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Antioxidant activity and radioprotective effects of oral administration of vitis vinifera L. seed in wistar rats following fractionated whole-brain irradiation.

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► ***Original article***

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ABSTRACT

Background: Chronic oxidative stress is hypothesized to precede radiation-induced brain injury in irradiated brains. This study investigated the antioxidant activity and radioprotective effects of Vitis vinifera L. seed in Wistar rats following fractionated whole-brain irradiation. ***Materials and Methods:*** Seventy-two young adult male Wistar rats were randomly selected and divided into three equal groups. Group A was the control group, while Groups B and C received a total dose of 40 Gy of ^{60}Co gamma radiation to the head. The radiation dose was fractionated at 5 Gy per exposure. In addition, group C rats were orally administered 100 mg/kg/day of Vitis vinifera L. seeds starting from one week before exposure and lasting until the time of sacrifice. At weeks 1, 2, 3, and 4 post-irradiations, the average brain weights, total brain protein concentration, and superoxide dismutase activity of the rat brains were determined. ***Results:*** The study recorded significant reductions in the average brain weights, total brain protein concentration, and brain superoxide dismutase activity in group B rats when compared to groups A and C ($P < 0.05$). However, the study found that group C rats had a higher total brain protein concentration ($P > 0.05$), correlative average brain weight ($P > 0.05$), and a significant increase in brain superoxide dismutase activity ($P < 0.05$) when compared with group A rats. ***Conclusions:*** Vitis vinifera L. seed has antioxidant and radioprotective effects on irradiated rat brains.

INTRODUCTION

Electromagnetic radiation is commonly used to treat brain malignancies [1,2]. However, this treatment can cause brain injury induced by ionizing radiation [3,4]. The severity of the injury can range from mild short-term memory loss to severe cognitive disabilities that can be life-threatening [5,6]. Prolonged exposure to high levels of electromagnetic radiation can impair the ability of cells to repair themselves [7]. This occurs because the radiation depletes the cells' natural antioxidant enzymes, causing chronic oxidative stress [8]. As a result, reactive oxygen species (ROS) produced by the photons can interact with cells and damage important organelles within them [9]. This can eventually lead to mutations that compromise the cell's functional phenotype [7]. Therefore, radiation-induced brain injury is often preceded by chronic oxidative stress [10]. Interventions that aim to reduce free radical cell fixation have been suggested to limit radiation-induced brain injury during treatment [9]. Mammalian cells contain enzymes known as superoxide dismutase (SOD), which play an essential role in eliminating harmful superoxide radicals [11]. SODs are the primary defense mechanisms against oxygen radicals in human cells [12,13]. The harmful effects of electromagnetic radiation-cell interaction and the consequent role of free radicals in radiation-induced brain injuries highlight the need for a readily available natural free radical scavenger that can help ameliorate ROS.

Vitis vinifera L., the common grape vine, originates from the Mediterranean region, southwestern Asia, and central Europe [14]. The seed of the plant is known for its antioxidative properties [16,17], which are attributed to the presence of active components such as flavonoids, proanthocyanidins, procyanidins, polyphenols, and anthocyanins [18,19]. Studies have revealed that these constituents are more effective in scavenging free radicals when compared to vitamins C, E, and carotene [20]. In a study by Castillo *et al.* [21] Vitis vinifera L. seed was reported to prevent chromosomal damage induced by X-ray in mice exposed to a dose of 48 cGy. A study by Devi *et al*. showed that administering Vitis vinifera L. seed significantly reduced the incidence of lipid peroxidation in the central nervous system of aged rats [22]. Vitis vinifera L. seed was reported to protect the heart and pancreas tissues of rats exposed to 5 Gy of gamma radiation from oxidative damage induced by ionizing irradiation [23]. In another study, Muresan

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et al. found that Vitis vinifera L. seed enhanced the plasma antioxidant capacity in pregnant Wistar rats [24]. Similarly, Ragab *et al*. showed that Vitis vinifera L. seed inhibited oxidative stress in rats' livers treated with Carbon Tetrachloride [25]. In addition, Vitis vinifera L. seed extracts were found to inhibit vascular impairment induced by oxidative stress in the pulmonary arteries of rats with diabetes [26].

Although a preponderance of evidence supports the hypothesis that Vitis vinifera L. seed is an antioxidant, there is a significant lack of research on its effects as an antioxidant on brains exposed to electromagnetic radiation. Hence, this study aims to investigate the antioxidant and radioprotective effects of Vitis vinifera L. seed in Wistar rats that have undergone fractionated whole-brain irradiation (fWBI). The findings of our work might provide evidence for a naturally available ROS scavenger that will assist the brain in its effort to mitigate radiation-induced brain injuries.

MATERIALS AND METHODS

Animal care and selection

Breeder Wistar rats were obtained from the animal facility at the Faculty of Basic Medical Sciences, Obafemi Awolowo University, Nigeria. The rats were allowed to mate and produce litters. Seventy-two male litters were weaned during the fourth week and nurtured until they weighed between 100-150g. The rats were randomly assigned to three different groups, namely groups A, B, and C, and were housed in well-ventilated plastic cages. They were exposed to natural light and dark cycles and were provided with pelleted rat chow and water ad libitum. Ethical approval with registration number SCP14/15/H/0661 was obtained from the Health Research and Ethical Committee of Obafemi Awolowo University, Ile-Ife-Nigeria on 3rd February 2016.

Study design

Seventy-two young adult male Wistar rats that weigh between 100-150 grams were randomly selected and divided into three groups of 24 rats each. The control animals were placed in group A while groups B and C were exposed to 40 Gy of gamma radiation from a ^{60}Co source. The radiation doses were delivered in 8 fractions at 5 Gy per exposure for four weeks. In addition to the ^{60}Co gamma radiation, group C rats were administered 100 mg/kg/day of Vitis vinifera L. seed. The Vitis vinifera L. seed was encapsulated in gelatin and was purchased from Swanson Health Products in Fargo, United States of America. Each Vitis vinifera L. seed capsule was emptied and dissolved in distilled water and administered to the rats through an oral cannula. The Vitis vinifera L. seed was administered daily from one week before radiation exposure and lasted until the time of sacrifice. At weeks 1, 2, 3, and 4 post-irradiations, six rats were randomly selected from each group and humanely sacrificed by cervical dislocation. The brains were then harvested, and the rat brain weight, quantification of total brain protein concentration, and the specific superoxide dismutase activities of the rat brains were routinely determined. The study utilized reagents that were procured from Sigma-Aldrich Co, USA.

Irradiator

The GAMMA BEAMX200 research irradiator manufactured by Best Theratronics Ltd. in Ontario, Canada was used for this study. It is located at the National Institute of Radiation Protection and Research at the University of Ibadan in Nigeria. The irradiator uses 60Co gamma radiation with a source-to-surface distance of 80 cm and a field size of 10 × 10 cm2.. The source's activity during irradiation was 292.3 TBq. The dose rates for each irradiation procedure were recorded as 15.11 mGy/s, 15.101 mGy/s, 15.090 mGy/s, 15.063 mGy/s, 15.041 mGy/s, 15.025 mGy/s, 14.993 mGy/s, 14.972 mGy/s respectively. Equation (1) was used to calculate the irradiation time for each procedure.

$$\text{Irradiation time} = \frac{\text{radiation dose}}{\text{dose rate}} \quad (1)$$

During irradiation, improvised cushioned wooden rails were manually designed to position the rats. Lead blocks were used to shield their bodies, which attenuated the absorbed dose to 263 mSv.

Protein determination

The Bradford method [36] was employed to determine the protein concentration. We added 0.05 ml of our enzyme sample to 5 ml of Bradford reagent, which contains 100 mg of Coomassie Brilliant Blue C-250 dissolved in 250 ml of 95% ethanol and 100 ml of 85% (w/v) phosphoric acid (Sp. gr. 1.75 g/ml). We then diluted the final solution to a volume of 1 liter with distilled water.

The spectrophotometer, manufactured by Thermo Fisher Scientific Inc, USA was used to measure the absorbance at 595 nm immediately. The protein concentration was determined by extrapolating from a standard curve using bovine serum albumin.

Superoxide dismutase activity (SOD) determination

We used the Mccord and Fridovich method [37] to measure the antioxidant activity. We added 30 mM EDTA, 2 mM of pyrogallol, and 75 milliliters of tris HCL buffer (pH = 8.2). The spectrophotometer recorded the increase in absorption at 420 nm for 3 minutes. A unit of enzyme activity is defined as the amount of enzyme that causes 50% inhibition of the rate of antioxidant activity of pyrogallol, which is determined by the change in absorbance per minute

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at 420 nm. Equation (2) represents an expression for evaluating the increase in absorbance per minute while equation 3 represents an expression for evaluating the % inhibition. The activity of SOD is expressed as γ mg/ml.

$$\text{Increase in absorbance per minute} = \frac{A_3 - A_0}{2.5} \quad (2)$$

Where A_o is the absorbance at 30 s and A_3 is the absorbance at 150 s.

$$\% \text{ inhibition} = 100 - \left(100\left(\frac{\text{increase in absorbance from substrate}}{\text{increase in absorbance for blank}}\right)\right) \quad (3)$$

A unit of SOD activity is the amount necessary to inhibit the oxidation of adrenaline by 50%, as shown in equation 4.

$$\gamma = \left(\frac{\text{absorbance}}{0.0028 * 0.1 * 1000}\right) (\gamma \text{ mg/ml}) \quad (4)$$

The specific superoxide dismutase activity was calculated by dividing the measured superoxide dismutase activity by the total protein concentration.

Statistical analysis

We presented the data as the mean with the standard error of the mean. To investigate the statistical significance, we performed a *t-test* using Stata (Version 12, USA) and a one-way analysis of variance (ANOVA) using GraphPad Prism (Version 5.03, GraphPad Inc, USA). A p-value of less than 0.05 was chosen to indicate a significant difference between the two groups.

RESULTS

The effects of 40 Gy fractionated irradiation on Rat brain weights in the presence or absence of Vitis vinifera L. seeds

The average weight of the brains in each group of rats was measured and recorded for four weeks after irradiation, as shown in table 1. Data analysis revealed significant differences in the average brain weight between groups A and B (P = 0.0033) and between groups B and C (p = 0.028). However, no significant difference was found in the average brain weight between groups A and C rats. The weekly comparison of the average brain weights within each rat group showed no significant difference, except in group C rats, where a significant difference was observed between week 1 and week 4 (p = 0.0032). Over the four-week study period, the results showed that group A rats had the highest average brain weight, while group B rats had the lowest.

The effects of 40 Gy fractionated irradiation on Rat brain protein concentration in the presence or absence of Vitis vinifera L. seeds

The protein concentration of the brains of all rat groups was measured and recorded at weeks 1, 2, 3, and 4 after irradiation (as shown in table 2). The data revealed variations in the average total brain protein concentration values for all the rat groups. Comparing groups, A and B rats showed significant differences at week 2 (p = 0.011). Significant differences were also recorded at week 1 (p = 0.016), week 2 (p = 0.030), and week 4 (p = 0.019) in group B rats when compared with group C rats. However, no significant difference was found in group A rats as compared to group C rats (p = 0.102). During the 4-week study period, the data showed that group C rats had the highest average total brain protein concentration value, while group B rats had the lowest.

Table 1. Average brain weight of rats in the presence (Group C) or absence (Groups A and B) of Vitis vinifera L. seed and exposure to 40 Gy fractionated whole-brain gamma irradiation (Groups B and C) from a ^{60}Co radiation source. The data represents the average brain weights recorded over four weeks' post-irradiation.

Weeks	Group A	Group B	Group C
Week 1	1.750±0.050	1.700±0.014	1.700±0.012
Week 2	1.755±0.002	1.700±0.050	1.750±0.038
Week 3	1.800±0.050	1.700 ±0.067	1.750±0.020
Week 4	1.800 ±0.031	1.750±0.050	1.800±0.048

Table 2. Average total brain protein concentration (mg/ml) in rat models in the presence (Group C) or absence (Groups A and B) of Vitis vinifera L. seed and exposure to 40 Gy fractionated whole-brain gamma irradiation (Groups B and C) from a ^{60}Co radiation source. The data represents the average brain weights recorded over four weeks' post-irradiation.

Weeks	Group A	Group B	Group C
Week 1	2.950±0.026	2.820±0.030	3.130±0.030
Week 2	3.130±0.018	2.890±0.012	3.135±0.005
Week 3	3.180±0.008	2.925±0.015	3.143±0.021
Week 4	3.200±0.021	2.980±0.020	3.305±0.015

The effects of 40 Gy fractionated irradiation on the specific superoxide dismutase activity of Rat brain, in the presence or absence of Vitis vinifera L. seeds

The study quantified the specific superoxide dismutase activity (SOD) of rat brains at weeks 1, 2, 3, and 4 post-irradiations (as shown in figure 1). The results revealed that group B had lower values of specific SOD activities across the four weeks when compared to group A and C. The values of SOD activities of rats were significantly reduced in group B at week 3 (p = 0.031) and week 4 (p = 0.025). In contrast, group C had significantly higher values of specific SOD activities when compared to group A over the four-week study period (p = 0.011, 0.038, 0.027, 0.031), respectively. Furthermore, group C experienced a significant increase in the values of specific SOD activities when compared to group B across the study period (p = 0.035, 0.012, 0.018, 0.022), respectively. Overall, the data revealed that group C recorded the highest values of SOD activities, while group B recorded the lowest values of SOD activities over the four-week study period.

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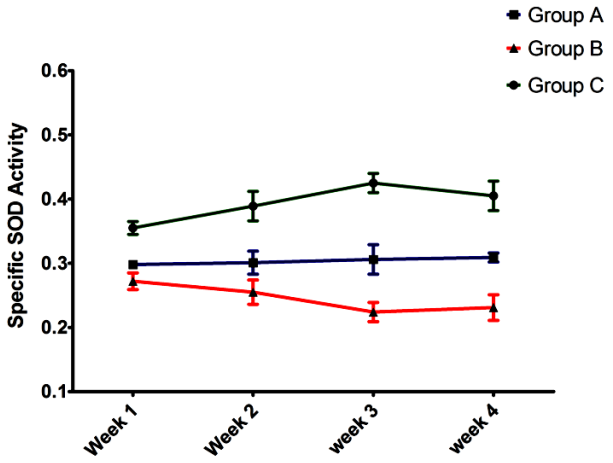

Figure 1. Specific superoxide dismutase activity of rat brains in the presence (Group C) or absence (Groups A and B) of Vitis vinifera L. seed and exposure to 40 Gy fractionated whole-brain gamma irradiation (Groups B and C) from a ^{60}Co radiation source. The data represents the average specific superoxide dismutase activity recorded over a 4-week post-irradiation.

DISCUSSION

The use of ionizing radiation has been proven effective in treating malignant brain diseases [2]. However, exposure to it can cause reversible and irreversible damage to the brain [3,4,13]. This damage occurs due to the high levels of radiation delivered in multiple fractions over a period of time [13]. The brain is a crucial organ that controls bodily functions, and any damage to it can lead to significant changes that may impact the quality of life and survival of the affected person [33,34]. Various agents, including Lithium, memantine, renin-angiotensin system blockers, peroxisomal proliferator-activated receptor agonists, small molecule compounds targeting the p53 isoform, and anti-inflammatory agents have been used to limit radiation-induced brain damage in patients [13,29,35-39]. These substances work by different mechanisms such as increasing the survival of glial cells or neurons, ameliorating the neurotoxic environment, or promoting the growth of new neurons in the hippocampus [29], radiation-induced brain damage is caused by the involvement of multiple cell types [13, 29]. As a result, there is a need for a substance that can provide comprehensive protection to various brain cells during radiation therapy. The role of free radicals and the complex interplay between different cell types in the process of radiation-induced brain damage suggest that using antioxidants may be a viable option to serve this purpose. Hence, this paper reports investigations on the anti-oxidative and radioprotective effects of *Vitis vinifera* L. seeds on irradiated rat brains.

The results of our study revealed that the exposure of rat brain cells to 40 Gy fractionated whole-brain gamma irradiation resulted in the loss of brain matter (table 1). Since the brain comprises neurons and glial cells, the loss of brain matter (groups B and C) may be attributed to radiation-induced apoptosis and necrosis [29,30]. Loss of brain matter can result in cognitive difficulties, memory loss, and impaired daily function, which have been observed in patients receiving brain radiation therapy [13,40,41]. Hence, the loss of brain matter presents a useful clinical marker for identifying patients who are at the risk of radiation-induced cognitive impairments. In addition, the results of our study showed that group C recorded higher values of average brain weights compared to group B, suggesting that Vitis vinifera L. seeds protected and preserved the brain matter of the rats. This further suggests that the Vitis vinifera L. seeds' may have mitigated ionizing-radiation effects that initiate the death of normal cells in the rat brain [25,26]. Moreover, the significant increase in the average brain weight of rats in group C at the end of the fourth week, compared to the first week, indicated that Vitis vinifera L. seeds improved the viability of the rat brain matter.

Cells depend on proteins for repair, structure and tissue building [42-44]. This study showed that exposure of rats' brain to 40 Gy fractionated whole-brain gamma irradiation resulted in reduced values of the total brain protein concentration (table 2). The reduced values of the total brain protein concentration that was recorded in group B compared to groups A and C may have occurred due to the depletion of the rat's brain proteins as a response to repairing the radiation-induced damage to the brain cells. However, the higher values of the total brain protein concentration that was recorded in group C rats, compared to group B rats, suggests that Vitis vinifera L. seeds protected against radiation-induced cell damage and hence the consequent preservation of the rat brain protein. Furthermore, the higher brain protein concentration that was recorded in group C compared to group A indicates that Vitis vinifera L. seeds stimulated protein synthesis in the rat brain. However, the weekly increase in the average total brain protein concentration value in group B shows that the radiation-induced loss of brain proteins in rats may be reversible. Our results support the hypothesis that the early delayed effects of radiation-induced brain injury may be reversible [13].

Animal models are commonly used in drug development to test the efficacy of new drugs and substances [29,45]. Several studies conducted on animals have shown that Vitis vinifera L. seeds have antioxidant properties that can prevent chromosomal damage, lipid peroxidation in the central nervous system and protect vital organs such as the heart, and liver [21-26]. In our study, we investigated the antioxidant effects of Vitis vinifera L. seeds on the

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brain by measuring the SOD activity of rat brains at different intervals after exposure to ionizing radiation. The study results showed that the exposure of rat's brain to 40 Gy fractionated whole-brain gamma irradiation resulted in reduced brain SOD activity (figure 1). The reduction in SOD activity in group B compared to Groups A and C is opined to result from the depletion of the antioxidant (SOD) proteins in the rat brain as it tries to counteract the superoxide molecules generated by electromagnetic radiation [9,11,32]. However, the higher levels of SOD activity in group C, compared to Group B, indicate that Vitis vinifera L. seeds preserved brain SOD in rats exposed to gamma radiation. Additionally, the superior levels of SOD activity that was recorded in group C rats, as compared to group A, demonstrated that Vitis vinifera L. seeds induced the production of SOD enzymes in the rat brains.

The study's major limitation is the use of a fixed dosage of 100 mg/kg/day of Vitis vinifera L. seeds. Further research is necessary to determine the optimal dosage for brain protection.

CONCLUSION

Vitis vinifera L. seeds have anti-oxidative properties and radioprotective effects on the brains of rats exposed to ionizing radiation. The anti-oxidative property is achieved by inducing the production of SOD enzymes, while the radioprotective effect is achieved by preserving the brain matter.

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Conflicts of Interest: The authors declare that there are no known competing financial interests or personal relationships that might have influenced the work reported in this study.

Ethical consideration: This study was approved by the Ethics Committee of the Obafemi Awolowo University (approval number: SCP14/15/H/0661).

Data availability statement: The data that support the findings of this study is available from the corresponding author upon reasonable request.

Author Contribution: Conceptualization: all authors. Methodology: all authors. Formal analysis: Abe A.A. Project administration: Abe A.A, Ayannuga O. A. Visualization: Abe A.A. Writing - original draft: Abe A.A. Writing - review and editing: all authors. Approval of final manuscript: all authors.

REFERENCES

1. DeNunzio NJ and Yock TI (2020) Modern radiotherapy for pediatric brain tumors. *Cancers,* ***12(6):*** *1533.*
2. Söderström H, Brocki K, Kleberg JL, Martinsson U & Ljungman G (2022) Neurocognitive functions before and after radiotherapy in pediatric brain tumor survivors. *Pediatric Neurology,* ***133:*** *21-29.*
3. Lassaletta Á, Morales JS, Valenzuela PL, Esteso B, Kahalley LS, Mabbott DJ & Calvo F (2023) Neurocognitive outcomes in pediatric brain tumors after treatment with proton versus photon radiation: a systematic review and meta-analysis. *World Journal of Pediatrics,* ***19(8):*** *727-740.*
4. Rübe CE, Raid S, Palm J & Rübe C (2023) Radiation-Induced Brain Injury: Age Dependency of Neurocognitive Dysfunction Following Radiotherapy. *Cancers,* ***15(11):*** *2999.*
5. Makale MT, McDonald CR, Hattangadi-Gluth J & Kesari S (2017) Brain irradiation and long-term cognitive disability: Current concepts. *Nature reviews Neurology,* ***13(1):*** *52.*
6. Haldbo-Classen L, Amidi A, Wu LM, Lukacova S, Oettingen GV, Gottrup H & Høyer M (2019) Long-term cognitive dysfunction after radiation therapy for primary brain tumors. *Acta Oncologica,* ***58 (5):*** *745-752.*
7. Liew H, Mein S, Debus J, Dokic I & Mairani A (2020) Modeling direct and indirect action on cell survival after photon irradiation under normoxia and hypoxia. *International Journal of Molecular Sciences,* ***21(10):*** *3471.*
8. Jiao Y, Cao F & Liu H (2022) Radiation-induced cell death and its mechanisms. *Health Physics,* ***123(5):*** *376-386.*
9. Wang B, Wang Y, Zhang J, Hu C, Jiang J, Li Y & Peng Z (2023) ROS-induced lipid peroxidation modulates cell death outcome: mechanisms behind apoptosis, autophagy and ferroptosis. *Archives of Toxicology, 1-13.*
10. Wang Y, Branicky R, Noë A & Hekimi S (2018) Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *Journal of Cell Biology,* ***217(6):*** *1915-1928.*
11. Rosa AC, Corsi D, Cavi N, Bruni N & Dosio F (2021) Superoxide dismutase administration: A review of proposed human uses. *Molecules,* ***26(7):*** *1844.*
12. Ighodaro OM & Akinloye OA (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine,* ***54(4):*** *287-293.*
13. Robbins M, Greene-Schloesser D, Peiffer AM, Shaw E, Chan MD & Wheeler KT (2012) Radiation-induced brain injury: a review. *Frontiers in Oncology*, ***2:*** *30551.*
14. Dong Y, Duan S, Xia Q, Liang Z, Dong X, Margaryan K, *et al.* (2023) Dual domestications and origin of traits in grapevine evolution. *Science,* ***379(6635):*** *892-901*
15. Adámez JD, Samino EG, Sánchez EV & González-Gómez D (2012) *In vitro* estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (Vitis vinifera L). *Food Control,* ***24(1-2):*** *136-141.*
16. Di Stefano V, Buzzanc C, Melilli MG, Indelicato S, Mauro M, Vazzana M, *et al.* (2022) Polyphenol Characterization and Antioxidant Activity of Grape Seeds and Skins from Sicily: A Preliminary Study. *Sustainability,* ***14(11):*** *6702.*
17. Pozzo L, Grande T, Raffaelli A, Longo V, Weidner S, Amarowicz R, Karamać M (2023) Characterization of Antioxidant and Antimicrobial Activity and Phenolic Compound Profile of Extracts from Seeds of Different Vitis Species. *Molecules,* ***28(13):*** *4924.*
18. Zhou DD, Li J, Xiong RG, Saimaiti A, Huang SY, Wu SX, *et al.* (2022) Bioactive compounds, health benefits and food applications of grape. *Foods,* ***11(18):*** *2755.*
19. Chengolova Z, Ivanov Y & Godjevargova T (2023) Comparison of Identification and Quantification of Polyphenolic Compounds in Skins and Seeds of Four Grape Varieties. *Molecules,* ***28(10):*** *4061.*
20. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA & Pruess HG (2000) Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology,* ***148(2-3):*** *187-197.*
21. Castillo J, Benavente-Garcia O, Lorente J, Alcaraz M, Redondo A, Ortuno A, Del Rio JA (2000) Antioxidant activity and radioprotective effects against chromosomal damage induced in vivo by X-rays of flavan-3-ols (Procyanidins) from grape seeds (Vitis vinifera): comparative study versus other phenolic and organic compounds. *Journal of Agricultural and Food Chemistry,* ***48(5):*** *1738-1745.*
22. Devi A, Jolitha AB & Ishii N (2006) Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. *Medical Science Monitor. Int Med J Exp Clin Res,* ***12(4):*** *BR124-9.*
23. Saada HN, Said UZ, Meky NH & Azime ASAE (2009) Grape seed extract Vitis vinifera protects against radiation-induced oxidative damage and metabolic disorders in rats. *Phytotherapy Research,* ***23(3):*** *434-438*
24. Mureşan A, Alb C, Suciu S, Clichici S, Filip A, Login C & Mocan T

[DOI: 10.61186/ijrr.22.3.663]

(2010) Studies on antioxidant effects of the red grapes seed extract from Vitis vinifera, Burgund Mare, Recaş in pregnant rats. *Acta Physiologica Hungarica,* ***97(2):*** *240-246.*
25. Ragab GM, El-Denshary, ES, Hassan AM, Abdel-Azeim SH, Hassan NS, Mannaa FA & Abdel-Wahhab MA (2013) Grape (Vitis vinifera) seed extract inhibits the cytotoxicity and oxidative stress in liver of rats treated with carbon tetrachloride. *Global Journal of Pharmacology,* ***7(3):*** *258-269.*
26. Pinna C, Morazzoni P & Sala A (2017) Proanthocyanidins from Vitis vinifera inhibit oxidative stress-induced vascular impairment in pulmonary arteries from diabetic rats. *Phytomedicine,* ***25:*** *39-44.*
27. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* ***72(1-2):*** *248-254.*
28. McCord JM & Fridovich I (1969) Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry,* ***244(22):*** *6049-6055.*
29. Turnquist C, Harris BT & Harris CC (2020) Radiation-induced brain injury: current concepts and therapeutic strategies targeting neuroinflammation. *Neuro-Oncology Advances,* ***2(1):*** *vdaa057.*
30. Yang X, Ren H & Fu J (2021) Treatment of radiation-induced brain necrosis. *Oxidative Medicine and Cellular Longevity, 2021.*
31. Rostom H & Shine B (2023) Basic metabolism: proteins. *Surgery (Oxford),* ***36(4):*** *153-158.*
32. Bakar NFA, Othman SA, Azman NFAN & Jasrin NS (2019, June) Effect of ionizing radiation towards human health: A review. *In IOP Conference Series: Earth and Environmental Science*, ***268 (1):*** *p. 012005; IOP Publishing.*
33. Gorgoraptis N, Zaw-Linn J, Feeney C, Tenorio-Jimenez C, Niemi M, Malik A & Sharp DJ (2019) Cognitive impairment and health-related quality of life following traumatic brain injury. *NeuroRehabilitation,* ***44(3):*** *321-331.*
34. Hshieh TT, Jung WF, Grande LJ, Chen J, Stone RM, Soiffer RJ & Abel GA (2018) Prevalence of cognitive impairment and association with survival among older patients with hematologic cancers. *JAMA oncology,* ***4(5):*** *686-693.*
35. Zanni G, Di Martino E, Omelyanenko A, *et al.* Lithium increases proliferation of hippocampal neural stem/progenitor cells and rescues irradiationinduced cell cycle arrest *in vitro*. *Oncotarget, 2015;* ***6(35):*** *37083-37097.*
36. Kale A, Piskin Ö, Bas Y, *et al.* Neuroprotective effects of Quercetin on radiation-induced brain injury in rats. *J Radiat Res, 2018;* ***59(4):*** *404-410.*
37. Moore ED, Kooshki M, Metheny-Barlow LJ, Gallagher PE, Robbins ME. Angiotensin (1-7) prevents radiation-induced inflammation in rat primary astrocytes through regulation of MAP kinase signaling. *Free Radic Biol Med, 2013;* ***65:*** *1060-1068.*
38. Cramer CK, Alphonse-Sullivan N, Isom S, *et al.* Safety of pioglitazone during and after radiation therapy in patients with brain tumors: a phase I clinical trial. *J Cancer Res Clin Oncol, 2019;* ***145 (2):*** *337-344.*
39. Kale A, Piskin Ö, Bas Y, *et al.* Neuroprotective effects of Quercetin on radiation-induced brain injury in rats. *J Radiat Res, 2018;* ***59(4):*** *404-410.*
40. Domingos C, Pêgo JM & Santos NC (2021) Effects of physical activity on brain function and structure in older adults: A systematic review. *Behavioural Brain Research*, ***402:*** *113061.*
41. Nasios G, Bakirtzis C & Messinis L (2020) Cognitive impairment and brain reorganization in MS: underlying mechanisms and the role of neurorehabilitation. *Frontiers in Neurology*, ***11:*** *147.*
42. Zhu J, Ren Z, Chen Y, Hu B (2016) The biological effects induced by high-charged and energy particles and its application in cancer therapy. *International Journal of Radiation Research*, ***14(1):*** *1.*
43. Zhu J, Ren Z, Chen Y & Hu B (2016) The biological effects induced by high-charged and energy particles and its application in cancer therapy. *International Journal of Radiation Research*, ***14(1):*** *1.*
44. Cox D, Raeburn C, Sui X & Hatters DM (2020, March) Protein aggregation in cell biology: An aggregomics perspective of health and disease. *Seminars in Cell & Developmental Biology,* ***(99):*** *40-54.*
45. Mogensen J (2021) Animal models in neuroscience. *Handbook of laboratory animal science*, *591-614.*

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