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Evaluation Of Protective Effect On Liver And Kidney Of Gamma-Irradiated Purslane (*Portulaca oleracea*) Against Paracetamol-Induced Toxicity In Rats.

Fawzia A Alshubaily*.

Biochemistry department, Faculty of science, King Abdulaziz University, Kingdom of Saudi Arabia

ABSTRACT

The present study was aimed to evaluate the effect of using gamma (γ)-radiation on the active contents of dried purslane and also to assess the effect of γ-irradiated purslane against paracetamol-induced liver and kidney toxicity in rats. The results indicated that γ-irradiation (10kGy) induced fluctuating change in the value of phenolic fractions with significant increase in the level of total phenolic and total flavonoids of purslane dried powder. Exposure of rats to over dose of paracetamol (PC) (2 g/kg b.wt. /day/ 15 days) resulted in significant hepato-nephrotoxicity evidenced by elevation of the activity of some liver enzymes, kidney function, inflammatory factors and lipid oxidation with inhibition of antioxidant status when compared to control group. By contrast, Co-administration of PC with either raw (RPP) or γ-irradiated purslane powder (GPP) (50 mg/kg/day/15 days) reduced the activity of liver enzymes, urea and creatinine levels, serum tumor necrotic factor-alpha (TNF-α), interleukin-6 (IL-6), level of malondialdehyde and activity of xanthine oxidase (XO) accompanied by significant elevation in the activities of xanthine dehydrogenase (XDH), superoxide dismutase (SOD) and catalase (CAT) and glutathione content (GSH) relative to PC-intoxicated rats. In conclusion, the results indicated that γ-radiation food processing can be used as effective technique that can increased the active contents of purslane. Also, the results revealed that the protective effects of GPP against PC-induced hepato-nephrotoxicity may be related to its antioxidant properties.

Keywords: Paracetamol, Gamma-radiation, Purslane, Antioxidants

Corresponding author

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INTRODUCTION

Paracetamol (PC) (known as acetaminophen in the United States) is widely used as analgesic and antipyretic drug. This drug can be safe at therapeutic levels but abuse and over doses of PC can lead to undesirable side effects such as hepatotoxicity and nephrotoxicity. The side effects of PC have been attributed to the formation of toxic highly reactive metabolite n-acetyl parabenzoquineimine (NAPQI). The production of this toxic metabolite leads to induction of cytochrome P450 (CYP450) isoforms (CYP2E1, CYP3A4, and CYP1A2), reduction of intracellular glutathione (GSH) and oxidative stress which in turn resulted in liver and kidney damage **(1)**. **Kanchana and Sadiq (2)** reported that medicinal plants consist a lot of natural products (flavonoids, terpenoids and steroids) andhavehepato- protective activity.Purslane (Portulacaoleracea) is one the natural plants that is widely used as a nutritional food. It has a wide range of health benefits and pharmacological effects including antibacterial, hypolipidemic, anti-aging, anti-inflammatory, anti-oxidative, analgesic, and wound healing activities **(3)**. Also, this medicinal plant has beneficial effect on hepatic, renal, and testicular tissue due to its antioxidant properties. These antioxidant properties are related to its bioactive components such as alkaloids, omega-3 fatty acids, coumarins, flavonoids, polysaccharide, cardiac glycosides, anthraquinone glycosides and containing β-sitosterol**(3)**. Moreover, it contains higher amounts of vitamin E&C than cultivated plants **(4)**. Commercial phytosanitary treatments have been required for insect quarantine barriers.**Jenjob et al. (5**) indicated that gamma radiation food processing has emerged as a potential economic alternative for insect disinfestations, sterilization, inhibition of sprouting, and for the extension of the storage life of fresh fruits and vegetables. Several studies showed that gamma radiation treatment increases the total phenolic content and antioxidant capacity of several plants and fruits such as date palm fruit and pomegranate peel **(6 and 7)**. Considering the above issues, the present study was undertaken to evaluate the effect of using γ- radiation as a phytosanitary treatmentson the total phenolic content of dried purslane. Also, the study aimed to assess the effect of γ-irradiated purslane against paracetamol -induced liver and kidney toxicity in rats.

MATERIAL AND METHODS:

Purslane plant was purchased from local market (Cairo, Egypt) and Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of purslane powder:

Purslane was washed with distilled water and evaporated residual moisture at room temperature, samples were cut into pieces then dried in oven at 45ºC for 72 h and ground to fine powder.

Gamma Irradiation treatment:

Purslane powder was packed in polyethylene bags and irradiated with gamma rays at dose level (10kGy), using Indian Gamma Cell (Ge 4000 A) 60Co source at a dose rate of 1.667 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Determination of total phenolic and flavonoids:

The total phenolic compounds of raw and γ-irradiated purslane were determined using the Folin-Ciocalteu reagent according to the method described by **Halicia et al. (8)**. The determination of total phenols was done in triplicate and expressed milligrams of gallic acid equivalent (GAE) per gram dry weight (DW). Fractionation of poly phenolic compounds was determined by HPLC according to the method of **Goupy et al. (9)**.

Total flavonoid compounds were determined using the aluminum chloride colorimetric assay based on **Zhisen et al. (10)** and were expressed as mg quercitin equivalent/g dry weight (DW).

Experimental design

Animals:

The experiment was conducted on twenty eight albino male rats (170 to 200g body weight (B.WT)). Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were allowed ad libitum.

Animals groups:

The animals were randomly divided into four groups, each consisted of seven rats.

Control group(Group C**):** rats fed on balanced diet and served as control,

Paracetamol group(Group PC**)**: rats were orally given PC (2 gm/kg b.wt. dissolved in 2 ml distilled water) **(11)** daily for 15 days.

Group PC&RPP: rats were administered raw purslane powder (50 mg/kg/day) **(12)** concurrently with oral paracetamol administration (2 gm/kg b.wt. dissolved in 2 ml distilled water) daily for 15 days.

Group PC&GPP: rats were administered γ-irradiated purslane powder (50 mg/kg/day)concurrently with oral paracetamol administration (2 gm/kg b.wt. dissolved in 2 ml distilled water) daily for 15 days.

At the end of the experiment, animals from each group were sacrificed 24 hrs post the last dose of treatment. Blood samples were withdrawn by cardiac puncture after slight anathesation of rats using diethyl ether and allowed to coagulate and centrifuged to get serum for biochemical analysis.

Biochemical analysis:

Serum urea was measured by enzymatic colourimetric method as described by **Coulomb and Farreau (13)** and serum creatinine was measured by the method of **Husdan and Rapoport (14)**. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to **Reitman and Frankel (15)** and serum gamma glutamyltransferase (GGT) was assessed according to **Rosalki (16)**. Detection of serum tumor necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6) was performed by ELISA technique (BioSource International,Camarillo, CA, USA) according to the manufacturer's instructions.

Liver and kidney was dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate the level of malondialdehyde (MDA) **(17)**, the activity of xanthine oxidase (XO) and xanthine dehydrogenase (XDH)**(18)**, glutathione content (GSH) **(19)** and the activity of superoxide dismutase (SOD)**(20)** and catalase (CAT) **(21)**.

Statistical analysis

Results were presented as mean ± SE (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. The statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) **(22)**. Differences between means were considered significant at $P < 0.05$.

RESULTS

The data of this work revealed that γ-irradiation of purslane dried powder (PDP) resulted in fluctuating change in the value of phenolic fractions with significant increase in the level of its total phenolic and total flavonoids by percent change 7.24% and 6.9%, respectively (Table 1&2).

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Table 1: Effect of γ-irradiation on total phenolic contents and total flavonoids of purslane dried powder.

Parameters	Dried purslanepowder		% Change
	Raw	Irradiated	
Total phenolic content (mg GAE/100g DW ± S.D)	338.23 ± 4.2	362.72± 4.4	7.24%
Total flavonoids (mg QE/100g DW ± S.D)	89.67± 2.3	95.92± 2.1	6.97%

Values are means of three replicates (± SD)

The serum levels of urea, creatinine, ALT, AST and GGT were found to be higher in the PC-group when compared with control group. Co-administration of PC with RPP or GPP to rats significantly reduced the level of kidney and liver function compared to PC-group **(Table 3)**.

The results indicated that PC leads to significant increase in the levels of inflammatory factors (TNF-α and IL-6) relative to control group. The levels of these factors were found to be lower in the group of rats that received RPP or GPP along with PC comparing to PC-group **(Table 4)**.

In the group treated with PC, the liver and renal tissues had significantly decreased activity of antioxidant enzymes (XDH, SOD, CAT) and GSH concentration compared to that of control. In addition, MDA level and XO activity were significantly higher in PC-treated animals than in control group animals. Whereas, treatment of rats with PC and RPP or PC and GPP induced significant increase in the level of hepatic and renal antioxidant parameters (XDH, SOD, CAT and GSH) and reduced the oxidative stress by decreasing the MDA level and XO activity when compared to the group treated with PC only **(Table 5)**.

Table 2: Effect of γ-irradiation on phenolic fractions of purslane dried powder (mg/100gm).

Phenolic compounds (mg/100gm)	Dried purslanepowder	
	Raw	Irradiated
Pyrogallol	0.21	0.30
Protocatchuic	4.82	4.26
Chlorogenic	17.82	18.64
Catechol	4.13	7.02
Caffien	3.51	3.73
Vanillic	7.48	9.62
Gallic	5.09	8.17
Ferulic	5.36	5.47
Ellagic	59.43	68.13
Salicylic	46.75	34.64
Benzoic	0.96	0.72
Coumaric	2.07	2.14
Cinnamic	0.30	0.26
Caffeic	5.49	5.58
Catechein	16.21	14.05

Table 3: Effect of raw and γ-irradiated purslane powder on liver and kidney function of paracetamol-administrated rats.

Parameters	Control	PC	PC&RPP	PC&GPP
AST (U/ml)	36.93 ± 1.55^{c}	66.72 ± 2.36^{a}	45.24 ± 1.62^{b}	44.11 ± 1.80^{b}
ALT (U/ml)	32.85 ± 2.23^{d}	70.36 ± 3.18^{a}	44.46 ± 2.76^{b}	39.52 ± 2.45^{c}
γ GT (U/ml)	4.52 ± 0.38^{c}	7.33 ± 0.56^{a}	5.94 ± 0.48^{b}	5.69 ± 0.54^{b}
Urea (mg/dl)	33.42 ± 2.27^{c}	47.58 ± 2.67^{a}	37.42 ± 2.15^{b}	36.16 ± 1.93^{b}
Creatinine(mg/dl)	0.90 ± 0.18^{c}	1.49 ± 0.27^{a}	1.17 ± 0.12^{b}	1.12 ± 0.11^{d}

Values are expressed as means ± S.E. (n=7).
Values in the same row with different superscript are significantly different at P<0.05.

RPP: raw purslane powder **GPP:** γ-irradiated purslane powder

Table 4: Effect of raw and γ-irradiated purslane powder on levels of tumor necrotic factor-alpha and interleukin-6 of paracetamol-administrated rats.

Parameters	Control	PC	PC&RPP	PC&GPP
TNF-α (pg/mL)	675.28 ± 52.47^{c}	887.16±56.11^{a}	754.36 ± 54.23^{b}	749.37 ± 52.82^{b}
IL-6 (pg/mL)	326.48 ± 25.52^{c}	514.18±39.56^{a}	401.28 ± 30.27^{b}	394.62 ± 31.42^{b}

Values are expressed as means ± S.E. (n=7).

Values in the same row with different superscript are significantly different at P<0.05.

RPP: raw purslane powder **GPP:** γ-irradiated purslane powder

Table 5: Effect of raw and γ-irradiated purslane powder on hepatic and renal antioxidant status of paracetamol-administrated rats.

Parameters		Control	PC	PC&RPP	PC&GPP
MDA (n mol/g tissue)	Liver	168.66±4.82^{c}	292.88±5.68^{a}	198.86±4.66^{b}	182.68±4.70^{b}
	Kidney	171.12±4.32^{c}	272.56±5.27^{a}	194.23±4.25^{b}	191.52±3.91^{b}
XO (mU/mgprotein)	Liver	2.42±0.16^{c}	4.48±0.19^{a}	2.96±0.15^{b}	2.86±0.14^{b}
	Kidney	1.62±0.09^{c}	2.76±0.11^{a}	1.86±0.12^{b}	1.83±0.10^{b}
XDH (mU/mgprotein)	Liver	3.43±0.06^{a}	1.50±0.07^{c}	2.76±0.08^{b}	2.88±0.06^{b}
	Kidney	2.78±0.08^{a}	1.36±0.07^{c}	2.34±0.06^{b}	2.40±0.07^{b}
GSH (mg/g tissue)	Liver	28.12 ±2.75^{a}	19.57±1.71^{c}	24.55±0.64^{b}	24.80±0.53^{b}
	Kidney	23.18± 1.56^{a}	13.86±1.32^{c}	18.86±1.25^{b}	19.11±1.25^{b}
SOD (U/mg protein)	Liver	48.69 ±2.75^{a}	30.24±2.54^{c}	41.27±2.25^{b}	42.10 ±2.35^{b}
	Kidney	28.46± 1.48^{a}	17.30±1.08^{d}	23.64±1.27^{c}	25.72±1.55^{b}
CAT (U/mg protein)	Liver	50.82±1.48^{a}	33.27±1.64^{d}	44.27±1.29^{c}	47.58±1.44^{b}
	Kidney	38.16±172^{a}	19.10±1.55^{d}	32.49±1.65^{c}	34.78±1.24^{b}

Values are expressed as means ± S.E. (n=7).

Values in the same row with different superscript are significantly different at P<0.05.
RPP: raw purslane powder **GPP:** γ-irradiated purslane powder

DISCUSSION

Application of γ-radiation food processing method in this work resulted in significant increase in the level of total phenolic and total flavonoids of PDP which indicated that irradiation treatments might cause some chemical changes in components of purslane plant (23). Also, γ-irradiation of PDP induced quantitative increased in some phenolic compounds such as elagic and salicylic these results in agree with the results of Sallam and Anwar (24). Hamza et al. (25)revealed that the changes in phenolic contents might be due to the ability of gamma-rays to induced decomposition of some large insoluble phenolic compounds into small soluble phenolic molecules.

The present study indicated that PC induced significant increase in liver and kidney function relative to normal rats.In accordance with previous studies of Anthony et al. (26) and Saxena et al. (27) the elevation inserum urea and creatinine in PC-groupmay be indicators of acute tubular necrosis caused by paracetamol toxicity.Mathur et al. (28) suggested that PC overdose imposed severe proximal tubular damage leading to apoptotic cell death through decrease in Nuclear factor erythroid 2-related factor (2Nrf2) and increase the level of urea and creatinine. Michaut et al. (29) reported that PC is often considered as a safe painkiller drug, even though, overdoses of this drug lead to acute liver failure and hepatic cytolysis. The hepatic cell injuries caused by PC toxicity induce disturbance of transport function of the hepatocytes resulting in the leakage of the plasma membrane, thus causing an increase in serum levels of liver enzymes (30). Upon concurrent administration of PC with either RPP or GPP with, the levels of these markers were significantly reduced compared to treatment with PC alone, indicating protection against liver and kidney damage caused by PC

toxicity. Such a restoration of increased serum levels of hepatic enzymes and kidney function reflects protection against liver and kidney damage caused by PC toxicity (31). Hanan et al. (32) reported that oral administration of purslane aqueous extract significantly attenuated carbon tetrachloride-induced increase in serum ALT activity and liver injury without any alteration in serum AST activity in mice.

The elevated effect of PC on the level of proinflammatory mediators (TNF--α andIL-6) in this investigation is in accordance with the findings of previous investigators (33). Tilg et al. (34) reported that the hepatotoxicity of acetaminophen is associated with elevated levels of pro-inflammatory cytokines such as TNF-α and IL-1b. The over production of these cytokines is associated with mediation of various inflammatory conditions. The proinflammatory cytokine (TNF- α) triggers a cascade of interleukins like IL-1 b, IL-6 and IL-8 (35). IL-1 b which is synthesized as 31 kDa precursor peptides and converted into mature cytokine of 17 kDa triggers inflammatory response resulting in tissue necrosis (33).Co-administration of PC along with RPP or GPP, in the present study, induced significant reduction in serum levels of TNF-α and IL-6 which may explain The anti-inflammatory effect of GPP (36). Lee et al. (37) stated that purslane contain omega-3 fatty acid that acts as anti-inflammatory agent may be a method of reducing long-term complications of liver fibrosis and cirrhosis Furthermore, flavonols as quercetin and catechin were found to inhibit the production of TNF-α and nitric oxide by lipopolysaccharide-activated macrophages. Similarly, apigenin, one of the most potent flavones, inhibits prostaglandin synthesis induced by IL-1and the production of IL-6 and IL-8 activated by TNF-α (38).

The finding of this study indicated that PC administration causes depletion of antioxidant parameters (XDH, SOD, CAT and GSH) and increases the level of lipid peroxidation in the liver and kidney of PC-rats suggesting the hepato-renal toxicity of PC (39). The oxidative damage induced by toxic dose of PC could be related to over formation of toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) (40). The detoxification of NAPQI is normally occurred by conjugation with reduced glutathione (GSH) with both oxidant scavenger and redox-regulation capacities (41). Insufficient glutathione resulting from depletion by toxic dose of PC caused limitation of detoxification and accumulation of NAPQI. The accumulated NAPQI covalently binds to the cysteinyl sulfhydryl groups (-SH) of cellular proteins forming NAPQI-protein adducts (42) and resulting in generation of reactive oxygen species (ROS) that affect the cellular membrane and induce lipid peroxidation and also cause hepatic necrosis (41). In the groups of rats received PC along with RPP or GPP, the levels of hepato-renal antioxidant parameters were higher and the levels of lipid peroxidation were lower than of those of rats received PC only. These results confirmed the antioxidative properties of RPP and GPP against tissue damage (43). Siriamornpun and Suttajit (44) obtained that purslane effectively detoxifies superoxide, either by superoxide dismutation or by directly reacting with it. Study of Sadeghi et al. (45) indicated thatsupplementation of dried aerial parts of purslane powder to diet positively affected the antioxidant enzymes activities, decreased oxidative damage to lipids, and improved antioxidant status in healthy broilers. It has been suggested that the antioxidant capacity of purslane may be related to its phenolic constituents (flavonoids, phenolic acids, and alkaloids) (46). Uddin et al. (47) reported that the compounds of purslane such as saponins, proteins, amino acids, melatonin, vitamin C, vitamin E, and trace mineral contents may contribute to antioxidant capacity of this plant. Purslane is a good source of glutathione which acts as a substrate for GPxin animal cells (48). Furthermore, purslane is known as rich source of coenzymeQ10 (49) that in its reduced form (ubiquinol) can be an important antioxidant to reduce the accumulation of free radicals, in particular reactive oxygen intermediates, and lessen the peroxidative damage in the body (50).

CONCLUSION

The results revealed that γ-radiation food processing technique could be considered as effective method that can be used for decontamination of PDP and one the same time increase its phenolic and flovonoids contents. On the other side, the results concluded that GPP has protective effects on the function and antioxidant activity of liver and kidney tissues against side effects that can be induced by over doses of paracetamol.

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