Research Journal of Pharmacognosy (RJP) 2(2), 2015: 19-25
Received: Nov 2014
Accepted: Dec 2014

Original article

Protective effects of *Nasturtium officinale* against gamma-irradiation-induced hepatotoxicity in C57 mice

M. Karami[1], A. Nosrati[2], M. Naderi[3], M. Makhloogh[4], S. Shahani[5*]

[1]*Department of Toxicopharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.*
[2]*Department of Pathology, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran.*
[3]*Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.*
[4]*Institute of Experimental Animal Research (IEAR), Mazandaran University of Medical Sciences, Sari, Iran.*
[5]*Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.*

Abstract

Background and objectives: *Nasturtium officinale* W.T.Aiton (Brassicaceae) is used as an edible vegetable in various parts of Iran. The aim of the present study was to investigate the protective activity of the methanolic extract of *Nasturtium officinale* against gamma-radiation-induced hepatotoxicity in terms of histopathological changes. **Methods:** Male C57 mice were divided into 10 groups. Groups 1 and 2 received saline solution intra-peritoneally (IP) for 15 days (subacute) and 2 h (acute) before whole body γ-irradiation (6 Gy). Groups 3 to 5 (subacute) and 6 to 8 (acute) received the extract at doses of 20 mg/kg, 50 mg/kg and 100 mg/kg body weight IP, respectively. Group 9 served as radiation group. Group 10 received nothing. Finally, sections of the liver tissue were evaluated for any histopathologic changes. Total phenolic and flavonoid contents were determined using Folin Ciocalteu and aluminium chloride methods. **Results:** Pre-treatment with 100 mg/kg body weight per day for 15 days and 2 h before γ-radiation significantly lowered incidence of inflammation (portal and periportal inflammation). Furthermore, liver cells necrosis, edema and congestion were slightly reduced. The total phenolic and total flavonoid contents of the extract were 11.3 ± 0.4 mg gallic acid equivalents and 9.4 ± 0.7 mg quercetin equivalents per gram of dried extract. **Conclusion:** This protection can be attributed to the presence of phenols and isothiocyanates in the extract of *N. officinale* which act as antioxidants and anti-inflammatory agents.

Keywords: Brassicaceae, gamma-radiation, liver, *Nasturtium officinale*

Introduction

Gamma-radiation induces oxidative stress and produces reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide and hydroxyl radicals that react with macromolecules such as DNA, proteins and lipids. Endogenous antioxidant defenses are insufficient to scavenge all of the free radicals induced by radiation [1,2]. Liver is sensitive to

*Corresponding author: so.shahani@yahoo.com, Tel: +9811-33543083-6, Fax: +9811-33543082

radiation and hepatic injury is a serious clinical complication of γ-irradiation [3]. ROS stimulate inflammatory cells to kill hepatocytes and other liver cells [4]. One study has demonstrated that recruited neutrophils attach to the portal vessels and to portal fibroblasts in the liver of irradiated rats [3].

Research for finding effective and less toxic radioprotective agents has focused on screening plant sources [5,6]. Phenolic and flavonoid compounds in vegetables and fruits act as antioxidants in the human diet and protect normal cells from free radicals and other ROS that are produced during exposure to ionizing radiation [7,8].

Nasturtium officinale W.T. Aiton (NO) is a perennial herb which belongs to Brassicaceae family and grows around water [9]. This plant is considered as a vegetable in Iran and other countries [10] and is rich in valuable nutrients. It has been used as expectorant, antiscorbutic, appetizer, anti-dyspeptic and cardioprotective agent in folk medicine [11,12]. Previous studies on the extracts of this plant have shown anticarcinogenic [13], anti-inflammatory [14], anticholesterolaemic, anti hyperlipidaemic [15] and antimycobacterial [16] effects. In addition, antioxidant effects of *N. officinale* have been reported in several studies [17-19]. Phytochemical analysis has shown that this plant is rich in glucosinolates [20], flavonoids and phenolic compounds [21]. A literature review has revealed that the radioprotective effect of NO has not been investigated; thus the present study has aimed to evaluate the hepatoprotective effects of NO extract in C57 mice irradiated by γ-rays.

Experimental

Animals

Adult male C57 mice (6 to 8 weeks), weighing 25-30 g were used for all experiments. They were housed individually in standard mice cages in a room under a 12-hour light- dark cycle at 22 ^{o}C (22±1 ^{o}C) and with 50±5 % relative humidity with *ad libitum* food and water. The animals were adapted to the condition for 7 days prior to the experiments [22]. The experiments were performed during the day time (08:00-16:00 hours). Each animal was used only once.

The project was approved by the Institutional Animal Ethical Committee (IAEC) of Mazandaran University of Medical Sciences.

Plant material and extraction

The aerial parts of *Nasturtium officinale* were collected from a riverside in Nur country, Mazandaran province, Iran. A voucher specimen (1006) was deposited at the Herbarium of the Faculty of Pharmacy, Mazandaran University of Medical Science, Sari, Iran.

Dried aerial parts of N.O (200 g) were powdered and extracted with MeOH by maceration at room temperature. The extract was concentrated by using a rotary evaporator (Heidolph, Germany) at 37 ^{o}C and dried.

Gamma irradiation

The Cobalt-60 Teletherapy Unit (Theratron 780, Canada) at Shahid Rajai radiotherapy centre, Babolsar, Iran, was used for irradiation. Unanesthetized mice were restrained in a well-ventilated Perspex box and their whole-body was exposed to 6 Gy gamma radiations at a distance (SSD) of 80 cm from the source to deliver the dose rate of 0.85 Gy/min.

Experimental design

Male C57 mice were divided into 10 groups with five mice per group. Groups 1 and 2 (as the negative controls) received 10 mL/kg body weight (b.w.) of saline solution intraperitoneally (IP) for 15 days (subacute) and 2 h (acute) before whole body irradiation, respectively. Groups 3 to 5 (subacute) and 6 to 8 (acute) received the extract at doses of 20 mg/kg, 50 mg/kg and 100 mg/kg b.w. IP, respectively. Group 9 (as the positive control) served as radiation group. Group 10 was a control group that received nothing.

Histological studies

Mice were sacrificed 2 h after irradiation and their livers were removed for histopathological

examination. The livers were completely excised and freed of any extraneous tissue. Multiple samples were then taken from each liver (mean 3 mm in thickness) and placed in 10% neutral buffered formalin. Then they were processed into 4-5 μm thick sections stained with hematoxylin-eosin (H&E) and observed under a photomicroscope (N-400ME, CEL-TECH Diagnostics, Hamburg, Germany).

Determination of total phenolic and flavonoid contents

Total phenolic content of the methanolic extract was determined by Folin-Ciocalteu method [23]. Calibration curve was plotted using various concentrations of gallic acid. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dried extract. The total flavonoid content was estimated using aluminium chloride colorimetric assay [24]. Quercetin was used to make calibration curve and flavonoid content was expressed as milligrams of quercetin equivalents per gram of dried extract.

Results and Discussion

Examined liver sections of group 1, 2 and 10 showed normal pattern of cellular structure (figure 1a). Meanwhile, group 9 which was exposed to 6 Gy of γ-radiation showed severe pathologic changes like scattered hepatocytes necrosis surrounded by mononuclear cells, spotty necrosis (figure 1b), portal and periportal inflammatory cells infiltration (figure 1c). Group 5 (subacute) and 8 (acute) which received 100 mg/kg b.w. of the extract showed considerable decrease in inflammatory cells infiltration, whereas liver cell necrosis, edema and congestion were slightly reduced (figure 1d). Group 4 which received 50 mg/kg for 15 days had shown little hepatoprotective activity as well. Group 3 (20 mg/kg b.w., subacute), 6 (20 mg/kg b.w., acute) and 7 (50 mg/kg b.w., acute) showed no remarkable pathological changes (table 1). The total phenolic and total flavonoid contents of the extract were 11.3±0.4 mg gallic acid equivalents and 9.4±0.66 mg quercetin equivalents per gram of dried extract.

Human exposure to ionizing radiation leads to production of free radicals, which play a key role in developing many diseases [1]. Liver is sensitive to radiation and clinical and pathological studies have revealed that radiation therapy can produce significant hepatic injury [3]. In order to find radioprotective compounds from natural sources, many studies have been conducted on radioprotection effect of plant extracts. The extracts of some edible plants including *Panax ginseng* [2], *Chamellia sinensis* [25], *Zingiber officinalis* [26], *Curcuma longa* [27], *Zataria multiflora* [28] and *Mentha piperita* [29] have shown radioprotective effects due to their antioxidant and anti-inflammatory potential.

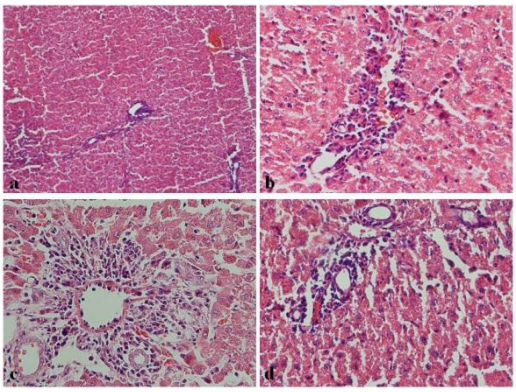

Figure 1. Microscopic photographs of liver sections stained with H&E. **(a)**: Normal liver parenchyma showing portal tracts and hepatocytes, (**b)**: Liver parenchyma after exposure to radiation, spotty necrosis, (**c)**: Liver parenchyma after exposure to radiation, marked portal and periportal inflammation, (**d)**: Liver parenchyma after treatment with methanolic extract of *Nasturtium officinale* at a dose of 100 mg/kg b.w. displaying decreased portal and periportal inflammation

The family Brassicaceae has several types of edible plants, which are rich sources of variety of nutrients and health promoting phytochemicals such as vitamins, carotenoids, minerals, glucosinolates and phenolic compounds [30,31]. Nowadays, particular attention has been paid towards edible plants, which are rich in secondary metabolites. The most of phenolic compounds in Brassicaceae family are flavonoids and cinnamic acid derivatives [31].

Table 1. Histopathological changes of liver cells after using *Nasturtium officinale* extract

	Score	Negative control	Positive control	20 mg/kg b.w		50 mg/kg b.w		100 mg/kg b.w	
				Acute	Subacute	Acute	Subacute	Acute	Subacute
Edema & Congestion	0	+							
	1+								
	2+						+	+	+
	3+		+	+	+	+			
Spotty necrosis	0	+							
	1+								
	2+						+	+	+
	3+		+	+	+	+			
Portal& Periportal inflammation	0	+							
	1+							+	+
	2+						+		
	3+		+	+	+	+			

Phenolics possess many useful properties for human health including anti-inflammatory, enzyme inhibition, antimicrobial, antialergic, antitumor and especially antioxidant activities [32]. Phenolics are able to scavenge ROS and have inhibitory effects on lipid peroxidation [33]. A number of flavonoids exhibit anti-inflammatory properties and may have potential for management of radiation injuries [6].

Based on phytochemical analysis on the extract of NO, flavonoids such as quercetin and kampferol derivatives and hydroxycinnamic acid derivatives have been identified as major phenolic compounds in the leaves of this plant [10,13]. The amount of phenoic and flavonoid compounds of NO extract in our study was found to be less than those reported in previous studies [17,19]. It seems that the plant from high altitudes has more phenolic and flavonoid contents compared to plants from lowlands [19].

In the present study, histopathological examinations of the liver in radiation group have shown severe changes like spotty necrosis, inflammation, edema and congestion. Pre-treatment with 100 mg/kg b.w. per day of the methanolic extract of NO for 15 days and 2 h before 6 Gy γ-radiation significantly lowered incidence of inflammation (portal and periportal inflammatory cells infiltration).

In one study, the hydroalcoholic extract of the aerial parts of NO showed potent anti-inflammatory activity in systemic and topical applications [14]. Several studies have shown that the extracts of NO have remarkable antioxidant activity [17-19]. Bahramikia *et al*. reported the powerful antioxidant activity of the hydroalcoholic extract of NO *in vitro* which might be attributed to scavenging of free radicals, metal chelating activity and lipid peroxidation inhibition. In their study, the amount of total phenolics and flavonoids were 96.2 mg gallic acid equivalents and 63.2 mg catechin equivalents per gram of dried extract, respectively [17].

When the Brassicaceae vegetables are cut or crushed, isothiocyanates are produced by hydrolysis of glucosinolates [13]. Phenethyl isothiocyanate (PEITC) is a breakdown product of gluconasturtin, an abundant glucosinolate in watercress (*Nasturtium officinale*) [34], which has been reported to possess various biological activities such as cytostatic, cytotoxic, antitumor and anti-inflammatory properties [35-37]. In other researches, PEITC has regulated the inflammatory responses in mast cells and has inhibited the production of IL6 and IL-1β [38].

The radioprotective property of NO can be attributed to the presence of phenolic compounds and isothiocyanates in the methanolic extract. It seems that the combination of antioxidant and anti-inflammatory effects of NO is responsible for its hepatoprotective activity.

In conclusion, our results have indicated that the methanol extract of *N. officinale* in high dose can

provide considerable hepatoprotection against 6 Gy γ-radiation even if it is given only 2 h prior to the exposure. In addition, we suggest that this plant could be used as an edible vegetable in the diet of people who are exposed to gamma-irradiation.

Acknowledgement
This work was supported by a grant from Research council of Mazandaran University of Medical Sciences, Sari, Iran.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Ran Y, Wang R, Gao Q, Jia Q, Hasan M, Awan MU, Tang B, Zhou R, Dong Y, Wang X, Li Q, Ma H, Deng Y, Qing H. Dragon's blood and its extracts attenuate radiation-induced oxidative stress in mice**.** *J Radiat Res*. 2014; 55(4): 699-706.

[2] Mansour HH. Protective effect of ginseng against gamma-irradiation-induced oxidative stress and endothelial dysfunction in rats. *Excli J*. 2013; 12: 766-777.

[3] Malik IA, Moriconi F, Sheikh N, Naz N, Khan S, Dudas J, Mansuroglu T, Hess CF, Rave-Frank M, Christiansen H, Ramadori G. Single-dose gamma-irradiation induces up-regulation of chemokine gene expression and recruitment of granulocytes into the portal area but not into other regions of rat hepatic tissue. *Am J Pathol*. 2010; 176(4): 1801-1815.

[4] Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: Present concepts. *J Gastroen Hepatol*. 2011; 1: 173-179.

[5] Yamini K, Gopal V. Natural radioprotective agents against ionizing radiation - an overview. *Int J Pharmtech Res*. 2010; 2(2): 1421-1426.

[6] Arora R, Gupta D, Chawla R, Sagar R, Sharma A, Kumar R, Prasad J, Singh S, Samanta N, Sharma RK. Radioprotection by plant products: present status and future prospects. *Phytother Res*. 2005; 19(1): 1-22.

[7] Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. Radioprotective effects of antioxidative plant flavonoids in mice. *Mutat Res*. 1996; 350(1): 153-161.

[8] Arora R. *Herbal radiomodulators: applications in medicine, homeland defense and space*. Cambridge: CABI, 2008.

[9] Carrasco G1, Moggia C, Osses IJ, Alvaro JE, Urrestarazu M. Use of peroxyacetic acid as green chemical on yield and sensorial quality in Watercress (*Nasturtium officinale* R. Br.) under soilless culture. *Int J Mol Sci*. 2011; 12(12): 9463-9470**.**

[10] Martínez-Sánchez A, Gil-Izquierdo A, Gil MI, Ferreres F. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. *J Agric Food Chem*. 2008; 56(7): 2330-2340.

[11] Blumenthal M, Goldberg A, Brinckmann J. *Herbal medicine: Expanded commission E monographs*. 1th ed. Boston: Integrative Medicine Communications, 2000.

[12] Fleming T. *PDR for Herbal Medicine*. 1st ed. Montvale: Medical Economics Company, 1998.

[13] Boyd LA, McCann MJ, Hashim Y, Bennett RN, Gill CIR, Rowland IR. Assessment of the anti-genotoxic, anti-proliferative, and anti-metastatic potential of crude watercress extract in human colon cancer cells. *Nutr Cancer*. 2006; 55(2): 232-241.

[14] Sadeghi H, Mostafazadeh M, Sadeghi H, Naderian M, Barmak MJ, Talebianpoor MS, Mehraban F. *In vivo* anti-inflammatory properties of aerial parts of *Nasturtium officinale*. *Pharm Biol*. 2014; 52(2): 169-174.

[15] Bahramikia S, Yazdanparast R. Effect of hydroalcoholic extracts of *Nasturtium officinale* leaves on lipid profile in high-fat

diet rats. *J Ethnopharmacol*. 2008; 115(1): 116-121.
[16] Camacho-Corona Mdel R, Ramírez-Cabrera MA, Santiago OG, Garza-González E, Palacios Ide P, Luna-Herrera J. Activity against drug resistant-tuberculosis strains of plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases. *Phytother Res*. 2008; 22(1): 82-85.
[17] Bahramikia S, Yazdanparast R. Antioxidant efficacy of *Nasturtium officinale* extracts using various in vitro assay systems. *J Acupunct Meridian Stud*. 2010; 3(4): 283-290.
[18] Ozen T. Investigation of antioxidant properties of *Nasturtium officinale* (watercress) leaf extracts. *Acta Pol Pharm*. 2009; 66(2): 187-193.
[19] Mazandarani M, Momeji A, Zarghami moghaddam P. Evaluation of phytochemical and antioxidant activities from different parts of *Nasturtium officinale* R.Br. in Mazandaran. *Iranian J Plant Physiology*. 2012; 3(2): 659-664.
[20] Engelen-Eigles G, Holden G, Cohen JD, Gardner G. The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.). *J Agric Food Chem*. 2006; 54(2): 328-334.
[21] Gill CIR, Halder S, Boyd LA, Bennett R, Whiteford J, Butler M, Pearson JR, Bradbury I, Rowland IR. Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. *Am J Clin Nutr*. 2007; 85(2): 504-510.
[22] Gay WI. *Methods of animal experimentation*. London: Academic Press, 1965.
[23] Miliauskas G, Venskutonis PR, Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem*. 2004; 85(2): 231-237.
[24] Dasilva MCA, Paiva SR. Antioxidant activity and flavonoid content of Clusia fluminensis Planch & Triana. *An Acad bras cienc*. 2012; 84(3): 609-616.
[25] Pal S, Saha C, Dey SK. Studies on black tea (*Camellia sinensis*) extract as a potential antioxidant and a probable radioprotector. *Radiat Environ Bioph*. 2013; 52(2): 269-278.
[26] Baliga MS, Haniadka R, Pereira MM, Thilakchand KR, Rao S, Arora R. Radioprotective effects of *Zingiber officinale* Roscoe (ginger): past, present and future. *Food Funct*. 2012; 3(7): 714-723.
[27] Nada AS, Hawas AM, Amin ND, Elnashar MM, Abd Elmageed ZY. Radioprotective effect of *Curcuma longa* extract on γ-irradiation-induced oxidative stress in rats. *Can J Physiol Pharm*. 2012; 90(4): 415-423.
[28] Hosseinimehr SJ, Mahmoudzadeh A, Ahmadi A, Ashrafi SA, Shafaghati N, Hedayati N. The radioprotective effect of *Zataria multiflora* against genotoxicity induced by γ-irradiation in human blood lymphocytes. *Cancer Biother Radio*. 2011; 26(3): 325-329.
[29] Baliga MS, Rao S. Radioprotective potential of mint: a brief review. *J Cancer Res The*r. 2010; 6(3): 255-262.
[30] Soengas P, Sotelo T, Velasco P, Cartea ME. Antioxidant properties of *Brassica* vegetables. *Func Plant Sci Biotechology*. 2011; 5(2): 43-55.
[31] Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in *Brassica* vegetables. *Molecules*. 2011; 16: 251-280.
[32] Podsedek A. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: a review. *Lwt-Food Sci Technol*. 2007; 40(1): 1-11.
[33] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Bio Med*. 1996; 20: 933-956.
[34] Wang LG, Lin XM, Fang Y, Dai W, Chiao FB, Puccio GM, Feng J, Liu D, Chiao JW. De-repression of the p21 promoter in prostate cancer cells by an isothiocyanate via

inhibition of HDACs and c-Myc. *Int J Oncol*. 2008; 33(2): 375-380.

[35] Cavell BE, Syed Alwi SS, Donlevy AM, Proud CG, Packham G. Natural product-derived antitumor compound phenethyl isothiocyanate inhibits mTORC1 activity via TSC2. *J Nat Prod*. 2012; 75(6): 1051-1057.

[36] Park HJ, Kim SJ, Park SJ, Eom SH, Gu GJ, Kim SH, Youn HS. Phenethyl isothiocyanate regulates inflammation through suppression of the TRIF-dependent signaling pathway of Toll-like receptors. *Life Sci*. 2013; 92(13): 793-798.

[37] Okubo T, Washida K, Murakami A. Phenethyl isothiocyanate suppresses nitric oxide production via inhibition of phosphoinositide 3-kinase/Akt-induced IFN-gamma secretion in LPS-activated peritoneal macrophages. *Mol Nutr Food Res*. 2010; 54(9): 1351-1360.

[38] Moon PD, Kim HM. Anti-inflammatory effect of phenethyl isothiocyanate, an active ingredient of *Raphanus sativus* Linne. *Food Chem*. 2012; 131(4): 1332-1339.