2014). This plant was used as a vegetable (Drury, 2012). Spice and medicinal plant has been known since the times of the ancient Egyptians and was popular in England during the middle Ages (Lanska, 2015).

Purslane has been described as a power food of the future because its high nutritive and antioxidants properties (Al-Howiriny, 2008). In addition contain Omega-3 fatty acid and β- carotene, ascorbic acid. One hundred grams of fresh purslane leaves (one serving) contains about 300–400 mg of α- linolenic acid, 12.2mg of α- tocopherol, 26.6 mg of
ascorbic acid, 1.9 mg of β- carotene, and 14.8 mg of glutathione, respectively (Liu et al., 2014).

Purslane provides highest dietary minerals such as potassium (494 mg/100 g), magnesium (68 mg/100 g), calcium (65 mg/100 g), phosphorus (44 mg/100 g), and iron (1.99 mg/100 g). Also, it contained (17.50-29.04%) protein, (5.00-12.00%) crude fibers, (17.80-23.01%) ash, on dry weight basis / 100g (El-Hadidy, 2014 and Obied et al., 2016).

Recent researches showed that it was exhibited a wide range of biological effects, including skeletal muscle relaxant effect (Parry et al., 1993) analgesic & anti-inflammatory effects (Chan et al., 2000), antifungal activity (Chang et al., 2000), anti-fertility and anti-aging, therapy increasing the level of superoxide dismutase (SOD) and decreasing the level of Malondialdehyde (MDA) in the brains of mices (Hao et al., 2009 and Ray, 2014).

It has shown other beneficial effects such as anti-diabetic (Gong et al., 2009), and wound healing properties (Rashed et al., 2003). Also, purslane contained active molecules for the treatment of some parasitic infectious diseases such as leishmaniasis and trypanosomiasis (Arruda et al., 2004 and Costa et al., 2007). Plant and seeds of purslane are used in diseases of the kidney and bladder (Nadkarni, 2011).

The present study aims to evaluation of anti hyperlipidemic effect of Purslane (Portulaca oleracea) extract in albino rats induced hypercholesteremic rats by the determin of TC, LDL, HDL, TG, the plasma levels of lipid peroxides and the total antioxidant capacity.

Materials and Methods:

Materials:

Fresh Purslane (Portulaca oleracea L.) plants were harvested from fields Assuit City, Assuit Government, Egypt prior to flowering period during 2016.

Methods:

Plant drying:

Green leaves were manually separated from plant, washed with water and then dried and left to dry for 15 min at room temperature. The
moisture content of the fresh leaves was immediately determined according to the procedures described in the A.O.A.C. (2008) and found to be 85.78 g water per 100 g of sample. Hot-air oven drying: One Kg of purslane leaves were dried at 70°C for 1 hour. The moisture content of the dried leaves was 7.18 g water per 100 g dried leaves A.O.A.C. (2010):

Plant extraction:

The air-dried leaves of P. oleracea L. (500g) was powdered and successive extracted with distilled water (1 g/10 ml) with constant stirring for 4 h and then filtered. The filtrates were combined and concentrate in a rotary evaporator under reduced pressure at 55°C, and then freeze dried in a lyophilize (Gao et al., 2007).

Chemicals and reagents:

Isooctyl- polyoxyethylene phenol (Triton WR) from Sigma Chemical Co., St. Louis, MO.

Biological experiment:

Experimental animals:

Forty adult male white albino rats, (Sprague dawley strain) weighing between 200 - 220 gram were obtained from the animal house of the Faculty of Medicine, Assiut University, Egypt. The animals were divided into 4 groups. Each group consisted of 10 rats as shown in table (2). Daily administrations were continued for (4) weeks. All groups were housed in wire cages under the normal Laboratory conditions and were fed on basal diets for one week to adaptation period. Body weight gain and feed intake were weighed weekly and by the end of the experimental feeding period.

The daily oral supplementation of one ml of either (200 - 400 mg/kg b. w. for POL) extracts suspension. The daily supplementation was lasting for four weeks. The first group of rats (Control) was fed on a basal diet Table (1), while the other groups were fed on a Portulaca oleracea L. extract suspension samples as shown in Table (2) according to Ilwy (2003). The rats were divided into 4 groups as following:

The basal diet used consisted of corn oil, salts mixture, vitamins mixture, corn starch according to Campell (1998) and Hegested et al.
The daily oral supplementation of one ml of either (200 - 400 mg/kg b. w. for purslane) extracts suspension.

**Table (1): Commercial basal diet for 100 gm diet.**

<table>
<thead>
<tr>
<th>Item</th>
<th>%</th>
<th>Item</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>67.8</td>
<td>Salt mixture</td>
<td>3.5</td>
</tr>
<tr>
<td>Casein</td>
<td>12.5</td>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10.0</td>
<td>Choline chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamins mixture</td>
<td>1.0</td>
<td>Total</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

**Table (2): Experimental diets of purslane extract for white albino rats.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>basal diet (Negative control)</td>
</tr>
<tr>
<td>2</td>
<td>Hyperlipidemic(non-treated) groups: basal diet + injection with 50 mg Triton (WR) / 100g</td>
</tr>
<tr>
<td>3</td>
<td>Hyperlipidemic + 200mg/ kg POL (LD) low dose</td>
</tr>
<tr>
<td>4</td>
<td>Hyperlipidemic + 400mg/ kg POL (HD) high dose</td>
</tr>
</tbody>
</table>

Control rats were given saline by the same route, dose, and frequency. Anesthesia was induced in a 2.5-1 glass chamber in which 15 ml isoflurane had been volatilized. Three injections each week for three weeks were essential to assure continuously high levels of lipid. The levels of cholesterol were higher than normal (Levine and Saltzman, 2006).

**Blood sampling:**

Weekly and at the end of the experiment, fasting blood samples were collected from the retro-orbital plexus from all animals in each group into clean and labeled tube contained heparin (10.0 IU/ml). Blood was centrifuged at (3500 r.p.m.) for 15 min. to separate plasma which
was stored in sealed tightly tubes at –20°C until biochemical assays were carried Hegested et al. (1991).

Biochemical analysis:

Total triglycerides levels in plasma were estimated calorimetrically according to the method of Wahlefeld (1974). Total cholesterol was estimated according to the method of Allian et al. (1974). High density lipoprotein cholesterol (HDL-c) was determined according to the method of Warnick et al., (1983). Low density lipoprotein cholesterol (LDL-c) was determined according to the method of Friedewald et al. (1998). Total antioxidants capacity was determined by the method of Satoh (1998) and Koracevic (2001).

Statistical method:

All the values were expressed as mean ± SD. The data were statistically analyzed by one way ANOVA followed by Dennett's t test (Duncan, 1995).

Results and Discussion:

Total Cholesterol:

The results given in Table (3) and Fig. (1) showed that the plasma of rats injected with Triton WR had highest concentration of cholesterol as high as (333.3 ± 32.3 mg/dl) of positive control which was higher than that found in plasma of control rats (185.5 ±7.45 mg/dl). This indicates that Triton is responsible for increasing plasma cholesterol. Addition of purslane extract to rats injected with Triton WR resulted in significant decline in the concentration of plasma cholesterol. 400mg/kg of purslane extraction caused greater reduction in plasma cholesterol (198.3 ±4.62 mg/dl) than that was resulted by dose of 200 mg/kg of purslane extraction (221.2 ±5.82 mg/dl).

Hypercholesterolemia produce oxidative stress in various ways and increases synthesis of arachidonic acid and prostaglandins. Reactive Oxygen Species (ROS) have been implicated in the development of hypercholesterolemia atherosclerosis (Kuppast et al., 2012).

The data are in agreement with Prasad (2005) showed that extract of purslane for 12 weeks significantly decreased the serum total cholesterol, LDL-c and VLDL-c in comparison with the
hypercholesterolemia group (non treated). Movahedian (2007) mentioned that addition of purslane leaves extract to the cholesterol-enriched diet in rabbits improved the total cholesterol level.

The lowering of cholesterol level properties may be due to the fiber found in this plant. Fiber changes the rate of the fat and carbohydrates absorption in the intestine (Bush, 1991). Certainly following the reduction in the level of serum cholesterol, the level of lipid liver lipidosis and the activity of liver enzyme are significantly reduced (Jalali et al., 2008).

Melatonin recently was identified in fresh purslane leaves. The melatonin concentration in purslane was found to exceed that reported in a number of other fruits and vegetables (Simopoulos et al., 2005 and Rodriguez et al., 2004). Melatonin has a variety of important functions including direct free radical scavenging and anti-inflammatory properties.

Table (3): Effect of different concentrations of purslane extract on lipid profile levels (mg /dl) in the different hyperlipidemia groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group1</td>
<td></td>
<td>185.5 ±7.45^c</td>
<td>175.56 ±13.63^c</td>
<td>42.92±2.61^b</td>
<td>101.98 ±07.3^c</td>
</tr>
<tr>
<td>Group2</td>
<td></td>
<td>333.3 ±32.3^a</td>
<td>334.86 ±23.03^a</td>
<td>22.38±2.46^c</td>
<td>239.14 ± 30.6^b</td>
</tr>
<tr>
<td>Group3</td>
<td></td>
<td>221.2 ±5.82^b</td>
<td>227.8 ±07.20^b</td>
<td>41.04±5.28^b</td>
<td>149.6 ±15.97^b</td>
</tr>
<tr>
<td>Group4</td>
<td></td>
<td>198.3 ±4.62^d</td>
<td>179.9 ±09.89^c</td>
<td>53.74±3.85^a</td>
<td>110.98 ±07.48^c</td>
</tr>
<tr>
<td>F. value</td>
<td></td>
<td>78.905**</td>
<td>125.105**</td>
<td>60.95**</td>
<td>60.30**</td>
</tr>
</tbody>
</table>

N=10 Means ± S.D. (Standard Deviation). * *(p< 0.01)
Fig. (1): Changes in the plasma total cholesterol levels in the different groups.

Triglycerides:

The results in Table (3) and Fig. (2) reveal that the plasma of rats that which were injected with Triton WR had highest concentration of triglycerides as high as (334.86 ±23.3 mg/dl) of positive control which was higher than that found in plasma of control rats (175.56 ±13.63 mg/dl). This indicates that Triton is responsible for increasing plasma triglycerides. Addition of *P. oleracea* extract to rats injected with Triton WR resulted in significant decline in the concentration of plasma triglycerides. Four hundred mg/kg of *P. oleracea* extract caused greater reduction in plasma triglycerides (179.9±9.89 mg/dl) than that resulted by addition of 200 mg/kg of *P. oleracea* extract (227.8 ±7.2 mg/dl).

The data are in agreement with Mohammed (2011) indicated that a significant decrease in serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), possibly due to its contents of polyunsaturated fatty acids, flavonoids, and polysaccharides.

The phytochemical constituents of *Portulaca oleracea* including steroids, vitamins, minerals, fatty acids, alkaloids and saponins (Naeem and Sohail, 2013).
Fig. (2): Changes in the plasma triglycerides levels in the different groups.

High density lipoprotein (HDL):

The results given in Table (3) and Fig. (3) found that high density lipoprotein (HDL) level showed significant differences among all the four experimental groups ($P < 0.01$).

The results of this study showed that the HDL levels in hyperlipidemic rats groups treated with either 200 mg/kg or 400mg/kg (3 and 4) were 41.04 ± 5.28 and 53.74 ± 3.85 mg/dl, respectively were significantly increased as compared with hyperlipidemic group (2) fed on basal diet without any treatment recorded (22.38 ±2.46 mg/dl). However, these levels were significantly increased than that of the control group of rats (42.92 ± 2.61mg/dl).

The data were in agreement with literature of Movahedian et al. (2007) indicated that, a decrease in the atherogenic index with respect of hypercholesterolemia groups, which is generally believed to be beneficial since the HDL level inversely correlated with coronary heart disease and reduction in this ratio is considered as an anti atherosclerotic factor. Treatment with extract of Portulaca oleracea Linn. Produced a significant decrease in the serum level of lipids in the dexamethasone induced hyperlipidemia in rats.
Our results are agreement with literature of Besong et al. (2011) observed that HDL-C level was increased in induced hypercholesterolemia rats treated with either 200mg/kg or 400mg/kg indicated that a significantly for 28 days.

These results were agreement with literature of Makni et al. (2008), stated that, increasing in HDL-c or HTR ratio is one of the most important criteria of anti-hypercholesterolemia agent.

The ability of Omega-3 fatty acids to raise the beneficial high density lipoprotein, decrease the thickness of the blood may be advantageous in the treatment of vascular diseases, cancers, and a number of chronic diseases and conditions throughout the human life. The high amount of phytostrols and alkaloids may be responsible for the hypolipidemic effect. It was found more effective in higher dose as compared to lower dose as an anti hyperlipidemic agent and also improves HDL- cholesterol levels.

Fig. (3): Changes in the plasma high density lipoprotein levels in the different groups.
Low density lipoprotein (LDL):

The results given in Table (3) and Fig. (4) noted that, rats were injected with Triton WR without any treatment the amount of low density lipoprotein (LDL) level in group (2) was (239.14 ± 30.6 mg/dl), while other groups treated with either 200 mg/kg or 400 mg/kg (3 and 4) were (149.6±15.97) and (110.98±7.48) mg/dl, respectively were significantly decreased as compared with hyperlipidemic groups (2).

Statistical analysis showed high significant differences among four studied groups at (P < 0.01).

A reduction in LDL would be advantageous clinically and in fact it was shown clearly that the present crude extract had an improving effect on the hypercholesterolemia induced by a high fat diet (Schaefer and Asztalos, 2006). Increasing in total cholesterol and LDL-C induced by the cholesterol enriched diet was reduced significantly by melatonin administration (Hoyos et al., 2000). The presence of these compounds in purslane leaf may be play a role in the observed hypocholesterolemic effects.

Additionally, the reduction of LDL-C by the supplementation of purslane reduction expected to be effective for the prevention of in HTR ratio is a major in importance predicting coronary arteriosclerosis and cardiovascular diseases, since an heart disease in human being, an increase in this ratio is increase of serum LDL-C level is considered to be a stronger risk factor for the occurrence of cardiovascular diseases than the increase of the total cholesterol level. In fact, the reduction of LDL-C is emphasized more for the therapy of hyperlipidemia (Simopoulos et al., 2013).
Fig. (4): Changes in the plasma low density lipoprotein levels in the different groups

Lipid peroxide (Malondialdehyde) levels:

The results given in Table (4) and Fig. (5) indicate that, the plasma of rats injected with Triton WR had highest concentration of lipid peroxide (Malondialdehyde) levels as high as \((11.2 \pm 0.89 \text{ mol/ml})\) of positive control which was higher than that was found in plasma of control rats \((4.02 \pm 0.40 \text{ mol/ml})\). This indicated that, Triton WR is responsible for increasing plasma lipid peroxide levels. Addition of \(P. \) oleracea extract to rats injected with Triton WR resulted in significant decline in the concentration of plasma lipid peroxide (Malondialdehyde) levels. Four hundred mg/kg of \(P. \) Olerace extract caused greater reduction in plasma lipid peroxide (Malondialdehyde) levels recorded \((2.64 \pm 3.45 \text{ mol/ml})\) than that resulted by addition of 200 mg/kg of POL extraction recorded \((6.50 \pm 1.43 \text{ mol/ml})\). These results showed that, significant differences among four groups at \((P < 0.01)\).

\(Portulaca \) oleracea induced significant reduction in MDA of liver and kidney (30.9 and 8.7\%), respectively \((\text{NCEP, 2002})\).

Many natural products are reported to influence the antioxidant systems and are good cytoprotective agents \((\text{Dragsted et al., 1997})\). SOD, CAT, GPx, GST, GR and GSH, play an important role in the biological systems to act against oxidative stress \((\text{Akyol et al., 2002})\).

The protective role of glutathione, as an antioxidant and detoxifying agent, has been demonstrated in various clinical studies \((\text{Simopoulos}, \ldots)\).
2004). It is a ubiquitous compound that is synthesized rapidly in the liver, kidney and other tissues, including the gastrointestinal tract.

**Table (4):** Effect of different concentrations of purslane extraction on lipid peroxide (nmol/ml) (Malondialdehyde) and total antioxidants capacity (m M/L) levels in different hyperlipidemia groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Group1</th>
<th>Group2</th>
<th>Group3</th>
<th>Group4</th>
<th>F. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipidperoxide (Malondialdehyde) (nmol/ml)</td>
<td>4.02 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.2 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.50 ±1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.64 ±3.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>73.15**</td>
</tr>
<tr>
<td></td>
<td>Total antioxidants capacity (m M/L)</td>
<td>0.58 ± 0.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2 ± 0.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.18 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.84 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.93**</td>
</tr>
</tbody>
</table>

N=5 Means ± S.D. * *(p≤ 0.01)

**Fig. (5) Changes in the plasma lipid peroxide levels in the different groups.**

**Total antioxidant capacity levels:**

The results given in Table (4) and Fig. (6) noted that, total antioxidant capacity level showed significant differences among all the four studied groups at (P < 0.01).
The results of present study showed that the mean total antioxidant capacity levels in hyperlipidemic rats groups which were treated with either 200 mg/kg or 400mg/kg (3 and 4) were $1.18 \pm 0.24 \text{ Mm/L}$ and $1.84 \pm 0.21$; respectively. There are significantly increased as compared with hyperlipidemic groups (2) fed on basal diet without any treatment recorded ($0.2 \pm 0.71 \text{ m M/L}$) and control group (1) fed on basal diet recorded ($0.2 \pm 0.71 \text{ mM/L}$) purslane may have a protective effect against oxidative stress caused by vitamin A deficiency (Arruda et al., 2004).

The purslane contains the major phytocmpounds, including flavanoids which may have been responsible for the observed antioxidant activity. The total phenol content of six cultivars of purslane ranged from 127 to 478 mg/ 100 g of fresh weight of plant (Oliverira et al., 2009).

Antioxidant activity of purslane increased levels of GPx, GR, GST, Catalase (CAT), and Superoxide dismutase (SOD) were found to correlate with elevated glutathione level and depressed Malondialdehyde (MDA) and Nitrate (NO) in rats (Obied et al., 2016).

Fig. (6) Changes in the plasma total antioxidants capacity levels in the different groups.

Conclusion:

The current study proved that, the efficiency of extract of Portulaca oleracea on induced hypercholesteroleemia rats and liver function.
References:


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فعالية مستخلص أوراق نبات الرجلة على مستوي الدهون المرتفع في فئران التجارب

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المستخلص العربي

لوحظ في العقود الأخيرة ارتفاع حالات الإصابة بأمراض القلب والأوعية الدموية بين المصريين. وتهدف الدراسة الحالية إلى التعرف على دور أوراق نبات الرجلة وتأثيره على بعض الأنشطة البيولوجية للقرود المصابين بارتفاع مستوي الدهون بالدم. وقد استخدم تركيزين من مستخلص أوراق نبات الرجلة في التجربة وهما 200 و 0.4 ملجم/ كجم من وزن جسم فئران التجارب المستخدمة في البحث، وتأثيرهما على مستوى سيرم الدم من الكوليسترول الكلي، الدهون الثلاثية، الكوليسترول المرتفع الكثاف، الكوليسترول المنخفض الكثاف، بيروكسيد الدهون، وكذلك اجمالي قدره المواد المضادة للأكسدة تم التحقق منها. وقد اثبتت النتائج أن هناك فروق ذات دلالة إحصائية (P<0.01) بين جميع المجموعات التجربية في مستوي الكوليسترول الكلي والدهون الثلاثية، الكوليسترول المرتفع الكثاف، الكوليسترول المنخفض الكثاف، بيروكسيد الدهون و قدره مضادات الأكسدة الكليه. وقد لوحظ ان تركيز 0.4 مجم / كجم من وزن الجسم من مستخلص أوراق نبات الرجلة أحدث انخفاض في جميع المعاملات السابقة باستثناء الكوليسترول المرتفع الكثاف، إجمالي قدره المواد المضادة للأكسدة. وبالتالي قد تكون المواد المضادة للأكسدة والتي سجلت في الاتجاه المعاكس.

الكلمات المفتاحية: نبات الرجلة - الدهون الثلاثية - الكوليسترول المرتفع الكثاف - الكوليسترول المنخفض الكثاف - بيروكسيد الدهون - اجمالي قدره المواد المضادة للأكسدة.