Egypt. J. Rad. Sci. Aplicc., Vol. **25**, No. 1-2, pp. 43-53 (2012)

Role of Betalains as Natural Antioxidant in Modulating Renal Disorders in γ-Irradiated Mice

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BETALAINS are natural antioxidants extracted from red beet (*Beta vulgaris* L.), prevent lipid oxidation and improve antioxidant defence system in the animal tissue.

This study investigates the protective role of betalains on γ-rays-induced renal disorders in mice. Thirty two mice were divided into four groups; the first (control group) received the vehicle only for 33 days (control), the second (betalains group) received betalains (80 mg/kg body weight/day) for 33 days, the third (irradiated group) received the vehicle for 30 days before exposed to 4 Gy γ-rays (one shot) and for 3 days after irradiation and the last (protected group) received betalains for 30 days before γ-irradiation and for 3 days after irradiation.

Gamma-rays-provoked oxidative stresses in renal tissue were indicated by significant increases of thiobarbituric acid reactive substances (TBARs), carbonyl content (PC) and total nitrate/ nitrite (NOx) as well as an increase of plasma renal tubular and glomerular markers; urea (Ur), creatinine (Cr) and γ-glutamyl transferase (γ-GT). In dissimilarity, γ-rays-induced significant decreases of renal reduced glutathione (GSH) level as well as peripheral blood indices; total red blood cells (RBC), haemoglobin (Hb), platelets (Pt) and total white blood cells (WBC) and renal enzymatic antioxidants; super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione *S*-transferase (GST).

The results indicate that the administration of betalains protects against renal disorders in mice irradiated by γ-rays.

Keywords: Betalains, renal distress, γ-rays, mice.

Ionizing radiation induces free radicals production such as hydrogen, hydroxyl, singlet oxygen and peroxyl radicals, in a cascade pathway. This irradiation can lead to mortality in mammals, so it is important to protect biological systems from radiation-induced tissue damage (Rzeszowska-Wolny *et al.,* 2009).

Natural plant extracts such as echinacea purpurea, betalains and gymnema Sylvester leaves have been shown to protect cells and tissues against ionizing radiation without adverse reactions (Abouelella *et al.,* 2007, El Gharras, 2012 and Sharma *et al.,* 2009). Accordingly, such plant extracts could be used as an adjunct to conventional radiotherapy. Betalains, are water-soluble pigments with high antiradical capacity (Gandia-Herrero *et al.,* 2010), which were isolated from red beet and possess antioxidant activity *in vivo* and *in vitro* (El Gharras, 2012 and Kanner *et al.,* 2001). It inhibits lipid peroxidation and haeme-decomposition (Cai *et al.,* 2003).

The present study was carried out to investigate whether betalains had modulating renal disorders in mice irradiated by γ-rays.

Materials and Methods

Animals

Male albino mice (18± 2 g) were housed at 22-24 °C and 60-70 % relative humidity in a 12 h light/12 h dark cycle. All mice were given normal diet and water *ad libitum*.

Chemicals and irradiation

Betalains (extracted from red beets) were purchased from Wuxi Talen Bioproduct Co., China. All another analytical chemicals were purchased from Sigma-Aldrich (St. Louis, USA).

Whole-body γ-irradiation was performed at National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt, using Gamma Cell-40 biological irradiator delivered at a radiation dose rate of 0.44 Gy/ min.

Experimental plan

Control group received the vehicle for 33 days. Betalains group: Mice were orally given betalains (dissolved in ½ ml of water) at a dose of 80 mg/ kg body weight/ day for 33 days by stomach tube according to Lu *et al.* (2009). Irradiated group: Mice were received the vehicle for 30 days before exposed to 4 Gy γ-rays and for 3 days after irradiation. Protected group (betalains+ γ-rays): Mice administered betalains dosage for 30 days before exposure to γ-irradiation and for 3 days after irradiation. Mice were decapitated 24 h at the end of the experimental time.

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Kidney organ was rapidly removed and washed in saline buffer and processed according to Varghese *et al.* (2009) for lipid peroxidation and antioxidant marker assays. Blood samples were prepared for blood indices by adding anticoagulant and for obtaining plasma by centrifugation at 1500 xg.

Renal lipid peroxidation (TBARs), PC, GSH and NOx estimations performed as renal oxidative stress parameters were assayed according to the procedures of Ohkawa *et al.* (1979), Levine *et al.* (1990), Hissin and Hilf (1973), and Miranda *et al.* (2001), respectively. Plasma levels of Ur, Cr and γ-GT were measured for assessment of the renal tubular and glomerular markers using method of Fawcett and Soctt (1960), Lustgarten and Wenk (1972) and Tietz (1994), respectively. Peripheral blood samples were analysed for total RBC, haemoglobin, platelets and total WBC were measured using Sysmex haematology analyser (model K4500, SYSMEX Shanghai Ltd., China). Determination of SOD, CAT, GPx and GST in kidney tissue were performed according to McCord (1985), Aebi (1984), Lawrence and Burk (1976) and Habig *et al.* (1974), respectively. Protein concentration was determined by the method of Bradford (1976).

Data are expressed as mean± S.E. of 8 mice per group. Statistical analysis was carried out using one way analysis of variance consider significant at $P< 0.05$ followed by student's *t*-test (Snedecor and Cochran, 1994).

Results

Administration of betalains for betalains group of mice resulted in non-significant changes in all blood, plasma and renal biochemical's parameters, tested.

TABLE 1. Renal oxidative stress parameters; thiobarbituric acid reactive substances (TBARs), carbonyl content (PC), total nitrate/nitrite (NOx) and reduced glutathione (GSH) in different mouse groups.

Mouse groups	TBARs (µg/g tissue)	PC (nmol/ mg protein)	NOx (nmol/g tissue)	GSH (mg/g tissue)
Control	1.6± 0.21[a]	1.4± 0.14[a]	14.7± 1.14[a]	13.1± 0.69[a]
Betalains	1.5± 0.33[a]	1.6± 0.18[a]	14.6± 1.27[a]	13.3± 0.72[a]
γ-rays (4 Gy)	5.3± 0.89[b]	4.5± 0.32[b]	38.2± 2.19[b]	8.3± 0.53[b]
Protected	2.1± 0.16[c]	2.1± 0.26[c]	16.6± 1.35[c]	11.1± 0.82[a]

[a–c]Means in the same column with different superscript letters differ significantly at $P< 0.05$.

A significant increase in TBARs, PC and NOx levels accompanied by a significant decrease in GSH level in γ-rays group compared with control group, suggest kidney oxidative stress. However, administration of betalains normalise these parameters, Table 1. Plasma levels of Ur, Cr and γ-GT were increased significantly in γ-rays groups. Protected group treated with betalains significantly normalise the examined renal oxidative stress parameters, Table 2.

TABLE 2. Plasma renal tubular and glomerular markers; urea (Ur), creatinine (Cr) and γ-glutamyl transferase (γ-GT) in different mouse groups.

Mouse groups	Ur (mmol/ L)	Cr (µmol/ L)	γ-GT (U/ L)
Control	1.43± 0.245[a]	0.65± 0.391[a]	0.71± 0.132[a]
Betalains	1.41± 0.256[a]	0.62± 0.414[a]	0.72± 0.214[a]
γ-rays (4 Gy)	3.88± 0.543[b]	1.23± 0.232[b]	2.84± 0.227[b]
Protected	1.63± 0.278[c]	0.93± 0.237[c]	1.16± 0.156[c]

Legends as in Table 1.

Compared with the control mice, the number of the RBC, WBC & Pt and Hb concentration were significantly reduced. However, oral intake of betalains attenuated the radiation-induced reduction in red, white and Pt cells and in Hb level, suggesting that betalains confers radioprotection (Table 3).

TABLE 3. Peripheral blood indices; total red blood cells (RBC), haemoglobin (Hb), platelets (Pt) and total white blood cells (WBC) in different mouse groups.

Mouse groups	RBC ($x10^6$/µL)	Hb (g/dl)	Pt ($x10^4$/ml)	WBC ($x10^3$/µL)
Control	5.6± 0.16[a]	10.1± 0.27[a]	28.4± 3.22[a]	6.1± 1.02[a]
Betalains	5.4± 0.10[a]	10.3± 0.42[a]	29.1± 3.46[a]	6.2± 1.14[a]
γ-rays (4 Gy)	3.4± 0.14[b]	8.2± 0.23[b]	17.8± 1.78[b]	2.7± 0.80[b]
Protected	5.1± 0.12[c]	9.2± 0.21[a]	24.3± 2.63[c]	5.8± 1.35[c]

Legends as in Table 1.

TABLE 4. Renal enzymatic antioxidants; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST) in different mouse groups.

Mouse groups	SOD (U/ mg protein)	CAT (U/ mg protein)	GPx (U/ mg protein)	GST (U/ mg protein)
Control	90.3± 7.42[a]	33.4± 2.31[a]	345.6± 34.33[a]	6.2± 0.46[a]
Betalains	88.5± 8.03[a]	31.7± 2.62[a]	314.3± 31.24[a]	6.1± 0.38[a]
γ-rays (4 Gy)	32.3± 3.12[b]	18.4± 3.18[b]	142.1± 17.26[b]	3.0± 0.27[b]
Protected	79.6± 6.21[c]	27.9± 2.11[c]	278.5± 18.44[c]	5.1± 0.21[c]

Legends as in Table 1.

γ-irradiation significantly suppressed the enzymatic activity of SOD, CAT, GPx and GST in renal tissue. Oral administration of betalains partially restored the four enzymatic antioxidants activity, Table 4.

Discussion

Red beet root and leaf are consumed in salad with other vegetables world-wide. They are a good source of natural antioxidants such as, betalains, flavonoids, polyphenols, vitamins and folic acid (Lee *et al.,* 2009); those act as reactive oxygen species (ROS) scavengers (Sepulveda-Jimenez *et al.,* 2004). Betalains have been defined as condensation products of betalamic acid with different amines and amino acids (Gandia-Herrero *et al.,* 2010); it has been appeared to be easily absorbed and detected in the urine of subjects who consumed red beet (Kanner *et al.,* 2001).

In human and animal body, ROS can be neutralized by antioxidant compounds. However, excessive ROS production and the depleted antioxidant defences lead to oxidative stress and induce oxidative damage, causing pathological dysfunction in the organism (Urso and Clarkson, 2003). The consequences of these oxidative damage are multiple and invariably advice. They include lipid peroxidation, resulting in the destruction of membrane lipids, and oxidative DNA damage, collectively leading to the loss of cell viability, either via necrotic or apoptotic pathways (Marnett, 2000). Cells could be injured and even killed under the most serious conditions of radiation exposure, when the content of ROS get uncontrolled by the cellular antioxidants (Pathak *et al.,* 2007).

In the present study, the significant increases in TBARs, PC and NOx levels in γ-rays group accompanied by a significant decrease in GSH level, suggest that oxidative stress occurs in kidney. Radiation-induced decline in GSH and augmentation in TBARs, PC and NOx levels in rats and mice tissues (Gharib and Fahim, 2007 and Sharma *et al.,* 2009).

Betalains found to protect mice against the oxidative stresses-induced decline in GSH (Sunila and Kuttan, 2005) and enhancement in TBARs and PC (Reddy *et al.,* 2005 and Tesoriere *et al.,* 2004). The increase in GSH levels may be due to the activation of protective response in the kidneys to counteract the excessive formation of ROS. It acts as radical scavenger due to redox-active sulphydryl group directly reacting with oxidant and transforms itself into oxidised GSH (Pathak *et al.,* 2007). These results suggest betalains from red beets are not only radio protective but are also effective as an antioxidant (Lu *et al.,* 2009).

Plasma levels of Ur, Cr and γ-GT were increased significantly in γ-irradiated mice due to impairment of kidney functions (El-Khafif *et al.,* 2003). Treatment with betalains significantly normalise renal oxidative stress parameters. Betalains protect liver γ-GT (Schwartz *et al.,* 1983) and improve renal function and excretion (Frank *et al.,* 2005).

Gamma-rays irradiation induced a marked reduction in RBC, WBC, Pt counts and Hb concentration in mice due to direct destruction of the mature circulating cells and the damage effects on a large number of blood forming stem cells (Aboueldlla *et al.,* 2007 and Tawfik *et al.,* 2006). Betalains partially restored these values. Its protective effect against oxidative damage was evaluated in red and white blood indices of human and mice (Hari Kumar and Kuttan, 2004 and Tesoriere *et al.,* 2006) and have a wide range of desirable biological activities, including antioxidant, anti-inflammatory and anti-cancer properties (Georgiev *et al.,* 2010).

Gamma-irradiation significantly suppressed the enzymatic activity of SOD, CAT, GPx and GST in renal tissue. Cells produce antioxidants such as CAT, SOD, GPx and GST as a part of cellular defence system against ROS-mediated cellular injury (Rahman *et al.,* 2006), that induced by ionizing radiation. Oral administration of betalains partially restored the four enzymatic antioxidants activity as reported in previous studies (Hari Kumar and Kuttan, 2004, Pradedova *et al.,* 2010 and Sunila and Kuttan, 2005). Betalains are phytochemicals that were classified as antioxidants (Butera *et al.,* 2002) suggest that they may behave as effective reducing species, whereas a clear antioxidant activity has been shown in biological environments such as membranes (Tesoriere *et al.,* 2003).

Conclusion

Betalains supplementation could prevent lipid peroxidation and improve antioxidant defence system in the renal tissue and plasma of γ-irradiated mice.

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(Received: 10/12/2012;

accepted: 31/01/2013)

دور البيتالانس كمضاد للأكسدة طبيعي في الحد من اضطرابات الكلي في الفئران البيضاء المشعة جاميا

منال درويش ، و غادة أحمد الطويل ، و حسين محمد صلاح مكاوي

قسمي البيولوجيا الإشعاعية و تشعيع الأغذية ، المركز القومي لبحوث و تكنوليوجيا الإشعاع ، ص. ب. ٢٩ مدينة نصر ، مصر.

البيتالانس (مضاد للأكسدة طبيعي) يستخرج من نبات البنجر الأحمر و يقي الليبيدات من الأكسدة و يحسن نظام الحماية بالإضافة إلي منع الأكسدة في النسيج الحيواني الحي.

تختبر هذه الدراسة دور البيتالانس في وقاية إصابة كلي الفئران البيضاء المعرضة لأشعة جاما. تم تقسيم الفئران إلي أربعة مجموعات: الأولي تجرعت المادة المذيبة فقط لمدة ٣٣ يوما ، الثانية تجرعت البيتالانس (٨٠ ملجرام/ كجم من وزن الفأر لمدة ٣٣ يوما) ، الثالثة عرضت لجرعة ٤ جراي من أشعة جاما ، و المجموعة الأخيرة تجرعت البيتالانس لمدة ٣٠ يوما قبل التعرض لأشعة جاما و لمدة ٣ أيام بعد التعرض للأشعة.

تم قياس مدي قدرة أشعة جاما علي إحداث ضغوط التأكسد في نسيج الكلي عن طريق رصد الزيادة الاحصائية لمستوي كل من: حامض الثيوبربتيورك (TBARs) و كاربونيل البروتين (PC) و النيترات/النيتريت (NOx) في نسيج الكلي ، الي جانب زيادة مستوي اليوريا (Ur) و الكرياتنين (Cr) و جاما-جلوتاميل ترانس فيريز (γ-GT) في بلازما الدم. كما سببت أشعة جاما نقصا إحصائيا لمستوي كل من: الجلوتاثيون المختزل (GSH) في نسيج الكلي. بالإضافة إلي نقص كل من: تعداد كرات الدم الحمراء (RBC) و الهيموجلوبين (Hb) و تعداد الصفائح الدموية (Pt) و التعداد الكلي لكرات الدم البيضاء (WBC). و كذلك نشاط إنزيمات متع التأكسد بالكلي: إنزيمات السوبر اكسيد ديسميوتيز (SOD) و الكتاليز (CAT) و الجلوتاثيون بيروكسيديز (GPx) و الجلوتاثيون-أس-ترانس فيريز (GST).

أظهرت النتائج أن تناول الفئران البيضاء للبيتالانس يقي من اضطرابات الكلي الحادثة في الفئران المعرضة لأشعة جاما.