*Bulgarian Chemical Communications, Volume 52, Special Issue D (pp. 84-88) 2020*

# Protective effect of gamma-irradiated extract of *Aronia Melanocarpa* L. in the gastro-intestinal tract of healthy mice models

G. D. Nikolova[1]*, Y. D. Karamalakova[1], S. Momchilova[3], V. Kancheva[3], P. Denev[4], A. Tolekova[2], V. G. Gadjeva[1]

[1] *Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria*

[2]*Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria*

[3]*Department of Lipid Chemistry, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., 9, 1113 Sofia, Bulgaria*

[4]*Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Science, 139 Ruski Blvd., 4000 Plovdiv, Bulgaria*

Received: February 27, 2020; Accepted: June 23, 2020

Over the last 20 years, antioxidant "food" therapy has been introduced as an effective method of reducing oxidative damage caused by various external factors. The antioxidants contained in *Aronia melanocarpa* L. berries can suppress oxidation reactions and regulate oxidative changes even in gastrointestinal tract diseases (GIT).

The aim of the study was to examine the influence of gamma ray irradiation at doses of 10 kGy and 25 kGy on the extract from dried berries of *A. Melanocarpa* L. on an experimental model of healthy mice, for its antioxidant activity and protective effect on the gastrointestinal tract. For this purpose, some markers of oxidative stress, such as reactive oxygen species (ROS) and nitric oxide NO· radicals were studied by EPR spectroscopy, before and after irradiation. The study was carried out with 24 mice divided in four groups: control group (n=6); 30% ethanol extract of non-irradiated *A. Melanocarpa* L. (n=6); 30% ethanol extract of 10 kGy *A. Melanocarpa* (n=6); and 30% ethanol extract of 25 kGy *A. Melanocarpa* L. (n=6). There was no statistically significant increase in the NO· levels ($p < 0.9$) between the groups treated with non-irradiated and irradiated with either 10kGy or 25kGy *A. Melanocarpa* L. The same dependence was observed in the results for ROS. There was no statistically significant difference (p=0.1) between the groups treated with non-irradiated and irradiated with 10 kGy and 25 kGy *A. Melanocarpa* L. Our results showed that irradiation with 10 kGy and 25 kGy does not impair the antioxidant and protective properties of the *A. Melanocarpa* L. extracts. Non-irradiated extract and irradiated extracts had a gastrointestinal protective effect.

**Keywords:** GIT, NO, ROS, oxidative stress, *Aronia Melanocarpa* L.

## INTRODUCTION

Since the mid-20th century, there have been many researches related to the safety of irradiated food. Most provide only a small link in the evidence chain, but some provide key evidence concluding that pasteurization (with less harmful effects) may be an alternative method of food preservation [1]. It is well known that the dose of radiation applied to a food product is measured in kilogray (kGy). Under standard conditions for the storage of dried spices and herbs, a range of $^{60}$Co irradiation is applied [2]. The gamma irradiation method is permitted for the decontamination of dried herbs and vegetable spices with a maximum total average absorbed dose of up to 10 kGy [3]. Exposure to gamma radiation at doses below 10 kGy has reduced the content of non-sporulating pathogens [1]. When the exposure to gamma irradiation is at doses above 10 kGy, it is effective for increasing food safety by reducing the pathogenic microorganisms and extending the shelf life of food by removing the microorganisms responsible for food spoilage. The gamma irradiation method is permitted for decontamination of dried aromatic herbs, spices and vegetable spices with a maximum total average absorbed dose of 10 kGy, but this limit has been raised by the FDA to doses of 30 kGy for these products [4]. Since every step of spices and herbs storage influences the final result of the products, the question is whether the irradiation of the starting material using ionizing radiation could possibly affect the composition of the plant material obtained and, therefore, its biological activities.

*Aronia Melanocarpa* L. fruits (*A. Melanocarpa; AM*) are widely used in the food industry for the production of juices, canned food, tinctures, fruit teas and nutritional supplements [5]. However, fresh, unprocessed *A. Melanocarpa* fruits are rarely consumed because of their bitter taste, as a result of the presence of a significant amount of polyphenols.

* To whom all correspondence should be sent:
E-mail: gnikkolova@gmail.com

In addition, the fruits are a rich source of anthocyanins, which include cyanidin 3-glucoside, 3-galactoside, 3-xyloside, 3-arabinoside, pelargonidine-3-galactoside and pelargonidine-3-arabinoside [3]. Polyphenols are biofactors that determine the high activity of *A. Melanocarpa*. Moreover, there is evidence that *A. Melanocarpa* fruits have the potential to inhibit the development of various cancers, and can suppress oxidation reactions and regulate oxidative changes even in gastrointestinal tract diseases (GIT) [3, 4].

The main goal of the herein reported study was to investigate whether gamma irradiation at doses of 10 kGy and 25 kGy influence the antioxidant activity of the extract from dried berries of *A. Melanocarpa L*. on an experimental model of healthy mice. For this purpose, we studied the oxidative stress biomarkers levels, reactive oxygen species (ROS), and nitric oxide NO• radicals by EPR spectroscopy, before and after irradiation. Possible protective effect of the extract on the gastrointestinal tract of the studied mice was also discussed.

## EXPERIMENTAL

### *Materials*

Carboxy PTIO.K potassium salt, dimethyl sulfoxide (DMSO), and other (HPLC grade) solvents and reagents were purchased from Sigma-Aldrich (Steinheim, Germany).

### *Animals*

The study was carried out with 24 mice divided in four groups: Control group (n=6); 30% *A. melanocarpa* non-irradiated (n=6); 30% 10 kGy *A. melanocarpa* (n=6); and 30% 25 kGy *A. melanocarpa* (n=6).

### *Black chokeberry fruits*

Black chokeberry fruits were supplied from Vitanea Ltd. (Plovdiv, Bulgaria) in the stage of full maturity, in 2017. Fresh fruits were frozen at -18˚C, lyophilized (Christ Alpha 1-4 LDplus, Martin Christ GmbH, Germany) and stored in a desiccator until use.

### *Gamma-irradiation of dried A. Melanocarpa fruits*

Freeze dried berries were irradiated at a cobalt-60 source with 8200 Ci activity. The chosen absorbed dose was 10 kGy and 25 kGy. All gamma-irradiated samples and untreated controls were pulverized and 0.5 g was mixed with 40 ml of extract - 30% ethanol acidified with 0.5% formic acid.

Samples were extracted on a magnetic stirrer for 1 h at room temperature [6]. The total polyphenol content of the investigated samples varied between 6935 ± 79 mg/100 g DW and the total content of anthocyanins was in the range of 1192 ±mg/100 g DW, at 10 kGy, as reference [7].

For the experiment IRC/w non-inbred albino male mice (25±1.5 g) were used. The mice were housed in polycarbonate cages in controlled conditions (12 h light/dark cycles), at a temperature of 18-23ºC and humidity of 40-70%, with free access to tap water and standard laboratory chow at the Suppliers of Laboratory Animals in the Medical Faculty, Trakia University.

The animal study was approved with Directive 2010/63/EU/ Ethical Committee for Animals of BABH and Trakia University, Stara Zagora, Bulgaria (131/6000-0333/09.12.2016). The experimental animals were randomly assigned to four groups, each of 6 mice: control, non-irradiated 30% ethanol AM extract, 10 kGy 30% ethanol AM extract and 25 kGy 30% ethanol AM extract. The mice were treated orally in the acute experiment according to Eftimov *et al.* [8].

The control group was pretreated orally with saline solution (30 mL/kg) for 2 h. The non – irradiated group was treated with 30% ethanol AM extract. Both irradiated groups - 10 kGy and 25 kGy were pretreated with the respective extract at a dose of 30 mL/kg of 30% ethanol AM extract. The mice were anesthetized and euthanized 2 h after the treatment. The freshly isolated gastrointestinal tract was collected on ice and homogenized.

The electron paramagnetic (EPR) measurements were performed on an X-Band, Emx$^{micro}$ spectrometer (Bruker, Germany). Spectral processing was performed using Bruker WIN-EPR and SimFonia software.

### *Levels of ROS*

The levels of ROS were determined according to Shi *et al*. [9] modified by us. The levels of ROS were calculated as double integrated plots of EPR spectra and results were expressed in arbitrary units.

### *NO• radicals*

Based on the methods published by Yoshioka *et al.* [10] and Yokoyama *et al.* [11] we developed and adapted the EPR method for estimation of the levels of NO• radicals. The levels of NO radicals were calculated as double integrated plots of EPR spectra and results were expressed in arbitrary units.

### *Statistical analysis*

Statistical analysis was performed with Statistica 7, StaSoft, inc. and the results were expressed as means ± S.E. $p < 0.05$ was considered statistically significant. To define which groups are different from each other we have used LSD post hoc test.

## RESULTS AND DISCUSSION

In recent years it has become very popular to use herbs as medicines in a significant proportion of cases for the treatment or prevention of digestive disorders. Regular consumption of fruits, herbs, vegetables and plant foods rich in antioxidants is associated with improving overall health and reducing the incidence of chronic diseases of the cardiovascular system, gastrointestinal tract and other [11, 12]. The plant antioxidants can scavenge the free radicals, unstable and reactive forms with an unpaired electron in the outer orbit. Free radicals can cause damage to the cells of the human body, inducing "oxidative stress" that leads to various chronic diseases. Some antioxidants have been able to provide hydrogen radicals and thus can act as scavengers of free radicals. Therefore, these compounds may prevent some crucial points in the development of the disorders [13]. The positive health effects from regular intake of plant foods are partly due to the presence of vitamins, minerals and fibers, but mostly due to the non-nutritional biologically active compounds such as polyphenols.

Phenolics are required for pigmentation, growth, resistance to pathogens and many other functions in plants. In the human body, phenols have a beneficial effect on health. Some of the reasons for the protection are their powerful antioxidant properties and free radical scavenging activity.

*A. Melanocarpa* berries are an extremely rich source of bioactive components, such as polyphenols, in particular anthocyanins and flavonoids, which are estimated to be 2-3 times higher than in comparable fruits, and considered as a very important nutritional antioxidant [14]. Phenolic compounds are a product of plant secondary metabolism. They are very different in structure and can be found in plants as simple molecules, such as phenolic acids, or as highly polymerized molecules, such as proanthocyanidins. A lot of studies indicated the high antiradical activity of chokeberry. Polyphenols have been shown to possess significant antioxidant properties by directly removing reactive oxygen species (ROS) and inducing cellular antioxidant systems to support in the fight against the oxidative damages.

In the present study, EPR methods were used to record free radicals in the samples tested. The results for the ROS levels (Fig. 1) in the studied groups showed a statistically significant difference only between the control group (p=0.000) and all groups treated with *A. Melanocarpa* (non–irradiated and irradiated at a dose of 10 kGy or 25 kGy).

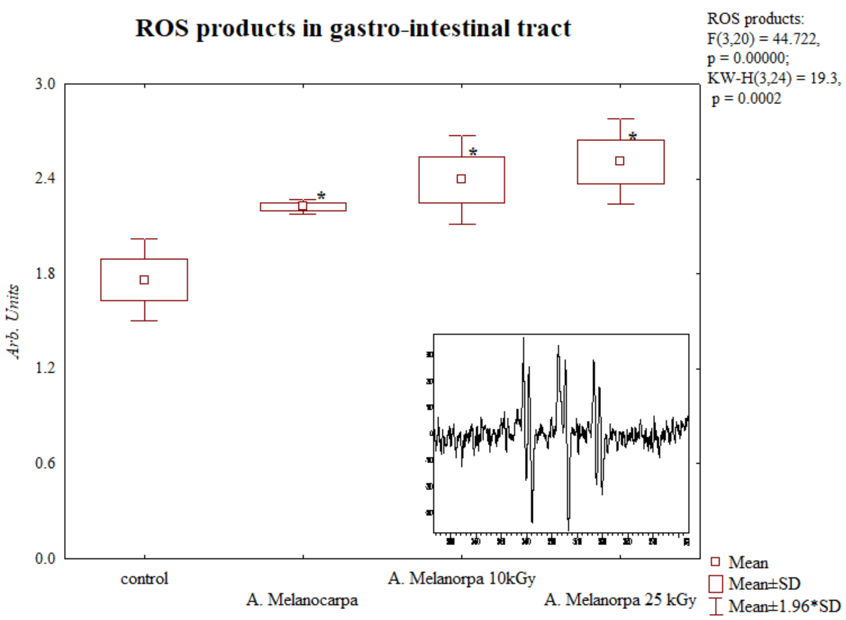


**Fig. 1.** ROS levels in the gastro-intestinal tract. Significant difference *p < 0.05 *vs* controls. The Scheffe post hoc test was used to determine the groups differing from each other.

Moreover, there was no statistically significant difference between the *A. Melanocarpa*-treated group (mean 2.20±0.05) and the groups treated with 10 kGy (mean 2.44 ± 0.07) and 25 kGy (mean 2.50 ± 0.06) *A. Melanocarpa* extracts.

Over the irradiation process, *A. Melanocarpa* is exposed to a controlled amount of radiation from a radioactive source, such as cobalt-60. Gamma rays penetrate the extracts evenly, quickly killing plant poisoning bacteria, harmful parasites and insects. The dose of radiation and the amount of radiation energy absorbed by black chokeberry are the most critical factors in the irradiation. Polyphenols without toxicity or side effects can help prevent and reduce the level of oxidative damage caused by free radicals and ionizing radiation. Plant polyphenols have been found to have significant radioprotective effects [3].

Despite many plants are being reported to have antioxidant potential, only a few of these antioxidant activities have been confirmed or investigated after a high dose of gamma irradiation [4]. In the presented study, we also evaluated reactive nitrogen species (RNS), such as the nitric oxide radicals (NO•). The results were the same as for the ROS levels (see Fig. 2), with a statistically significant difference between the control group (mean 2.28±0.08) and all groups treated with *A. Melanocarpa* extract (mean for non–irradiated A.M. 3.55 ± 0.07; mean for 10 kGy AM 3.64 ± 0.1; and mean for 25 kGy A.M. 3.69±0.2).

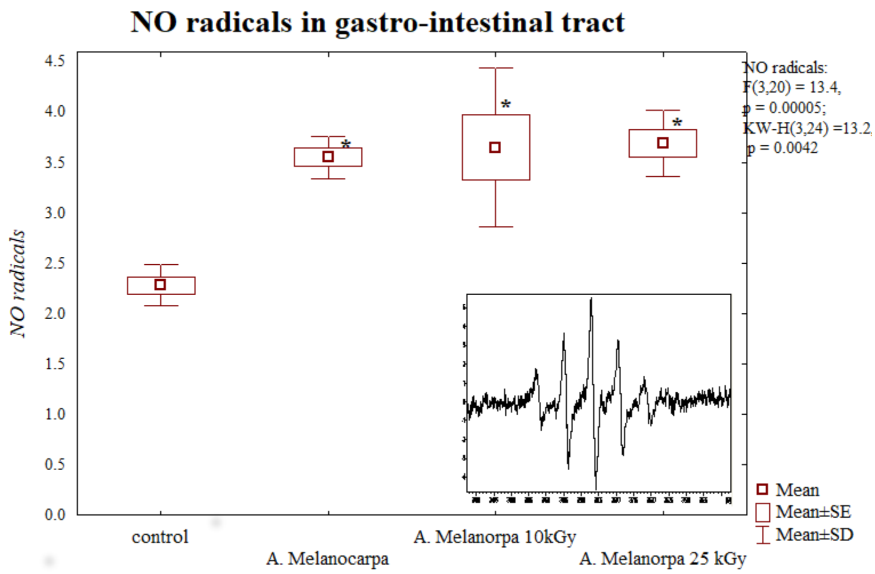


**Fig. 2.** NO radicals in the gastro-intestinal tract. Significant difference *p < 0.05 *vs* controls. The *Scheffe post hoc test* was used to determine the groups differing from each other.

Ionizing radiation induces cells to produce excessive ROS/RNS, leading to increased lipid peroxidation, disrupting redox homeostasis in cells and living tissues, and reducing the activity of enzymatic and non-enzymatic antioxidants *in vivo*. From the results presented in this study for levels of NO radicals and ROS, there were no statistically significant differences between the non-irradiated *A. Melanocarpa* extract and those irradiated with either 10 kGy or 25 KGy. These results of ours are supported by the study of Alloun *et al.* [3] who concluded that after irradiation at doses from 5 to 30 kGy the plant polyphenols had an anti-radiation and anti-oxidative stress effect by restoring the redox balance of the system.

The role of *A. Melanocarpa* antioxidants in gastrointestinal tract (GIT) diseases is important; the food (nutrition) contains various prooxidants, and increased the levels of lipid peroxides. Thus, prooxidants are likely to cause oxidative stress in the gastrointestinal tract, gastric ulcer and develop cancer of the stomach, colon and rectum [15].

In turn, antioxidants can suppress such oxidative stress and related diseases in the GIT before being absorbed. Moreover, chokeberry juice significantly and in a dose-dependent manner reduces the number and area of indomethacin-induced gastric ulcers in rats [3, 16]. In addition, administration of indomethacin resulted in extensive lipid peroxidation, indicating that ROS product and NO radicals are involved in the development of mucosal damage [17, 18]. The polyphenols from *A. Melanocarpa* are the least well absorbed. Aronia composition has significantly high concentrations of chlorogenic and non chlorogenic acids, but esterification impairs their intestinal absorption. At the same time, there is considerable experimental evidence of the efficacy of chokeberry products in a wide range of pathological conditions mediated by uncontrolled oxidation processes [1, 19]. Furthermore, the first site of the antioxidant action of *A. Melanocarpa* polyphenols is the gastrointestinal tract, where proanthocyanidins and their metabolites might act as radical scavengers [20]. The mechanisms of the *in vivo* antioxidant activity of *A. Melanocarpa* polyphenols after absorption extend far beyond radical scavenging and include inhibition of ROS and RNS formation, prooxidant inhibition, and restoration of antioxidant enzymes and possibly cellular signaling to regulate the levels of enzyme and antioxidant compounds [1]. Moreover, it might be hypothesized that irradiation at 10kGy and 25kGy does not impair the antioxidant and protective properties of the *A. Melanocarpa* extract.

## CONCLUSION

From the presented results we can conclude that irradiation at doses of 10 kGy and 25 kGy does not impair the antioxidant properties of the *A. Melanocarpa* extract. We suppose that non-irradiated extract *A. Melanocarpa* L. and irradiated at doses of 10 kGy and 25 kGy *A. Melanocarpa* L. extracts might be used as gastrointestinal tract protectors.

***Acknowledgements:*** *This work was supported by the scientific projects № 5/2017 and 1/2018 Medical Faculty, Trakia University and from the National Science Fund of Bulgaria, Grant DN19/14 from 12.12.2017.*

***Conflict of Interest:*** *The authors declare that they have no conflict of interest.*

## REFERENCES

1. P. N. Denev, C. G. Kratchanov, M. Ciz, A. Lojek, M. G. Kratchanova. *Comprehensive Rev. in Food Sci. and Food Safety*, **11** (2012).
2. D. M. Kasote, S. S. Katyare, M. V. Hegde, H. Bae, *Int. J. Biol. Sci*., **11**(8) (2015**).**
3. . Alloun, O. Benchabane, M. Hazzit, F. Mouhouche, A. Baaliouamer, A. Chikhoune, A. Benchabane, *Acta Chromatographica*, **31**(1), 57 (2019).
4. A. Sidor, A. Drożdżyńska, A. Gramza-Michałowska, *Trends in Food Sci. & Technol.*, **89,** 45 (2019).
5. C. D. Caulfeld, J. P. Cassidy, J. P. Kelly, *J. Am. Assoc. for Lab. Animal Sci*., **47**(6), 61(2008).
6. P. Denev, M. Kratchanova, I. Petrova, D. Klisurova, Y. Georgiev, M. Ognyanov, I. Yanakieva, *Hindawi: Journal of Chemistry,* Article ID 9574587, 11 pages, (2018).
7. P. Denev, D. Klisurova, D. Teneva, M. Ognyanov, Y. Georgiev, S. Momchilova, V. Kancheva, *Bulg. Chem. Commun*., **51(**A), 270 (2019).
8. M. Eftimov, St. Valcheva-Kuzmanova, *J. Biomed.*, **11**, 77 (2018).
9. H. Shi, Y. Sui, X. Wang, Yi. Luo, L. Ji, *Comp. Biochem Phys. - Part C, Toxic. & Pharm*. **140**, 115 (2005).
10. T. Yoshioka, N. Iwamoto, K. Lto, *J. Am. Soc. Nephrol*. **7**, 961 (1996).
11. K. Yokoyama, K. Hashiba, H. Wakabayashi, K. Hashimoto, K. Satoh, T. Kurihara, N. Motohashi, H. Sakagami, *Anticancer Res*., **24**, 3917 (2004).
12. M. Ekor, *Frontiers in Pharmacology*, **4**, 177 (2014).
13. H. Yuan, Q. Ma, L. Ye, G. Piao, *Molecules,* **21**(5), 559 (2016).
14. L. Jakobek, M. Šeruga, P. Krivak, *Int. J. Food. Sci. Nutrition*, **62**(4), 345 (2011).
15. S. E. Kulling, H. M. Rawel, *Planta Medica*, **74**(13), 1625 (2008).
16. M. J. Bermúdez-Soto, F. A. Tomás-Barberán, M. T. García-Conesa, *Food Chem*., **102**(3), 865 (2007).
17. N. Martins, L. Barros, I. C. Ferreira, *Trends in Food Sci & Technol*, **48**, 1-12 (2016).
18. H. Liao, M. Zhu, Y. Chen, *Trends in Food Sci. & Technol.* (2019).
19. C. Chrubasik, G. Li, S. Chrubasik, *Phytotherapy Res.*, **24**(8), 1107 (2010).
20. A. Speciale, F. Cimino, A. Saija, R. Canali, F. Virgili, *Genes & Nutrition*, **9**(4), 404 (2014).