Original article

Blueberry extract attenuates γ-radiation-induced hepatocyte damage by modulating oxidative stress and suppressing NF-κB in male rats

Maha I. Alkhalf [a,b], Fares K. Khalifa [c,d,*]

[a] Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Applied Biochemistry Department, Faculty of Science – AL Faisaliah Campus-University of Jeddah, Jeddah, Saudi Arabia
[c] Biochemistry Department, Science Faculty in Alsolimania, King Abdulaziz University, Saudi Arabia
[d] Biochemistry and Nutrition Department, Women College, Ain Shams University, Egypt

ABSTRACT

Radiation exposure is known to produce many harmful effects in biological systems, and these effects are often mediated by oxygen free radicals. Because blueberries are rich in antioxidant compounds such as anthocyanins and phenolic acids, we divided forty adult rats into four treatment groups of 10 (G1–4) as follows: G1 rats were used as a control, G2 rats were irradiated with 8 Gy at 2 Gy/week at a dose rate of 0.5 Gy/min, G3 rats were administered blueberry extract (200 mg/kg) and G4 rats were administered blueberry extract during the same irradiation period. In subsequent determinations, γ-irradiated rats had increased levels of cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL), and significantly elevated liver enzyme activities, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and total bilirubin. In contrast, significant reductions in albumin, total protein and globulin were observed, whereas gamma irradiation decreased activities of the antioxidant enzymes glutathione (GSH), catalase (CAT), xanthine dehydrogenase (XDH) and superoxide dismutase (SOD). We also observed incremental increases in DNA fragmentation percentages and histopathological changes in liver tissues. Serum pro-inflammatory cytokine levels (IL-6, IL-10 and TNF-α) were significantly elevated and hepatic NF-κB was upregulated. In G4 rats, treatments with blueberry extract restored liver pro-oxidant status, reduced cytokine levels, ameliorated histopathological parameters and reduced DNA damage. In conclusion, γ-radiation exerts toxic effects in the rat livers, and blueberry extract is protective against these.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Exposures to environmental hazards including ionising radiation, dietary factors, drugs and herbicides have prompted investigations of natural substances that protect human health. Radiation-related disorders are a current health problem with broad spectrum medical, social and economic consequences (Said and Nada, 2012). All types of radiation generate ions that form reactive oxygen species (ROS) and other radicals (Esposito et al., 2014). Remediation using plant based agents is increasingly considered, although efficacy in the management of free radical related pathological conditions remains a primary consideration of such nutraceuticals (Kalra, 2003).

Ionizing radiation has a variety of advantageous uses in medication including radiotherapy as a main treatment for extensive diversity of tumors, diagnosis and staging of diseases and malignancies, but its side effects on the normal tissues restrict the efficiency of therapy. It is well known that ionizing radiations stimulate oxidative stress on target tissues, mainly through the generation of reactive oxygen species (ROS) resulting in inequity of the cellular pro-oxidant and antioxidant, attack various cellular macromolecules such as DNA, lipids, and proteins, that leads to cell death (Boerma and Hauer-jensen, 2011). The utilization of a diet rich in polyphenol compounds may minimize the complications of metabolic disorders. Since the polyphenolic compounds, including proanthocyanidins, have strong antioxidant properties, they can protect against oxidative stress (Vendrame et al., 2014).

Blueberries (*Vaccinium corymbosum L.*) are rich in antioxidant compounds such as proanthocyanidins, flavonols, anthocyanins and the phenolic acids ferulic, pcoumaric, chlorogenic and caffeic acids (Yuan et al., 2016). In particular, the health benefits of blueberry anthocyanins (BA) have been demonstrated, and have been shown to reduce the risk cardiovascular disorders, neurodegenerative diseases and some cancers. BA also reportedly inhibits the production of pro-inflammatory molecules and oxidative stress products (Del Bo et al., 2016). Moreover, blueberries ameliorate inflammation, ageing, hepatic disorders, diabetes mellitus, neuronal and cardiac disorders and cancers. Herein, we characterised the hazards of γ-radiation by assessing biochemical parameters and histological changes in livers of adult male albino rats following γ-radiation exposures, and demonstrated the protective effects of blueberry antioxidants.

2. Materials and methods

2.1. Animals

A total of 40 mature male albino rats weighing 120–150 g were purchased from the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt). Rats were acclimatised for 3 days, and were housed one per cage in wire bottomed stainless steel cages in a temperature controlled room (25 °C ± 5 °C) with relative humidity of 50% ± 10% and a 12-h light/12-h dark cycle.

2.2. Gamma-radiation

Whole body γ-radiation of rats was performed using a [137]Cesium source Gamma Cell-40 biological irradiator at the NCRRT, Nasr City, Cairo, Egypt. Rats were administered 8 Gy of γ-radiation at 2 Gy/week and at a dose rate of 0.5 Gy/minute. This dose is sub-lethal in rats according to Adaramoye et al. (2010).

2.3. Blueberry extract

Blueberry extract was purchased from life extension company, USA.

2.4. Experimental design

Animals were randomly assigned to four experimental groups (10 rats/group) as follows:

Group (1): Rats of the control group received no treatment.
Group (2): Rats received a total of 8-Gy whole body γ-irradiation at 2 Gy/week and at a dose rate of 0.5 Gy/min for 4 weeks.
Group (3): Animals were orally administered aqueous suspensions of blueberry extract (200 mg/kg) via gavages daily for four weeks, as described by Pang et al. (2010).
Group (4): Rats were administered blueberry extract (200 mg/kg) daily via gavages through the radiation period described for G2 rats.

2.5. Biochemical analyses

At the end of the experimental period, rats were anaesthetised with light ether and blood samples were obtained via heart puncture using a sterilised syringe. Blood samples were centrifuged at 1000g for 15 min.

Lipid profile indices, including total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL), and low density lipoprotein-cholesterol (LDL), were assayed using commercial kits from Diamond Diagnostics. The indicators of liver activity aspar-tate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein and albumin were all were determined using commercial kits from Biodiagnostic, Egypt. Globulin levels were calculated by subtracting individual albumin values from corresponding total protein values. Activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were assayed according to Janknegt et al. (2007) and Aebi (1995) respectively. Glutathione contents (GSH) were determined using the methods described by Habig et al. (1974). Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) activities were determined using commercial kits as described by Kamiński and Jezewska (1979).

2.6. Serum cytokines

The cytokines interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) were assayed in serum commercial ELIZA kits for rats (Mabtech AB, Sweden) according to the manufacturer's instructions.

2.7. Real time polymerase chain reaction determinations of hepatic NF-kB expression

For determining the expression of hepatic NF-κB gene, Total RNA was extracted from liver tissues using QIAGEN Total RNA kits (Germany) according to the manufacturer's instructions. Briefly, add 10 μL of 20 μg primer/mL template stock to give 200 ng of primer in that tube. The PCR reactions included 10 min at 95 °C (activation), followed by 40 cycles at 94 °C for 15 s (denaturing) and 60 °C for 1 min (annealing/extension). The primers sequences used are grouped amounts of RNA (2 μg) were reverse transcribed into cDNA the NF-kB forward and reverses primers ACAAATGGGCTA-CACCGAAG and ATGGGGCATTTTGTTGAGAG, respectively (Livak and Schmittgen, 2001).

2.8. DNA fragmentation assays

Rat livers were homogenised in extraction buffer and incubated for 1 h at room temperature, and were then digested overnight. An equivalent amount of phenol equilibrated with 1 mol/L Tris buffer (pH 8.0) was then added, and the tube was put on an device for 1 h. After centrifugation, the extraction repeated with an equal volume of phenol/chloroform. DNA was precipitated by addition sodium acetate and ethanol. The precipitated DNA was rinsed with ethanol, and finally re-suspended. To detect DNA fragmentation, 10 mg of each DNA was electrophoretically fractionated on 1.5% agarose gel with 0.5 mg/ mL ethidium bromide. The DNA in the gel was visualized and photographed under UV light (Okamura et al., 2000).

2.9. Histopathological examinations of liver sections

Liver tissues from rats of all treatment groups were immersed in formalin solution (10%), were dehydrated in alcohol and were then embedded in paraffin. Sections of 5 μm were then cut, and were deparaffinised and stained with hematoxylin and eosin (H and E) for examinations under a light microscope.

2.10. Statistical analyses

Statistical analyses were performed using SPSS. Data are presented as means ± standard errors of the mean (SE) and differences between groups were identified using analysis of variance (ANOVA test). Differences were considered significant when $p < 0.05$.

Table 1
Effects of Blueberry extracts and/or γ -irradiation on total cholesterol, triglyceride, HDL and LDL levels in rats.

Groups	Parameters			
	T Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Control group	153.7 ± 6.9	101.9 ± 6.2	43.5 ± 3.9	71.9 ± 4.1
Irradiated rats	288.4[*] ± 11.3	222.4[*] ± 16.5	151.9[*] ± 8.1	88.3[*] ± 4.2
Blue berry rats	121.2[**] ± 8.6	91.6[**] ± 4.3	49.1[**] ± 3.4	54.6[**] ± 3.7
Blue berry and radiation	210.3[**] ± 7.4	118.2[**] ± 8.1	81.4[**] ± 3.2	69.2[**] ± 4.9

[*] Means ± SD are significant (P < 0.05) compared with normal control group (1).
[**] Means ± SD are significant (P < 0.05) compared with Irradiated group (2).

3. Results

During the study, clinical observation results demonstrated that rats in control group were in a good state as compared to irradiated groups.

As seen in Table 1:Exposure to γ-radiation led to increases in serum total cholesterol, TG, LDLc, HDLc and concomitant treatments with blueberry extract ameliorated the associated dyslipidaemia.

AST, ALT and ALP levels were significantly ($p < 0.05$) higher in irradiated rats than in control rats (Table 2), but were significantly reduced towards normal values by treatments with blueberry extract ($p < 0.05$).

Total bilirubin levels were significantly increased in rats of the irradiation group compared with the other treatment groups. In contrast, radiation exposures reduced total protein, albumin and globulin levels significantly ($p \leq 0.05$) compared with control values (Table 3). Treatments with blueberry extracts ameliorated all departures from normal values and restored these serum proteins to near normal levels in irradiated rats.

The data in Table 4 show significant increases in serum XO activity and significant reductions in xanthine dehydrogenase (XDH) activity in irradiated rats, and concomitant treatments with blueberry extract improved these activities. Moreover, radiation significantly reduced serum GSH contents and SOD and CAT activities compared with those in the control group. Treatments with blueberry extract significantly influenced these antioxidant enzyme activities in irradiated rats, and again almost restored these to normal values.

Determinations of serum cytokine levels (IL-6, TNF-α and IFN-γ) listed in Table 5 revealed significant induction following irradiation ($p < 0.05$), whereas serum IL-10 levels were significantly reduced ($p < 0.05$) compared to control group. Blueberry supplementation of irradiated rats restored serum cytokines levels towards those seen in control animals.

In quantitative determinations of hepatic NF-κB mRNA expression (Fig. 1), whole body gamma irradiation led to significant increases relative to those in control rats and supplementation with blueberry extract significantly suppressed these increases compared with the irradiated group.

Fig. 2 to further elucidate the effects of blueberry extracts in irradiated rats, we performed DNA fragmentation assays (Fig. 1). These experiments showed considerable DNA fragmentation in irradiated rats (lane 2) and no DNA fragmentation in control and blueberry supplemented rats (lane 1 and lane 3). In addition, treatments with blueberry extract during the irradiation period were moderately protective against DNA fragmentation (lane 4) (see Fig. 3).

Table 2
Effects of blueberry extract and/or γ-irradiation on AST, ALT and ALP activities in rats.

Groups	Parameters		
	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	18.5 ± 0.174	21.9 ± 1.24	43.4 ± 2.98
Irradiated group	113.7[*] ± 8.2	129.7[*] ± 8.77	98.3[*] ± 7.43
Blue berry group	19.9[**] ± 0.81	19.5[**] ± 1.18	41.2[**] ± 0.85
Blue berry/radiated group	52.1[**] ± 3.42	67.2[**] ± 6.33	64.8[**] ± 3.55

[*] Means ± SD are significant (P < 0.05) compared with normal control group (1).
[**] Means ± SD are significant (P < 0.05) compared with Irradiated group (2).

Table 3
Effects of blueberry extract and/or γ-irradiation on serum total bilirubin, albumin, protein and globulin levels.

Groups	Parameters			
	T. Bilirubin (mg/dl)	Albumin (g/dl)	T. Protein (g/dl)	Globulin (g/dl)
Control	0.41 ± 0.18	3.7 ± 0.14	6.7 ± 0.65	3.0 ± 0.21
Irradiated group	1.69[*] ± 0.02	1.9[*] ± 0.08	3.9[*] ± 0.18	2.0[*] ± 0.25
Blue berry group	0.50 ± 0.16	3.9[**] ± 0.15	6.5[**] ± 0.41	2.6[**] ± 0.27
Blue berry/radiated group	0.88[**] ± 0.13	2.8[**] ± 0.11	5.7[**] ± 0.22	2.9[**] ± 0.21

[*] Means ± SD are significant (P < 0.05) compared with normal control group (1).
[**] Means ± SD are significant (P < 0.05) compared with Irradiated group (2).

Table 4
Effects of blueberry extract and/or γ-irradiation on serum xanthine oxidase (XO), xanthine dehydrogenase (XDH), superoxide dismutase (SOD), catalase (CAT) and gluthathione (GSH) levels.

Groups	Parameters				
	XO (mU/mg protein)	XDH (mU/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (mg/g tissue)
Control	2.15 ± 0.07	3.28 ± 0.06	42.59 ± 0.86	52.12 ± 1.71	25.95 ± 0.36
Irradiated group	3.24[*] ± 0.08	2.12[*] ± 0.04	39.17[*] ± 0.97	49.20[*] ± 0.64	21.43[*] ± 0.84
Blue berry group	2.87[**] ± 0.09	2.93[**] ± 0.03	42.05[**] ± 0.83	36.72[**] ± 0.99	23.77[**] ± 1.65
Blue berry/radiated group	2.12[**] ± 0.03	2.9[*] ± 0.02	41.79[**] ± 0.66	43.72[**] ± 0.61	22.13[**] ± 0.18

[*] Means ± SD are significant (P < 0.05) compared with normal control group (1).
[**] Means ± SD are significant (P < 0.05) compared with Irradiated group (2).

Table 5
Effects of blueberry extract and/or γ-irradiation on serum IL-6, IL-10, TNF-α and IFN-γ levels.

Groups	Parameter			
	IL-6 Pg/mg	IL-10 Pg/mg	TNF-α Pg/mg	IFN-γ IU/mL
Control	35.8 ± 3.29	134.38 ± 7.21	34.72 ± 4.82	48.11 ± 11.85
Irradiated group	133.54[*] ± 3.29	62.76[*] ± 6.63	122.87[*] ± 5.44	111.64[*] ± 12.05
Blue berry group	32.76[**] ± 4.82	129.98[**] ± 8.11	39.45[**] ± 4.95	44.76[**] ± 10.76
Blue berry/radiated group	58.76[**] ± 3.91	92.99[**] ± 8.43	63.98[**] ± 3.98	67.15[**] ± 11.88

[*] Means ± SD are significant ($P < 0.05$) compared with normal control group (1).

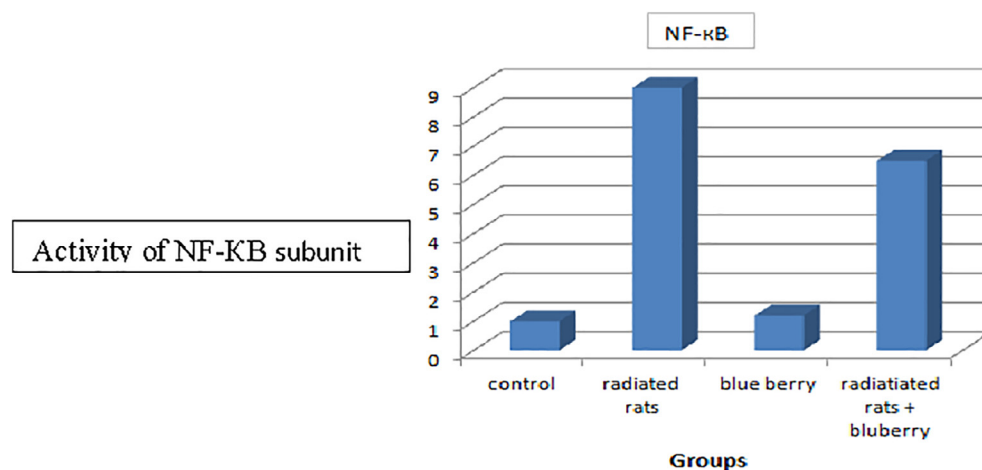[**] Means ± SD are significant ($P < 0.05$) compared with Irradiated group (2).

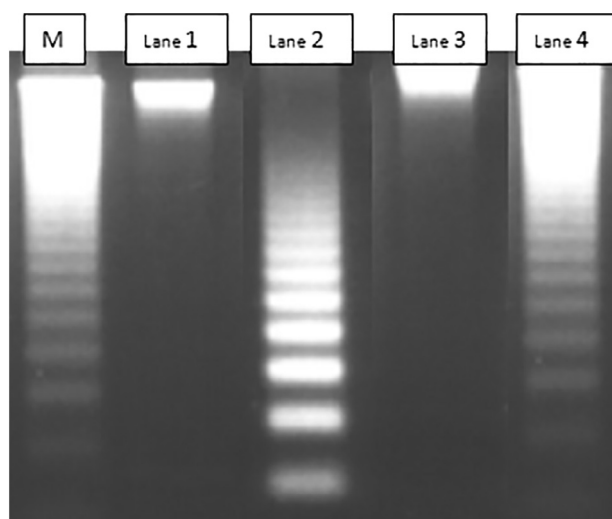**Fig. 1.** The effect of different treatments on hepatic NF-κB mRNA expression.

Fig. 2. DNA fragmentation patterns were monitored in irradiated and/or blueberry supplemented rats; M, marker; Lane 1, control group; Lane (2), irradiation groups; Lane (3), blueberry extract group; Lane (4), irradiated and blueberry supplemented group.

4. Discussion

Berry fruits are considered super foods because of their high levels of bioactive natural compounds, including phenolic acids and esters, flavonols, anthocyanins and procyanidins (Ferlemi and Lamari, 2016). Exposure to radiation during radiotherapy causes systemic damage to cellular structures by generating free radicals, in particular leading to increases in lipid peroxidation and declines in antioxidant activity (Ramadan et al., 2001). In the present study, irradiation led to dyslipidemia in rats, as shown previously by Mansour (2006) who demonstrated irradiation-related increases in serum cholesterol, HDL, LDL and TG levels in rats. These conditions are indicative of hyperlipidemia and are accompanied by increased synthesis of various lipids, including cholesterol and triglyceride. Radiation-induced hyperlipidemia was also related to increases in liver enzymes that synthesise fatty acid and mobilise fats from adipose tissues to the blood stream Mahmoud (1996).

Diets that are enriched in blueberries reportedly improve dyslipidaemia, and in a previous study, plasma TG and total cholesterol (TC) concentrations were significantly reduced in rats treated with blueberry extract (Vendrame et al., 2014). The anti-dyslipidaemic effects of blueberries include increased expression of key enzymes, such as lipoprotein lipase (LPL) and fatty acid synthase (Wei et al., 2011), which are involved in TG and cholesterol metabolism. Furthermore, sterol regulatory element-binding transcription factor (SREBP) and peroxisome proliferator-activated receptor (PPAR) were associated with the effects of blueberry consumption on lipid profiles (Vendrame et al., 2014), and consumption of 1%, 2% and 4% blueberry-supplements for 8 weeks significantly reduced TC and LDL-C concentrations in pigs (Kalt et al., 2008).

The present observations of liver injury were in agreement with previous studies (Mansour, 2006; El-Missiry et al., 2007). In particular, increases in ALT and ALP were consistent with increases in liver enzyme activities. Nada (2008) previously suggested that changes in serum enzyme activities after irradiation reflect either release from radiosensitive tissues or increased synthesis, and are associated with extensive damage to liver parenchyma. Accordingly, free radicals and lipid peroxides contribute to the
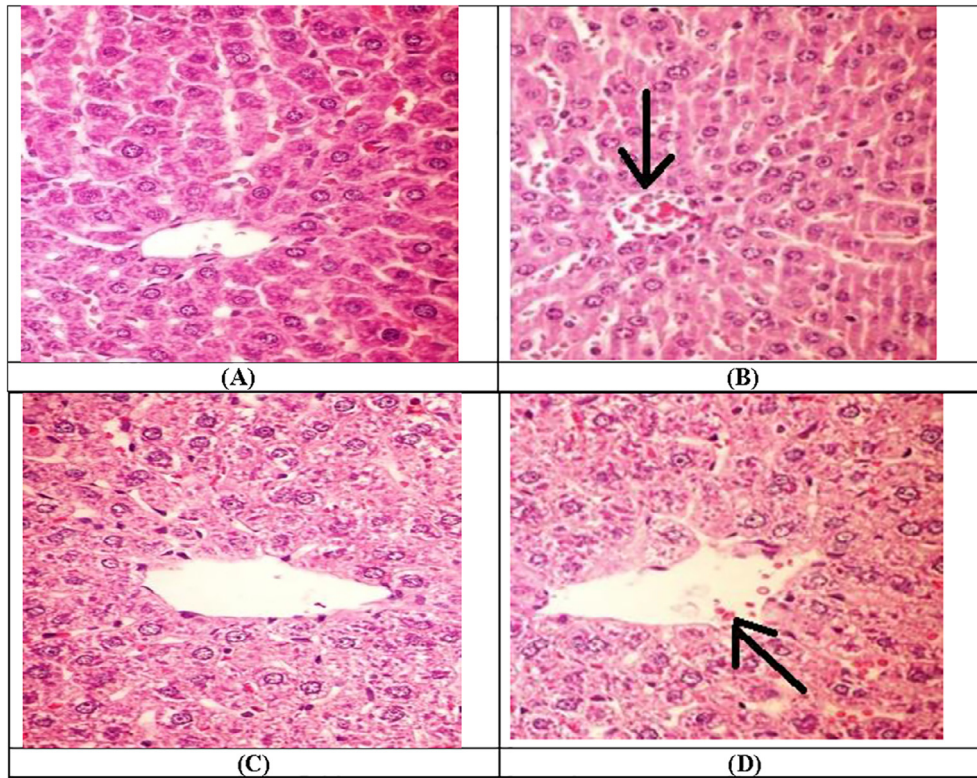
Fig. 3. Haematoxylin & eosin (H & E) stained liver sections from (A) control animals, with normal histological architecture and cell structure; (B) irradiated rats, focal hepatic haemorrhage in dilated and congested central veins; (C) blueberry extract supplemented rats, normal histological architecture and cell structure; (D) irradiated rats supplemented with blueberry extract, reduced haemorrhage and normal cell structure.

release of cytosolic enzymes such as aminotransferases (AST and ALT) and ALP, and blueberry extract reportedly restored AST, ALT and ALP levels to normal levels (Neto, 2007). Wang et al., (2010) showed that blueberry supplementation reduces hepatocyte injury and lipid peroxidation and protects liver tissues against carbon tetrachloride-induced hepatic fibrosis. Similarly, Osman et al. (2007) demonstrated that blueberry protects against galactosamine-induced acute liver injury.

In the present investigation, radiation reduced total serum protein levels and increased serum bilirubin concentrations in rats, likely reflecting increased red blood cell (RBC) and/or haemoglobin breakdown, as indicated by haematological alterations and hepatic damage. Moreover, serum bilirubin levels were reportedly elevated due to increased free radical production during hepatic damage (Liu et al., 2015). Moreover, whole body irradiation (5 Gy) reduced antioxidant enzyme activities of GSH, CAT and SOD, likely due to irradiation-induced oxidative stress, and these observations confirm previous experiments (Yasemine et al., 2013), and may reflect ROS-induced protein denaturation and enzyme inactivation. GSH levels are depleted by free radicals, and contribute to the formation of thiol radicals and oxidised glutathione (GSSG; Takikawa et al., 2010). Herein, we show that the harmful effects of radiation are associated with changes to the xanthine oxidoreductase (XOR) system, with increased conversion of xanthine dehydrogenase (XDH) to XO and elevated superoxide ($O2^-$) levels.

In a previous study, radiation exposures affected expression levels of the cytokines TNF-α, IL-1a, IL-1b, IL-6, type I IFN and IL-12 (Di Maggio et al., 2015). In agreement, our determinations of serum pro-inflammatory cytokines showed reduced serum levels of anti-inflammatory cytokine IL-10 in irradiated rats, and demonstrated corresponding protective effects of blueberry extracts. Accordingly, BA previously inhibited the production of pro-inflammatory molecules (Del Bo et al., 2016), and Vendrame

et al. (2014) showed that dietary supplementation with 8% blueberries for 8 weeks decreases plasma concentrations of IL-6 and TNF-α to obese Zucker rats. Similarly, supplementation with 4% whole blueberry powder deceased IL-10 and TNF-α in inflamed adipose tissues from high fat diet-fed mice (DeFuria et al., 2009).

It is widely accepted that free radicals induce NF-κB, which subsequently transactivates tumour necrosis factor alpha (TNF-α), thus causing hepatotoxicity by stimulating mitochondrial oxidant stress and upregulating chemokines and adhesion molecules. Interleukin- 6 (IL- 6) is also considered central to hepatic acute phase responses (Hosseinimehr et al., 2003). NF-kB plays central roles in immune and inflammatory responses by binding specific sequences in promoter regions of target genes and inducing the expression of various pro-inflammatory molecules such as TNF-a, IL-1b and IL-6 (Di Maggio et al., 2015). In a study by Li and Karin (1999), exposure to radiation resulted in increased NF-kB DNA binding activity, in part reflecting degradation of the major NF-kB inhibitor IkB-α. The present data reveal significant upregulation of hepatic NF-kB and downregulation of p53 in irradiated rats, suggesting decreased apoptotic activities and initiation of inflammatory pathways in hepatic cells. Thus, because the transcription factor NF-kB is potently activated by oxidative stress (Mattson et al., 2000), the present increases in oxidative stress likely enhanced NF-kB production following irradiation.

Finally, we observed considerable DNA fragmentation in irradiated rat tissues, and the associated DNA lesions were likely caused by ROS, which were formerly shown to cause DNA strand-breaks and cross linking between proteins and DNA Park et al. (2002). On the other hand, an improvement of DNA was noted in the present results following blueberry extract application and such enhancement may due to the effect of blueberry extract by DNA repairing system and enhancing protein synthesis (Kalpana et al., 2010).

5. Conclusion

Supplementation with blueberry extracts improves antioxidant defence systems and immune-system parameters in rats exposed to γ-radiation. Our data indicate that these effects are mediated by enhanced antioxidant enzyme activities and reduced lipid peroxide contents, leading to histopathological hepatoprotection against the deleterious effects of γ-radiation. Taken together, the present experiments warrant further consideration of blueberries as potent plant sources of antioxidants with protective effects against radiation-induce injury.

References

Adaramoye, O.A., Adedara, I.A., Popoola, B., Farombi, E.O., 2010. Extract of Xylopia aethiopica (Annonaceae) protects against gamma-radiation-induced testicular damage in wistar rats. J. Basic Clin. Physiol. Pharmacol. 21 (4), 295–314.

Aebi, M.E., 1995. Catalase. In: Bergmeyer, J., Grabl, B.M. (Eds.), Methods of Enzymatic Analysis vol. III. Enzymes oxidoreductases. 3rd ed. Verlag-Chemie, Weinheim, pp. 273–286.

Boerma, M., Hauer-Jensen, M., 2011. Preclinical research into basic mechanisms of radiation- induced heart disease. Cardiol. Res. Pract. 2011, 1–8.

DeFuria, J., Bennett, G., Strissel, K.J., Perfield, J.W., Milbury, P.E., Greenberg, A.S., Obin, M.S., 2009. Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. J. Nutrition 1139 (8), 1510–1516.

Del Bo, C., Roursgaard, M., Porrini, M., Loft, S., Møller, P., Riso, P., 2016. Different effects of anthocyanins and phenolic acids from wild blueberry (Vaccinium angustifolium) on monocytes adhesion to endothelial cells in a TNF-α stimulated proinflammatory environment. Mol. Nutrition Food Res. 1 (60 (11)), 2355–2366.

Di Maggio, F.M., Minafra, L., Forte, G.I., Cammarata, F.P., Lio, D., Messa, C., Gilardi, M. C., Bravatà, V., 2015. Portrait of inflammatory response to ionizing radiation treatment. J. Inflammation 12 (1), 14. 18.

El-Missiry, M.A., Fayed, T.A., El-Sawy, M.R., El-Sayed, A.A., 2007. Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicol. Environ. Saf. 66 (2), 278–286. 28.

Esposito, D., Chen, A., Grace, M.H., Komarnytsky, S., Lila, M.A., 2014. Inhibitory effects of wild blueberry anthocyanins and other flavonoids on biomarkers of acute and chronic inflammation in vitro. J. Agric. Food Chem. 62 (29), 7022–7028. 17.

Ferlemi, A.V., Lamari, F.N., 2016. Berry leaves: an alternative source of bioactive natural products of nutritional and medicinal value. Antioxidants 5 (2), 17. 1.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139. 25.

Hosseinimehr, S.J., Tavakoli, H., Pourheidari, G., Sobhani, A., Shafiee, A., 2003. Radioprotective effects of citrus extract against γ-irradiation in mouse bone marrow cells. J. Radiation Res. 44 (3), 237–241.

Janknegt, P.J., Rijstenbil, J.W., VandePoll, W.H., Gechev, T.S., Buma, A.G., 2007. A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae. J. Photochem. Photobiol. B: Biol. 87 (3), 218–226. 26.

Kalra, E.K., 2003. Nutraceutical-definition and introduction. The AAPS J. 5 (3), 27–28. 1.

Kalt, W., Foote, K., Fillmore, S.A., Lyon, M., Van Lunen, T.A., McRae, K.B., 2008. Effect of blueberry feeding on plasma lipids in pigs. Br. J. Nutrition 100 (1), 70–78. 1.

Kalpana, K.B., Devipriya, N., Thayalan, K., Menon, V.P., 2010. Protection against X-ray radiation induced cellular damage of human peripheral blood lymphocytes by an aminothiazole derivative of dendrodoine. Chem. Biol. Interact. 186, 267–274.

Kamiński, Z.W., Jezewska, M.M., 1979. Intermediate dehydrogenase-oxidase form of xanthine oxidoreductase in rat liver. Biochem. J 181 (1), 177–182. 1.

Li, N., Karin, M., 1999. Is NFkB the sensor of oxidative stress? FASEB J. 13, 137–143.

Liu, S.X., Yang, H.Y., Li, S.Y., Zhang, J.Y., Li, T., Zhu, B.Q., Zhang, B.L., 2015. Polyphenolic compositions and chromatic characteristics of bog bilberry syrup wines. Molecules 20 (11), 19865–19877. 4.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (−Delta Delta C (T)) Method. Methods 25, 402–408.

Mahmoud, K., 1996. Response of Certain Biochemical Parameters to Whole Body Gamma Irradiation in Albino Rats and Synergestic Effect of Pharmacological Agent. Fac Sci Alex Univ Alexendria, PhD.

Mansour, H.H., 2006. Protective role of carnitine ester against radiation-induced oxidative stress in rats. Pharmacol. Res. 54(3), 165–171. 30.

Mattson, M.P., Culmsee, C., Yu, Z., Camandola, S., 2000. Roles of nuclear factor κB in neuronal survival and plasticity. J. Neurochem. 74(2), 443–456. 1.

Nada, A.S., 2008. Modulating efficacy of rosemary extracts in rats exposed to oxidative stress. Egypt. J. Radiation Sci. Appl. 21 (2), 499–514.

Neto, C.C., 2007. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. Mol. Nutrition Food Res. 51 (6), 652–664. 1.

Okamura, T., Miura, T., Takemura, G., Fujiwara, H., Iwamoto, H., Kawamura, S., Kimura, M., Ikeda, Y., Iwatate, M., Matsuzaki, M., 2000. Effect of caspase inhibitors on myocardial infarct size and myocyte DNA fragmentation in the ischemia–reperfused rat heart. Cardiovascular Res. 45 (3), 642–650. 1.

Osman, N., Adawi, D., Ahrné, S., Jeppsson, B., Molin, G., 2007. Endotoxin-and D-galactosamine-induced liver injury improved by the administration of Lactobacillus, Bifidobacterium and blueberry. Digestive Liver Dis. 39 (9), 849–856. 30.

Pang, W., Jiang, Y.G., Yang, H.P., Cheng, D.M., Lu, H., Lu, S.J., 2010. Cytoprotective effect of blueberry extracts against oxidative damage of rat hippocampal neurons induced by H2O2. Zhongguo ying yong sheng li xue za zhi= Zhongguo yingyong shenglixue zazhi= Chin. J. Appl. Physiol. 26 (1), 51–54.

Park, J.E., Yang, J.H., Yoon, S.J., Lee, J.H., Yang, E.S., Park, J.W., 2002. Lipid peroxidation-mediated cytotoxicity and DNA damage in U937 cells. Biochimie 84 (12), 1198–1204. 1.

Ramadan, L.A., Shouman, S.A., Sayed-Ahmed, M.M., Ola, H., 2001. Modulation of radiation-induced organs toxicity by cremophor-el in experimental animals. Pharmacol. Res. 43 (2), 185–191. 1.

Said, R.S., Nada, A.S., El-Demerdash, E., 2012. Sodium selenite improves folliculogenesis in radiation-induced ovarian failure: a mechanistic approach. PloS one. 7, (12). 6 e50928.

Takikawa, M., Inoue, S., Horio, F., Tsuda, T., 2010. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. J. Nutrition 140 (3), 527–533. 1.

Vendrame, S., Daugherty, A., Kristo, A.S., Klimis-Zacas, D., 2014. Wild blueberry (Vaccinium angustifolium)-enriched diet improves dyslipidaemia and modulates the expression of genes related to lipid metabolism in obese Zucker rats. Br. J. Nutrition 111 (2), 194–200.

Wang, Y.P., Cheng, M.L., Zhang, B.F., Mu, M., Zhou, M.Y., Wu, J., Li, C.X., 2010. Effect of blueberry on hepatic and immunological functions in mice. Hepatobiliary Pancreat Dis Int. 9 (2), 164–168. 15.

Wei, X., Wang, D., Yang, Y., Xia, M., Li, D., Li, G., Zhu, Y., Xiao, Y., Ling, W., 2011. Cyanidin-3-O-β-glucoside improves obesity and triglyceride metabolism in KK-Ay mice by regulating lipoprotein lipase activity. J. Sci. Food Agric. 1;91(6), 1006–1013.

Yasemin, B., Cihan, A.O., Suleyman, S., Gokalp, E., 2013. Rotective role of royal jelly against radiation-induced oxidative stress in rats international. J. Hematol. Oncol. 23 (2), 79–87.

Yuan, Z., Zhang, J., Tu, C., Wang, Z., Xin, W., 2016. The protective effect of blueberry anthocyanins against perfluorooctanoic acid-induced disturbance in planarian (Dugesia japonica). Ecotoxicol. Environ. Saf. 31 (127), 170–174.