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Full Length Research Paper

Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action

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Aqueous juice from purslane (*Portulaca oleracea*) was screened for its antioxidant activity in adult male Waster albino rats. The antioxidant activity was determined by measuring reduced glutathione, catalase, superoxide dismutase, glutathione reductase, glutathione-S-transferase and glutathione peroxidase, as well as the inhibition in lipid peroxidation, nitric oxide in liver, kidney and testis of rats. Liver and kidney function were also determined. Administered rats with aqueous juice of purslane, resulted in marked improvement in all studied parameters. On the basis of the above results, it can be concluded that purslane is a promising natural product, which could be useful for the prevention of cardiovascular, neurodegenerative and other chronic diseases caused by oxidative stress.

Key words: Purslane, antioxidant enzymes, oxidative stress, rat.

INTRODUCTION

Purslane (*Portulaca oleracea*) is a nutritious vegetable used for human consumption, and it was mentioned in Egyptian texts from the time of the Pharaohs (Mohamed and Hussein, 1994). Purslane is eaten raw as a salad and also is eaten cooked as a sauce in soups or as greens. Purslane provides a rich plant source of nutritional benefits (Sudhakar et al., 2010). It is one of the richest green plant sources of omega-3 fatty acids and a-linolenic acid (Simopoulos and Salem, 1986). In areas where this 'weed' is eaten, there is a low incidence of cancer and heart disease, possibly due to purslane's naturally occurring omega-3 fatty acids (Simopoulos, 1991). Purslane has been used as an antiseptic, anti-diuretic, vermifuge in oral ulcer and urinary disorders.

Recent researches show that it exhibits a wide range of biological effects, including skeletal muscle relaxant effect (Parry et al., 1993), analgesic and anti-inflammatory effects (Chan et al., 2000), antifungal activity (Oh et al., 2000) and antifertility effect (Verma et al., 1982). Also, it has shown other beneficial effects such as antidiabetic (Gong et al., 2009) and wound healing properties (Rashed et al., 2003). In addition, purslane may have a protective effect against oxidative stress caused by vitamin A deficiency (Arruda et al., 2004). Also, purslane contains active molecules for the treatment of some parasitic infectious diseases such as leishmaniasis and trypanosomiasis (Costa et al., 2007).

Little information has been published regarding the antioxidant activity of purslane. The current study aimed to evaluate the beneficial effect of Purslane on hepatic, renal and testicular function, as well as antioxidant effect that may make it one of the more important foods of the future.

MATERIALS AND METHODS

Experimental animals

Adult male Wister albino rats weighing 120 to 150 g were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt).

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Animals were kept in wire bottomed cages, in a room under standard condition of illumination with a 12 h light-dark cycle at 25 ± 1°C. They were provided with water and balanced diet *ad libitum*. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

Plant juice

The fresh purslane herb, free of blemishes or obvious defects, was collected from the Delta of Nile during August 2010. An aqueous juice of the purslane herbs prepared by mashing in a proportion of 1:5 (w/v) and Left for about 24 h. After Mashing, the resulting crude extract was filtered and the filtrate was kept at 20°C for future use.

Experimental protocol

To study the effect of purslane, twelve adult male albino rats were randomly divided into two groups, six rats of each. Group (I) served as control and received saline (0.2 ml saline/ rat) by oral administration via epigastric tube. Group (II) received oral administration of 1.5 ml/kg purslane aqueous juice for 12 days.

The animals of the two groups were cervically dislocated after blood samples were collected from retro-orbital plexus. Blood stranded for half an hour and then centrifuged at 500 g for 15 min at 4°C to separate serum and stored at -70°C until analysis. Pieces of liver, kidney and testis were weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose (Tsakiris et al., 2004). The homogenate was centrifuged at 500 g for 10 min at 4°C. The supernatant (10%) was used for the various biochemical determinations.

Biochemical estimations

Liver function test

Colorimetric determination of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine, according to the method of Reitman and Frankel (1957).

The color of which was measured at 546 nm. γ-glutamyl transpeptidase (γGT) and alkaline phosphatase were assayed in liver homogenate, using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the method that was described by Szasz (1969) and Belfield and Goldberg (1971), respectively. Also, Total bilirubin (TB) in serum, was assayed according to the method of Schmidt and Eisenburg (1975).

Kidney function test

Uric acid (UA), blood urea nitrogen (BUN) and serum creatinine (Cr) were assayed in serum, using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the methods that were described by Fossati et al. (1980), Fawcett and Scott (1960) and Szasz et al. (1979), respectively.

Determination of malondialdehyde and nitrite/nitrate

Malondialdehyde (MDA) and nitrite/nitrate (NO) were assayed colorimetrically in liver homogenate, according to the method of Ohkawa et al. (1979) and Berkels et al. (2004), respectively. Where MDA determined by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) formed. Nitric oxide was determined in acid medium and in the presence of nitrite, the formed nitrous acid diazotise sulphanilamide is coupled with N-(1–naphthyl) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm.

Estimation of reduced glutathione and anti-oxidant enzymes

The hepatic, renal, testicular and serum reduced glutathione (GSH) levels were determined by the methods of Ellman (1959). The method is based on the reduction of Elman's reagent (5,5` dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm. In addition, the activity of hepatic, renal and testicular antioxidant as catalase (CAT) was determined catalase reacts with a known quantity of H_2O_2, according to the method of Aebi (1984). The reaction is stopped after exactly 1 min with catalase inhibitor. In the presence of peroxidase (HRP), the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the activity of catalase in the original sample.

Superoxide dismutase (SOD) activity was assayed by the method of Nishikimi et al. (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Also, the activity of glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) activities were determined by the methods of Habig et al. (1974), Paglia and Valentine (1967) and Factor et al. (1998), respectively.

Statistical analysis

The obtained data were presented as means ± standard error. Statistical analysis was performed using an unpaired Student's t-test using a statistical package program (SPSS version 17.0).

RESULTS

The results of the present study showed that purslane aqueous extract administration caused a significant reduction in AST, γ-GT, ALP and bilirubin (-7.4, -10.1, -31.0 and 13.3%) respectively, while ALT was non-significantly changed indicating that purslane administration support the function of liver (Table 1).

The results of kidney function tests in purslane group, showed that purslane administration caused significant increase in uric acid (28.0%) with significant decrease in urea and creatinine (33.2 and 28.0%), respectively, showing the beneficial effect of purslane (Table 2). Purslane administration induced significant reduction in MDA of liver and kidney (30.9 and 8.7%), respectively, with significant reduction in testicular nitrite/nitrate by -27.9%, indicating that purslane has antioxidant properties (Table 3). Data in Table 4, demonstrates the potential effect of purslane administered to normal rats as antioxidant.

Purslane caused a significant increase in glutathione

Table 1. Effect of purslane aqueous juice on liver function of rats.

Parameter / Groups	ALT (u/l)	AST (u/l)	γ-GT (u/l)	ALP (iu/l)	Bilirubin (mg/dl)
Control group	71.13 ± 1.05	60.58 ± 1.49	35.22 ± 0.65	3.13 ± 0.14	2.64 ± 0.09
Purslane group	70.12 ± 0.88	56.08 ± 0.24*	31.66 + 0.37*	2.16 ± 0.11*	2.29 ± 0.08*

Values are means ± S.E (n = 6). *: significant change at $p < 0.05$ with respect to control group.

Table 2. Effect of purslane aqueous juice on kidney function of rats.

Parameter / Groups	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/%)
Control group	69.63 ± 4.01	4.10 ± 0.19	0.50 + 0.01
Purslane group	89.13 ± 2.46*	2.74 ± 0.19*	0.36 ± 0.01*

Values are means ± S.E (n = 6). *: significant change at $p < 0.05$ with respect to control group.

Table 3. Malondialdehyde and nitrite/nitrate content in liver, kidney and testes of rats treated with purslane aqueous juice.

Parameter / Groups	Hepatic MDA (nmol/g tissue)	Renal MDA (nmol/g tissue)	Testicular MDA (nmol/g tissue)	Hepatic nitrite/nitrate (µmol/ g tissue)	Renal nitrite/nitrate (µmol/ g tissue)	Testicular nitrite/nitrate (µmol/ g tissue)
Control Group	1027.20 ± 47.79	1426.28 ± 28.59	548.64 + 15.96	128.54 ± 2.39	146.85 ± 7.73	97.61 ± 2.46
Purslane Group	710.06 ± 33.13*	1302.82 ± 46.81*	553.77 ± 8.71	113.90 ± 7.73	148.50 ± 3.66	70.41 ± 2.80*

Values are means ± S.E (n = 6). *: significant change at $p < 0.05$ with respect to control group.

Table 4. Reduced glutathione, catalase and superoxide dismutase levels in liver, kidney and testes of rats, treated with purslane aqueous juice.

Groups / Parameter	Control group	Purslane group
Hepatic GSH (mmol/g tissue)	36.69 ± 1.10	39.91 ± 0.62*
Renal GSH (mmol/g tissue)	53.02 ± 0.87	54.05 ± 0.99
Testicular GSH (mmol/g tissue)	18.08 ± 0.65	26.21 ± 0.78*
Hepatic CAT (u/g tissue)	1.21 ± 0.04	1.40 ± 0.01*
Renal CAT (u/g tissue)	0.84 ± 0.04	1.51 ± 0.07*
Testicular CAT (u/g tissue)	0.76 ± 0.17	1.14 ± 0.01*
Hepatic SOD (u/g tissue)	1.06 ± 0.01	1.56 ± 0.05*
Renal SOD (u/g tissue)	0.74 ± 0.02	2.53 ± 0.02*
Testicular SOD (u/g tissue)	0.43 ± 0.01	0.54 ± 0.01*

Values are means ± S.E (n = 6). *: significant change at $p < 0.05$ with respect to control group.

content of liver and testes by 8.8 and 45.0%, respectively. In addition, catalase and superoxide dismutase activity, increased significantly in all examined organs. The increase in catalase was by 15.7, 79.8 and 50% respectively, in liver, kidney and testes. Also, superoxide dismutase increased in liver, kidney and testes (47.2, 242.9 and 25.6% respectively).

The antioxidant mechanism of purslane can be seen by

Table 5. Effect of purslane aqueous juice on hepatic, renal and testicular glutathione reductase, glutathione peroxidase and glutathione-S-transferase.

Groups / Parameter	Control group	Purslane group
Hepatic GR (µmol/ g tissue)	102.48 ± 8.97	152.05 ± 5.43*
Renal GR (µmol/ g tissue)	158.75 ± 3.07	150.71 ± 3.40
Testicular GR (µmol/ g tissue)	72.34 ± 3.88	136.89 ± 9.02*
Hepatic GPx (u/g tissue)	1289.34 ± 109.98	1296.89 ± 99.76
Renal GPx (u/g tissue)	1080.74 ± 98.66	1945.34 ± 140.39*
Testicular GPx (u/g tissue)	1144.32 ± 66.07	1202.27 ± 91.39
Hepatic GST (µmol/h/ g tissue)	0.68 ± 0.05	1.32 ± 0.03*
Renal GST (µmol/h/ g tissue)	0.69 ± 0.01	1.15 ± 0.02*
Testicular GST (µmol/h/ g tissue)	0.31 ± 0.02	0.48 ± 0.02*

Values are means ± S.E (n = 6). *: significant change at $p < 0.05$ with respect to control group.

the data depicted in Table 5. Purslane caused significant increase in glutathione reductase, glutathione peroxidase and glutathione-S-transferase, in all tested organs except that of renal GR and hepatic and testicular GPx where glutathione reductase increased in liver and testes by 48.4 and 89.2%, respectively. In addition, glutathione peroxidase increased significantly in kidney (80.1%), also, GST showed significant increase in each of liver, kidney and testes (94.1, 66.7 and 54.8%), respectively.

DISCUSSION

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

Purslane has potential as an animal feed, in aquaculture (Simopoulos et al., 1995) and in the food processing industry (Wenzel et al., 1990). Thus, purslane is a plant with good nutritional and medicinal potential and it is used for its beneficial effects. Hao et al. (2009) reported that purslane can be used as a medicinal plant where it is used for anti-aging, thereby increasing the level of SOD and decreasing the level of MDA in the brains of mice treated with D-galactosamine. Also, Gong et al. (2009) demonstrated the effect of purslane in diabetic rats and found that purslane extract, decreased the serum glucose level and increased the insulin level in rats model. Purslane is best used for human consumption as a green vegetable rich in minerals and Omega-3 fatty acids (Mohamed and Hussein, 1994). Omega-3 fatty acid is a precursor of a specific group of hormones (prostaglandins) and may offer protection against cardiovascular disease, cancers and a number of chronic diseases and conditions throughout the human life.

Purslane is a potent antioxidant and is reported to contain omega-3 fatty acids (Mohamed and Hussein, 1994). The decreased enzyme activities in the current study were possibly due to the antioxidants present in purslane which act against oxidative stress. The decreased activity of the liver enzymes, ALT, AST, γ-GT and ALP in purslane treated group, indicates its protective role against liver damage. Decreased levels of urea, uric acid and creatinine in the purslane treated animals, may be due to its antioxidant potential (Shirwaikar et al., 2003).Many natural products are reported to influence the antioxidant systems and are good cytoprotective agents (Dragsted et al., 1997). SOD, CAT, GPx, GST, GR and GSH, play an important role in the biological systems to act against oxidative stress (Akyol et al., 2002).

The protective role of glutathione, as an antioxidant and detoxifying agent, has been demonstrated in various clinical studies (Simopoulos, 2004). It is a ubiquitous compound that is synthesized rapidly in the liver, kidney and other tissues, including the gastrointestinal tract. In animal cells, glutathione acts as a substrate for glutathione peroxidase, which reduces lipid peroxides that are formed from polyunsaturated fatty acids (PUFA) in the diet and as a substrate for glutathione-S-transferase, which conjugates electrophilic compounds. Many evidences showed that glutathione obtained from the diet is directly absorbed by the gastrointestinal tract and thus dietary glutathione can readily increase the antioxidant status in humans (Jones et al., 1989).

The antioxidant enzymes such as GPx, GR, SOD and GST, take part in maintaining GSH homeostasis in tissues (Abdel-Moneim et al., 2010). Also, increased levels of GPx, GR, GST, CAT and SOD, were all found to correlate with elevated glutathione level and depressed MDA and NO in rats, showing the antioxidant activity of purslane.

In conclusion, Purslane is beneficial for hepatic, renal

and a testicular tissue as well as it has an antioxidant properties and nutritive value.

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