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In vivo Hepato-protective Properties of Purslane Extracts on Paracetamol-Induced Liver Damage

Mohammed Abdalla H[1] **& Soad Mohamed AG**[2]

[1] *Department of Biochemistry, Faculty of Pharmacy, October 6th University,Cairo, Egypt*

[2] *Department of Chemistry, Faculty of Science (Girl's), Al-Azhar University, Cairo, Egypt*

ABSTRACT

This investigation is designed to evaluate the potential hepato-protective effect of ethanolic and aqueous extracts of air-dried leaves of Purslane against paracetamol-induced hepato-toxicity, in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic application. Paracetamol (1g/kg bw) administration to rats resulted in massive elevation in serum and hepatic lactate dehydrogenase (LDH) activity and thiobarbituric acid reactive substances (TBARS) as well as in serum tumor necrosis factor–alph (TNF-α) levels, with a significant decrease in serum and hepatic protein thiols (Pr-SHs) and reduced glutathione (GSH) superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood and liver. Oral administration of both the leaves extracts at a concentration of 150 mg/kg bw daily for 15 days showed significant protection against an induced increase in serum and hepatic LDH and TBARS as well as serum tumor necrosis factor–alph (TNF-α) levels. The treatment also resulted in a significant increase in serum and hepatic Pr-SHs as well as GSH, SOD and GPx in blood and liver. The results of the present study suggest that the ethanolic and aqueous extracts of Purslane leaves can generate antioxidants.The effect was more pronounced in ethanolic extract compared to aqueous extract.

Keywords: LDH, TBARS, Pr-SHs , TNF-α, GSH, SOD, GPx, serum, liver, paracetamol, Purslane leaves, hepato-protective

INTRODUCTION

Purslane, in Arabic '*Rejlah*' (*Portulaca oleraceae* L.), a member of Portulacaceae family, is widespread as a weed and has been ranked the eight most common plant in the world (Oiu *et al.*, 2000). It is eaten as a salad and vegetable all around the world and used medicinally for a variety of conditions that include headache, stomach ache, painful urination, enteritis, and mastitis. These conditions are usually treated with the fresh herb used as a poultice or the expressed juice is used(Leung & Foster Steven,1996). Purslane contains numerous common nutrients including vitamins and minerals (Mohammad, Mohammad & Farhad,2004), fatty acids (Xin *et al.*, 2008) whose concentration in purslane is the highest found among leafy vegetables, glutathione, glutamic acid, and aspartic acid. It has been described as a 'power food' of the future because of its high nutritive and anti-oxidant properties (Al-Howiriny, 2008). Other

Correspondence author: Mohammed Abdalla Hussein; Email: *abdallamohammed_405@hotmail.com*

constituents include a mucilage composed of a neutral fraction with structure determined, dopamine and dopa, coumarins, flavonoids, alkaloids, saponins(Sakai *et al.*, 1996). Moreover, purslane is known in folk medicine in some parts of China as a hypotensive and anti-diabetic (Hou *et al.*, 2008; Meng & Wu, 2008). *In vivo* and *in vitro* tests have been conducted with *P. oleracea* to determine, for example, its anti-inflammatory, analgesic and anti-oxidant activity (Xiaoling,1999). But there are no reports of the effect of purslane on liver damage. This study investigated the hepato-protective activities of the ethanolic and aquouse extracts of purslane. The present investigation is designed to evaluate the potential hepato-protective effect of ethanolic and aqueous extracts of air-dried leaves of purslane against paracetamol-induced hepatotoxicity, in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic application.

MATERIALS AND METHODS

Plant material

Fresh leaves of purslane were collected from Horbite farms village, El-sharkia, Egypt.

Preparation of ethanolic extract

Air-dried leaves of the plant (1.5 kg) were crushed to coarse powder and extracted exhaustively in a Soxhlet with 95% ethanol. The extract was concentrated under reduced pressure to yield a viscous mass. The ethanolic extract was kept in airtight containers in a deep freeze maintained at 40 °C until further use.

Preparation of aqueous extract

The aqueous extract of air-dried leaves of the plant was prepared by dissolving a known amount of air-dried leaves powder in distilled water using a magnetic stirrer. It was then filtered and evaporated to dryness under reduced pressure. An aqueous suspension, which is the form customarily, used in folk medicine, was prepared to facilitate easy handling. The drug solutions were prepared freshly each time and administered intragastrically. The dosage schedule for the drug was once a day.

Animals

Adult albino rats weighing around 180-200gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatised to animal house conditions. Animals were provided with a standard diet and water *ad libtum*. Rats were kept under constant environmental condition and observed daily throughout the experimental work.

Experimental set up

This experiment was carried out to examine the prophylactic potential of ethanolic and aqueous extracts of purslane leaves, given repeatedly for 2 weeks against paracetamol hepatotoxicity *in vivo*.

Groups of animals each consisting of 6 rats were treated daily for 14 days as follows. A suspended solution of 3g % was prepared for intragastric intubation of rats.

Group I: Normal (was given similar volume of tween 80, 1% p.o.)

Group II: Control (was given a similar volume of saline p.o.)

Group III: Was treated with ethanolic extract (150mg/kg bw) suspended in tween 80 orally in a single daily dose (Al-Howiriny, 2008).

Group IV: Was treated with aqueous extract (150 mg/kg bw) dissolved in saline orally in a single daily dose (Al-Howiriny, 2008).

Group V: Was treated with vitamin C (1 g/kg bw) suspended in tween 80 orally in a single daily dose (Luo *et al.*, 1995).

At day 13, that is, one day before the last treatment, animals of all groups were fasted for 18 hours. At day 14, one hour after the last dose of drug treatment, all animals in groups II, III, IV and V recived paracetamol (1 g / kg bw) (Kostrubsky, 1995).

I-T reatment of blood samples

After 15 days of treatment blood samples were withdrawn from the retro-orbital vein of each animal and each sample was collected into 2 tubes, one heparinised and the other non-heparinised. The non heparinised blood samples were allowed to coagulate and then centrifuged. The separated sera were used for the estimation of serum LDH, TNF-α, TBARS, Pr-SHs and total protein levels. The heparinised blood samples were divided into 2 aliquots. The first aliquot was used for determination of GPx activity. The second was haemolysed using bidistilled water and the haemolysate of each sample was divided into two portions and treated with chloroform / ethanol (3:5 V / V) mixture to precipitate; the resultant supernatant was used for the determination of SOD activity. The second portion was deproteinised with meta-phosphoric acid and the clear supernatant was used for the estimation of GSH level. Haemoglobin levels were determined in the heparinised blood samples and used in the calculation of the enzyme activity.

II-Preparation of liver samples

Animals were killed by cervical dislocation, and the livers were rapidly removed. A part of each liver was weighed and homogenised for the estimation of GSH, LDH, SOD and GPx activities and TBARS, Pr-SHs, total protein and albumin levels.

Biochemical assays

Serum and hepatic LDH, TBARS, Pr-SHs and GSH levels in blood and hepatic were carried out by the methods described by Matthew, Flick & Stephen (2002), Uchiyama & Mihara (1978), Koster, Biemond & Swaak (1986) and Chanarin (1989), respectively. Blood and liver superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were carried out following Paglia & Valentine (1967) and Glumin *et al.*(2006), respectively. Serum tumor necrosis factor–alph (TNF-α) was done according to Corti *et al.*(1992) Liver GSH was estimated according to the method of Sedlak & Lindsay (1968). Blood haemoglobin was determined according to the method of Van Kampen & Zijlstra (1961). The protein content of liver tissue was measured by applying the method of Lowry *et al.* (1951).

Statistical analysis

All the grouped data were statistically evaluated with SPSS / 7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for seven separate determinations.

RESULTS

Tables 1 and 2 show the concentrations of serum and liver LDH, TBARS, Pr-SHs and serum TNF-α of control and experimental groups of rats. Paracetamol (1 g / kg), orally given to rats markedly increased serum and hepatic LDH activity. Also, the levels of serum and hepatic TBARS and serum TNF-α in paracetamol-injected rats were significantly higher than in control rats, whereas paracetamol-injected rats treated with the purslane extract (ethanolic or aqueous) restored the altered values to near normalcy. The decreased concentration of serum and hepatic Pr-SHs was observed in paracetamol-injected rats. Administration of purslane extract (ethanolic or aqueous) tends to bring the Pr-SHs level to near normal.

Table 1. Levels of lactate dehydrogenase (LDH), lipid peroxides (TBARS), protein thiols (Pr-SHs) and tumor necrosis factor–alph (TNF-α) in serum of normal and experimental groups of rats

Groups	*LDH (U/l)*	*TBARS nmol/ml*	*Pr-SHs µmol/l*	*TNF-α Pg/ml*
Normal 1 % tween 80	140.22 ± 10.07	1.76 ± 0.08	550.49± 26.43	68.53± 3.22
Control (Paracetamol 1g/kg bw)	537.46 ± 17.61*	7.35 ± 1.64*	410.61± 16.44*	84.52± 4.81*
Ethanolic extract 150 mg/kg bw	254.80 ± 11.45@	2.44 ± 1.05@	510.33± 22.5@	71.86± 3.71@
Aqueous extract 150 mg/kg bw	310.27 ± 23.64@	4.21 ± 0.92@	476.46± 15.27@	76.39± 2.86@
Vitamin C 1g/kg bw	261.23 ± 19.71@	3.55 ± 0.21@	495.11± 27.64@	78.51± 3.99@

Paracetamol was given orally as a singal dose of 1g/kg bw to 18-hour fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose for each was given 1 hour before parcetamol administration.Values are given as mean ± SD for groups of six animals each.
* Significantly different from normal group at $p< 0.05$.
@ Significantly different from control group at $p< 0.05$.

Table 2. Activity of lactate dehydrogenase (LDH) and levels of lipid peroxides (TBARS) and protein thiols (Pr-SHs) in liver of normal and experimental groups of rats

Groups	*LDH (U/g protein)*	*TBARS nmol/g protein*	*Pr-SHs µmol/g protein*
Normal 1 % tween 80	305.10 ± 21.66	246.43 ± 12.43	123.71 ± 6.32
Control (Paracetamol 1 g/kg bw)	722.51 ± 26.35*	868.11 ± 32.47*	61.43 ± 4.95*
Ethanolic extract 150 mg/kg bw	331.61 ± 24.67@	299.57 ± 16.88@	95.81 ± 5.36@
Aqueous extract 150 mg/kg bw	405.56 ± 33.46@	356.54 ± 25.42@	73.43 ± 4.29@
Vitamin C 1g/kg bw	346.37 ± 11@	310.48 ± 10.77@	85.64 ± 5.67@

Paracetamol was given orally as a singal dose of 1 g/kg bw to 18-hour fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose for each was given 1 hour before paracetamol administration. Livers were isolated 24 hours after paracetamol administration. LDH activity was determined in liver cytosols while TBARS and Pr-SHs were determined in liver homogenate. Values are given as mean ± SD for groups of six animals each.
* Significantly different from normal group at $p< 0.05$.
@ Significantly different from control group at $p< 0.01$.

Table 3. Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of normal and experimental groups of rats

Groups	*GSH (mg %)*	*SOD (U/g Hb)*	*GPx (U/g Hb)*
Normal 1 % tween 80	51.27 ± 4.66	13.95 ± 1.14	167.84 ± 6.10
Control (Paracetamol 1 g/kg bw)	32.11 ± 3.72*	6.98 ± 1.08*	113.15 ± 5.24*
Ethanolic extract 150 mg/kg bw	46.37 ± 4.28@	11.79 ± 0.48@	152.87 ± 6.14@
Aqueous extract 150 mg/kg bw	39.16 ± 3.56@	9.47 ± 0.93@	136.55 ± 6.12@
Vitamin C 1g/kg bw	41.85 ± 3.72@	8.90 ± 0.87@	147.82 ± 8.63@

Paracetmol was given orally as a singal dose of 1 g/kg bw to 18-hour fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose for each was given 1 hour before paracetmol administration. Blood samples were collected 24 hours after paracetamol adminstation. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per minute for SOD and the obtained values were divided by the haemoglobin (Hb) concentration.Values are given as mean ± SD for groups of six animals each.

* Significantly different from normal group at $p < 0.05$.

@ Significantly different from control group at $p < 0.01$.

Furthermore, vitamin C, a proven liver protecting agent, also significantly inhibited the lipid peroxidetion of liver. The effect was more pronounced in the ethanolic extract of purslane.

Tables 3 and 4 show the concentration of blood and liver reduced glutathione (GSH), activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) of control and experimental groups of rats. The decreased concentration of blood and hepatic GSH was observed in paracetamol control rats. Administration of purslane extract (ethanolic or aqueous) tends to bring the GSH level to near normal. The activities of SOD and GPx in liver were significantly lower in paracetamol-injected rats compared to paracetamol injected rats treated with purslane extract (ethanolic or aqueous). Vitamin C, on the other hand, also exhibited a significant hepatoprotective effect on the enzymes tested. Similar effects were found in the group of rats administered with ethanolic extract of purslane.

The addition of ethanolic extract of purslane to EAT cell line in different doses of 25, 50, 100 µg/ml showed a reduction in the cell viability by 75, 80, 95%, respectively. However, the addition of the same doses of the aqueous extract showed a reduction in the cell viability by 35, 40, 45%, respectively.

The ethanolic extract has an obvious superior effect on aqueous extract at three doses levels. It was also obvious that doubling the dose of extract did not increase the percent of inhibition of viability of the cells.

DISCUSSION

Paracetamol (4'-Hydroxyacetanilide) is a oral analgesic and antipyretic drug. It is metabolised extensively by the liver *via* three main pathways: sulfonation, glucuronidation and oxidation (Tatsuya ,1995). The process produces a highly reactive arylating compound called N-acetyl-p-benzoquinoneimine (NAPQI). When more NAPQI

Table 4. Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) on liver of normal and experimental groups of rats

Groups	*GSH (mg/g protein)*	*SOD (U/g protein)*	*GPx (U/g protein)*
Normal 1 % tween 80	11.33 ± 2.07	147.56 ± 4.34	259.19 ± 9.47
Control (Paracetamol 1g/kg bw)	5.75 ± 1.62*	68.49 ± 4.33*	101.36 ± 5.29*
Ethanolic extract 150 mg/kg bw	10.22 ± 1.58@	137.25 ± 5.33@	235.22 ± 9.47@
Aqueous extract 150 mg/kg bw	7.31 ± 0.77@	108.65 ± 4.71@	198.46 ± 8.43@
Vitamin C 1g/kg bw	9.20 ± 1.64@	121.46 ± 6.41@	217.65 ±10.34@

Paracetamol was given orally as a singal dose of 1 g/kg bw to 18-hour fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose for each was given 1 hour before paracetamol administration. Livers were isolated 24 h after paracetamol administration. GPx and SOD activities were determined in liver cytosols while GSH was determined in liver homogenate.. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration.Values are given as mean ± SD for groups of six animals each.

* Significantly different from normal group at $p< 0.05$.

@ Significantly different from control group at $p< 0.01$.

is formed, it can then be conjugated to GSH; the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins (Mladenovica *et al.*,2009).

From our findings, the Purslane supplementation was consumed as prophylaxis to confront paracetamol-mediated hepatotoxicity. However, co-administration of purslane with liver insult agent or after the occurrence of liver damage will be more informative in elaborating purslane's ability to regenerate hepatocytes and recuperate liver function. It is evident that the ethanolic and aqueous extracts of purslane were able to reduce all the elevated biochemical parameters as a result of hepatotoxin challenge, indicating improvement of the functional status of the liver. Oral administration of both the aerial parts extracts at a concentration of 150 mg/kg bw daily for 15 days showed a significant hepato-protecive effect. The effect was more pronounced in ethanolic extract compared to aqueous extract. Large amounts of phenolic compounds, (coumarins, flavonoids, alkaloids, and saponins) in ethanolic extract may contribute towards the antioxidant properties (Sakai *et al.*,1996). The preliminary studies conducted in this work revealed the non-toxic nature of purslane on normal rats. Hepatic necrosis following massive paracetamol administration is well documented(Mladenovica *et al.*,2009). Drastic elevation in the activity of serum and liver cytosolic LDH were shown in the current study after administration of paracetamol (1g/kg bw). Paracetamol toxicity was reported to be associated with increased release of LDH in experimental animals (Strubelt & Younes,1992).

Increased lipid peroxidation, as evidenced by the elevated levels of thiobarbituric acid reactive substances (TBARS) in serum and hepatic tissues was demonstrated in the present study. These results are in harmony with those of other

investigators who reported the association between paracetamol toxicity and lipid peroxidation (Wendel, Jaeschke & Gloger, 1996). A toxic dose of paracetamol can modulate the lipid composition and fluidity of biomemberanes by activation of Ca^{2+}-phospholipase that results in diminished structural integrity and also by alteration of membrane bound enzymes such as Ca^{2+}-ATPase activity(Ray *et al.*,1996). In the present study, serum and hepatic TBARS levels were significantly lower in the purslane extracts -treated groups compared to the paracetamol-treated group. The above result suggests that the purslane extracts (ethanolic and aqueous) may exert antioxidant activities and protect the tissues from lipid peroxidation. The protective effects due to treatment with purslane extracts strongly indicated the possibility of the extracts being able to prevent and / or mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and hence restore these enzymes levels (Al-Howiriny *et al.*,2004).

Serum and hepatic protein thiols (Pr-SHs) contents were markedly decreased after paracetamol administration, as shown in the current investigation. Such depletion is presumed to be a direct oxidation of the thiol groups of contiguous amino acids with the formation of protein-protein disulphides (Kyle *et al.*,1990).

Serum tumor necrosis factor–alph (TNF-α) content was markedly increased after paracetamol administration, as shown in the current investigation. These results are in harmony with those of Mladenovica *et al.* (2009) who showed that hepatocytes treated with paracetamol release factors which activate proinflammatory cytokines as TNF-α and interleukins.

The hepato-protective effect on purslane extracts were demonstrated through correcting the value of serum TNF-α that were significantly raised by paracetamol administration. This result indicate that purslane extracts have a membrane stabilising effect (Asai & Miyazawa, 2001). Additionally, there is growing evidence that the hepato-protective effect of extracts takes place directly at the level of hepatocytes by lowering intracellular levels of cholesterol and cytotoxic bile acids (Wohaieb & Godin, 1987). Liu *et al.* (1993) suggested that the decrease in blood and liver GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress and tissue injury. Increased oxidative stress, resulting from a significant increase in aldehydic products of lipid peroxidation has probably decreased hepatic GSH content. In the present study, the elevation of GSH levels in blood and liver was observed in the purslane extracts -treated rats (O'Brien *et al.*,2000). This indicates that the purslane extracts (ethanolic and aqueous) can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects. Reduced activities of SOD and GPx in blood and liver have been observed in paracetamol-treated rats. Several authors have reported the decrease in SOD and GPx activities in paracetamol-treated animals (Ahmed & Khater, 2001. The results indicates that the purslane extracts (ethanolic and aqueous) can either increase the biosynthesis of SOD and GPx or reduce the oxidative stress leading to less degradation of SOD and GPx, or have both effects. The effect was more pronounced in ethanolic extract compared to aqueous extract.

The preliminary phytochemical screening of purslane revealed the presence of flavonoids. Flavonoids (or bioflavonoids) are natural products that are capable of modulating the activity of enzymes (SOD and GPx) and affecting the behaviour of many cell systems. They also possess a significant anti-hepatotoxic, anti-allergic, anti-inflammatory, anti-osteoporotic, and even anti-tumor and anti-oxidant activities (Rathee, Hassarajani & Chattopadhyay,

2006). Furthermore flavanoids have a preventive role in cancer therapy via the effect on signal transduction in cell proliferation (Rebecca *et al.*, 1998).

The anti-inflammatory mechanisms exerted by purslane probably play a major role in dampening liver damage. This effect may be due to purslane containing omega-3 fatty acids (Simopoulos *et al.*,2009). Omega-3 fatty acids help reduce inflammation as well as inhibit lipopolysaccharide (LPS). Free radical damage plays a key role in LPS-induced hepatic oxidative stress / metabolism; the implication of blood phagocytes or kupffer cells as free radical sources in this pathology has been reported. LPS is known to enhance the formation of reactive oxygen species such as superoxide radicals, H_2O_2 and their secondary product malondialdehyde (MDA) through the activity of NADPH oxidase or xanthine oxidase(Jahn, Baymes & Spiteller,1999). The combination of these two oxygen species creates a more active and aggressive form of oxygen, OH. LPS also generates free radicals intracellularly through the ischemia-reperfusion syndrome secondary to the decrease in tissue blood flow and alters the activity of the major physiological sources of radicals in liver microsomes, peroxisomes, mitochondria, even by induction of nitric oxide synthase. Free radicals cause serious injury to vital tissue and organs, especially to membrane phospholipids, connective tissues and cell nucleic acids (Al-Omar, Christine & Ibrahim, 2004).

CONCLUSION

Effects of purslane extracts (ethanloic and aquouse) on lipid peroxidation have not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. In conclusion, the results of this study demonstrated that purslane extracts possess a potent hepato-protective action upon paracetamol-induced hepatic damage in rats. This may be due to its antioxidative activity with its ability to scavenge free radicals and inhibit lipid peroxidation, all of which are capable of hepatocellular injury.

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