Egypt. J. Rad. Sci. Applic. **Vol. 35,** No. 1-2, pp. 49-55 (2022)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

N-Acetyl Cysteine Effects on Radiation-induced Brain Injury in Rats: Redox, Inflammatory and Apoptotic Modulations

Rasha R. Rashed, Engy R. Rashed[#]
Drug Radiation Research Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.

IN THE PRESENT study, the authors investigated the potential in vivo neuro-protective/therapeutic effects of N-acetyl cysteine (NAC) as an adjuvant supportive agent along head and neck irradiation protocols aiming at minimizing the radiation-induced neurotoxicity to non-tumor bystander brain tissues. Experimental animals were randomly assorted into four experimental groups; normal control, cranial irradiated, irradiated pre-treated with NAC and irradiated rats that received NAC post-irradiation. Redox, inflammatory and apoptotic alterations of brain tissues were assessed post animals' sacrifice. Cranial irradiation induced significant oxidative stress, inflammatory and apoptotic reactions in rat brain. However, administration of NAC for two weeks prior to irradiation effectively attenuated the radiation impact on the brain oxidative stress, inflammation and apoptosis. On the other hand, the neuro-protective effects offered by pre-treatment with NAC were much more promising than those observed when NAC was administered following irradiation; especially in the case of the apoptotic changes. In conclusion, NAC played a neuro-protective role rather than a corrective one, suggesting a sort of an "off-label use" for NAC as a radio protector against irradiation bystander effects on non-tumor brain tissues.

Keywords: Apoptosis, Cranial irradiation, Inflammation, N-acetyl cysteine, Oxidative stress.

Introduction

Head and neck radiotherapy is an irreplaceable tool for various types of brain tumors (McTyre et al., 2013). However, cranial irradiation (CI) has been reported to induce a number of neuronal disorders especially those related to cognitive dysfunctions; such as learning ability, spatial memory and dementia, resulting in negative impacts on patients' life quality (Wilke et al., 2018). Earlier studies have reported that the radiation-induced neuronal injuries are a consequence of the structural modifications in glial cell populations, cranial micro-circulation, and hippocampal neurogenesis inhibition (Pipova et al., 2020). This is in addition to the CI-induced neuro-inflammatory reactions which possess a pivotal role in irreversible brain milieu dis-regulation, blood-brain barrier (BBB) disruption, and neuronal demyelination (Jenrow et al., 2013). Thus, establishing an effective management, neuro-protective protocol would minimize CI-induced brain injuries and their consequent complications (Makale et al., 2013; Abdel-Naby et al., 2021).

N-acetylcysteine (NAC); a thiol amino acid, was reported to act as a free radicals scavenger, to replenish brain tissue glutathione (GSH) content and to inhibit neuronal lipid peroxidation (Samuni et al., 2013; Dhillon et al., 2022). Moreover, NAC is reported to possess neurovascular-protective effects that have a positive effect in neurological disorders treatment via the NAC-induced down-regulation of oxidative stress and inflammatory cascades (Bavarsad-Shahripour et al., 2014; Bhatti et al., 2018) in experimental cisplatin-induced neurotoxicity (Abdel-Wahab & Moussa, 2019). Furthermore, NAC has been shown to be able to cross the BBB and improve brain functions (Farr et al., 2003; Prakash et al., 2015). It also increases the brain tissue content of brain-derived neurotrophic factor (BDNF) which maintains the neuronal survival and stimulates the neurogenesis process (Bavarsad-Shahripour et al., 2014; Fredriksson et al., 2023).

[#]Corresponding author e-mail: engyrefaat@yahoo.com
Received 15/12/2022; Accepted 19/12/2022
DOI: 10.21608/ejrsa.2022.181084.1145

Based on the aforementioned information, this study was designed to evaluate and point out the optimum regimen; protective and/or therapeutic, that could maximize the benefits of using NAC to attenuate the radiation-induced injuries of normal non-tumor tissues.

Materials and Methods

Chemicals

N-acetyl cysteine (NAC) was purchased from Sigma-Aldrich Chemical Company (Saint Louis, Missouri, USA). All the other reagents, solvents and co-enzymes used in the current study were of high purity or of analytical grades.

Animals

Animals used in the present work were male rats of Wister strain; weighing 150±20 g, purchased from the breeding unit of the Egyptian National Research Center. The current study was carried out following the guidelines of the Research Ethics Committee of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt that is complied with the relevant legislation stated in the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, 1996).

Cranial irradiation process

Gamma irradiation has been carried out at the NCRRT, Cairo, Egypt, using the laboratory irradiator (Canadian GAMMACELL®220; Nordion International Inc., Ontario, Canada) with Cobalt-60 source. Rats were cranially irradiated at a single dose level of 20 Gy (Thabet et al., 2021) delivered at a dose rate of 1.8 KGy/h. Rats were anesthetized by sodium pentobarbital injection; 35 mg/kg by intraperitoneal route (Buell & Harhing, 1989). Each anaesthetized rat was placed inside a lead jacket attenuator of 10 cm thickness with its head exposed to ensure the protection of the other body parts from radiation.

Experimental design

In the current study, rats were randomly divided into four experimental groups; each of 8 rats. Group I: ***Control***; sham irradiated rats, group II: ***Rad***; rats that received a single dose of cranial irradiation, group III: ***NAC+Rad;*** rats that received daily doses of NAC for two weeks prior to the cranial irradiation process and group IV: ***Rad+NAC***; rats received a single dose of cranial irradiation; as in group II, followed by two weeks of daily treatment with NAC; as in group III. In the current study, NAC pure powder was dissolved in drinking water in a concentration of 20 mg/ml, to allow the optimum dissolution of the powder and avoid the oral tube clogging. The solution was then administered in a daily dose of 100 mg/kg body weight (Abdel-Wahab & Moussa, 2019) via oral gavage for 14 successive days either prior to or following cranial irradiation. Animals of all groups were sacrificed by decapitation; under deep urethane anesthesia, two weeks following irradiation or sham irradiation.

Assessment of brain redox status

In the whole brain homogenate, the degree of lipid peroxidation was assessed according to Uchiyama & Mihara (1978) as malondialdeyde; the endproduct of the lipid peroxidation process. In addition, the brain content of reduced glutathione (GSH) was determined according to the method of Beutler et al. (1963) together with the activity of glutathione peroxidase (GPX) which was assayed based on the method of Paglia & Valentine (1976). In addition, the brain total nitrate/nitrite (NOx) was measured according to the method described by Miranda et al. (2001).

Assessment of brain tissue total protein, inflammatory and apoptotic changes

Brain tissue contents of each of TNF-α; SUNLOG® BIOTECH CO. LTD, China, (Catalogue number: SL0722Ra) and IL-1β; Abcam® Co., Cambridge, USA; respectively was determined. Furthermore, the brain homogenate content of Caspase-3; SUNLOG® BIOTECH CO. LTD, China, (Catalogue number: SL0152Ra) as well as the brain mitochondrial content of the anti-apoptotic marker Bcl-2; Biovendor® Laboratory and Medicine Inc., Cezch republic, were assessed using rat-specific ELISA kits, according to the manufacturers' instructions. Total brain homogenate and mitochondrial fraction protein concentrations were quantified according to the method described by Lowry et al. (1951).

As an indicator of the inflammation-induced cerebral edema percentage; cerebral edema index was determined; then the wet and dry weights of the brain were recorded. The brains were then weighed before and after 48 h of incubation in dry air oven at 100˚C (Zhan & Yang, 2006), then the tissue water content of the brain samples was calculated using the following formula:

Cerebral edema index= [Wet tissue weight - Dry tissue weight] / Wet tissue weight X 100

Data analysis

Statistical data analysis was carried out using GraphPad Software, La Jolla, CA (Prism, version 6) by one-way analysis of variance (ANOVA) test that was followed by Tukey–Kramer posttest for comparisons among groups' means. p-values were considered statistically significant at $P< 0.05$. The data obtained were presented as the means ± standard error of the means (SEM).

Results

The results presented in Table 1 showed a significant oxidative stress in rat brain tissue following cranial irradiation, as indicated by the depletion of its GSH content, the rise in MDA and NOx concentrations and the marked inhibition of tissue GPx activity, when compared to the sham-irradiated group. Administration of NAC for two weeks prior to irradiation offered a protective effect for the brain; that was indicated by the restoration of brain GSH content and GPx activity, as well as the significant decrease in tissue contents of NOx and MDA as compared to the irradiated untreated rats. However, NAC administration post-irradiation had no effect on any of the oxidative stress markers except for the brain NOx content that showed a significant decrement in ***Rad+NAC*** group as compared to the irradiated group (Table 1).

Regarding the inflammatory reactions triggered by the cranial irradiation in rats' brain tissue, they have been evidenced by the marked rise in brain contents of the pro-inflammatory cytokines TNF-α and IL-1β as compared to the values recorded for the sham-irradiated group (Table 2). Nevertheless, pre-irradiation NAC administration offered a promising anti-inflammatory effect; as it attenuated the irradiation-induced inflammation showing a reduction in brain tissue TNF-α and IL-1β concentrations in comparison to ***Rad*** group. Another indicator of the degree of the inflammatory reaction induced by radiation was the cerebral edema index; which showed a marked increment in ***Rad*** group; in comparison the sham-irradiated control group, and has been reversed significantly in ***NAC+Rad*** group as compared to the untreated irradiated group (Table 2). However, NAC treatment following cranial irradiation offered a restoration of the brain contents of TNF-α and IL-1β, but showed no effect on the cerebral edema index.

TABLE 1. Effects of pre- and post-irradiation administration of N-acetyl cysteine (NAC) on radiation-induced rat brain redox status

Parameters / Groups	GSH (mg/g tissue)	MDA (nmol/mg prt)	NO (nmol/mg prt)	GPx (U/mg prt)
Control	32.03 ± 4.08	2.46 ± 0.37	15.18 ± 1.98	2.08 ± 0.14
Rad	13.67 ± 1.19 *	8.24 ± 0.98 *	38.61 ± 4.22 *	0.41 ± 0.34 *
NAC+Rad	25.09 ± 3.02 **	4.06 ± 0.57 *,**	20.30 ± 2.43 **	1.68 ± 0.27 **
Rad+NAC	15.71 ± 2.17 *	6.97 ± 0.77 *	25.17 ± 3.01*,**	0.62 ± 0.05 *

* Denotes significant difference from control group and ** denotes significant difference from Rad group at $P<0.05$. Control: normal sham irradiated group, Rad: cranial irradiated group, NAC+Rad: pre-irradiation NAC treated group, Rad+NAC: post-irradiation NAC treated group.

TABLE 2. Effects of pre and post-irradiation administration of N-acetyl cysteine (NAC) on radiation-induced rat brain inflammatory and apoptotic status

Parameters / Groups	Brain TNF-α (pg/mg prt)	Brain IL-1β (pg/mg prt)	Cerebral Edema Index (%)	Brain Caspase-3 (ng/mg prt)	Brain Bcl-2 (pg/mg prt)
Control	2.18 ± 0.30	41.47 ± 5.06	42.33 ± 4.62	28.65 ± 3.96	4.68 ± 0.52
Rad	6.36 ± 0.94 *	98.61 ± 8.75 *	70.80 ± 5.83 *	77.69 ± 8.74 *	0.86 ± 0.10 *
NAC+Rad	4.09 ± 0.58 **	58.66 ± 6.72 **	42.43 ± 4.89 **	43.00 ± 5.14 **	2.26 ± 0.29 **
Rad+NAC	4.78 ± 0.64 **	64.91 ± 7.18 **	65.41 ± 7.43 *	79.10 ± 8.51 *	1.14 ± 0.08 *

* Denotes significant difference from control group and ** denotes significant difference from Rad group at $P<0.05$. Control: normal sham-irradiated group, Rad: cranial irradiated group, NAC+Rad: pre-irradiation NAC treated group, Rad+NAC: post-irradiation NAC treated group.

As for the irradiation-induced neuronal apoptosis, it has been observed in ***Rad*** group; indicated by the rise in brain caspase-3 and the decrement in the mitochondrial Bcl-2 contents, when compared to the sham-irradiated group (Table 2). On the other hand, the apoptotic changes were corrected significantly in ***NAC+Rad*** group, but not in ***Rad+NAC*** group, in comparison to ***Rad*** group.

Discussion

Being an established tool for the tumor management, irradiation has been drawing attention of scientists to investigate its tumor cytotoxic and bystander effects. Early studies have reported irradiation-induced inhibition of hippocampal neurogenesis (Greene-Schloesser et al., 2013, Warrington et al., 2013) with the proposal of oxidative stress, neuro-inflammation, and neuronal apoptosis involvement as the main components of radiation-induced tissue injuries (Huo et al., 2012; Zhang et al., 2017). Such injuries are almost unavoidable in non-tumor bystander tissue; aiming to whose avoidance several studies have been carried out. In the present work the authors targeted each of the above named components of radiation-induced brain injuries; using NAC as a protective or therapeutic intervention.

On the other hand, N-acetyl cysteine (NAC) has been reported to improve some neurological disorders including bipolar depression, schizophrenia, unipolar depression and radiation-induced cognitive dysfunction (Shungu, 2012; Abdel-Naby et al., 2021). Moreover, NAC offered neuroprotective outcomes in about twenty clinical trials that employed NAC as a supportive therapy in a variety of psychiatric diseases (Sandhir et al., 2012). Such promising applications encouraged us to investigate an additional "off-label" use for NAC against radiation-induced neuronal injuries.

In the present study, cranial irradiation induced oxidative stress, inflammation and apoptosis in rat brain tissues were investigated. Oxidative stress was indicated by the rise in MDA brain content, GSH depletion and inhibition of GPx activity. As for the inflammatory reactions, they were reflected by the rise of brain contents of the pro-inflammatory cytokines TNF-α and IL-β as well as the brain water contents. On the same line, the radiation-induced apoptosis was evidenced herein by the rise in brain caspase-3 expression and the decrement in mitochondrial Bcl-2 contents.

However, herein, NAC administration prior to, but not following, cranial irradiation offered protective effects to brain against each of the oxidative stress, inflammation and apoptosis. Generally, the increased reactive oxygen species (ROS) production following irradiation is reported to be the prime injurious event, which induces consequent inflammation and apoptosis. Thus, the NAC effects that were observed in the present work could be mainly attributed to its strong antioxidant activity. In earlier studies, NAC was reported to act as anti-inflammatory and anti-apoptotic (Park et al., 2015; Hwang & Kim, 2022)

NAC represents a typical xenobiotic that directly takes its place in a variety of physiological and pathological biochemical reactions (Sahin & Alatas, 2013), acting as a potent antioxidant and anti-iflammatory agent. Moreover, recent studies have reported the ability of NAC to cross the blood–brain barrier (BBB), to attenuate some types of brain neuropathies and dysfunctions, to offer effective management of vascular and neuronal disorders, and to modulate each of the neurotrophic, glutamatergic, and inflammatory pathways (Bavarsad-Shahripour et al., 2014; Prakash et al., 2014; Saleh, 2015; Fredriksson et al., 2023).

On the cellular level, the inflammatory insult triggers the production of pro-inflammatory cytokines by leukocytes; which act as local signaling mediators among immune cells. However, their over-production may induce a rise in their blood levels, where they target remote organs and tissues such as the brain resulting in the activation of an inflammatory cascade (Monica et al., 2001). The pro-inflammatory cytokines; TNF-α, IL-Iβ and IL-6, have been reported to play a crucial role in the inflammatory pathways taking place between the brain and the immune system, and have drawn the attention in earlier studies (Engblom et al., 2002; Riazi et al., 2010) for being directly involved in neuronal injuries. In addition, inflammation consists of increased vascular permeability and extravasation of serum components and this holds true for peripheral nervous system as well as CNS tissue. The peripheral inflammatory process was accompanied by microglial activation and TNF-α

increase in the hippocampus (Riazi et al., 2010).

Generally, it has been reported that following brain trauma each of oxidative stress, neuro-inflammation and increased brain edema regularly occur (Yuceli et al., 2020); the latter is usually attributed to the increased BBB permeability following the ROS-induced endothelial injury. As for its anti-apoptotic effects observed in the current study, it has been previously reported in the study by Park et al. (2015); in which NAC reduced the levels of caspase-3 and the pro-apoptotic mediator Bax in cultured human epithelial cells.

Conclusion

NAC offered a neuro-protective role rather than a corrective one, suggesting a sort of possible "off-label future use" for NAC as a radio-protector against irradiation bystander effects on non-tumor brain tissues.

Disclosure statement: The authors report no conflicts of interests among them.

Acknowledgement: The authors address their gratitude and thanks to the gamma-irradiation facility workers at the NCRRT for their efforts in the process of animals' irradiationcarried out during the present study.

References

Abdel-Naby, D.H., Deghiedy, N.M., Rashed, R.R., El-Ghazaly, M.A. (2021) Tailoring of chitosan/diacrylated pluronic system as a versatile nanoplatform for the amelioration of radiation-induced cognitive dysfunction. *International Journal of Biological Macromolecules*, **193**(Pt B), 1507-1521.

Abdel-Wahab, W.M., Moussa, F.I. (2019) Neuroprotective effect of N-acetylcysteine against cisplatin-induced toxicity in rat brain by modulation of oxidative stress and inflammation. *Drug Design, Development and Therapy,* **13**, 1155–1162.

Bavarsad-Shahripour, R., Harrigan, M.R., Alexandrov A.V., (2014) N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities. *Brain and Behavior*, **4**(2), 108–122.

Beutler, E., Duron, O., Kelly, B.M. (1963) Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine*, **61**, 882–888. (PMID: 13967893)

Bhatti, J., Nascimento, B., Akhtar, U., Rhind, S.G., Tien, H., Nathens, A., Luis Teodoro da Luz, L.T., (2018) Systematic review of human and animal studies examining the efficacy and safety of N-acetylcysteine (NAC) and N-acetylcysteine amide (NACA) in traumatic brain injury: impact on neurofunctional outcome and biomarkers of oxidative stress and inflammation. *Frontiers in Neurology,* **8**, 744. doi: 10.3389/fneur.2017.00744.

Buell, M.G., Harhing, R.K. (1989) Proinflammatory effects of local irradiation on rat gastrointestinal tract. *Digestive Diseases and Sciences,* **34**, 390–399.

Dhillon, T., Kumar, A., Kumar, V. (2022) Neuroprotective effect of N-acetylcysteine against monocrotophos-induced oxidative stress in different brain regions of rats. *Applied Biochemistry and Biotechnology,* **194**(9), 4049-4065.

Engblom, D., Saha, S., Ericsson-Dahlstrand, A., Jakobsson, P.J., Blomqvist, A. (2002) Prostaglandins as inflammatory messengers across the blood-brain barrier. *Journal of Molecular Medicine (Berl),* **80**(1), 5–15.

Farr, S.A., Poon, H.F., Dogrukol-Ak, D., Drake, J., Banks, W.A., Eyerman, E., Butterfield, D.A., Morley, J.E. (2003) The antioxidants α-lipoic acid and N-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. *Journal of Neurochemistry,* **84**(5), 1173–1183.

Fredriksson, I., Jayaram-Lindström, N., Peter W. Kalivas, P.W., Melas, P.A., Steensland, P. (2023) N-acetylcysteine improves impulse control and attenuates relapse-like alcohol intake in long-term drinking rats. *Behavioural Brain Research,* **436**, 114089. https://doi.org/10.1016/j.bbr.2022.114089.

Greene-Schloesser, D., Robbins, M.E., Peiffer, A.M., Shaw, E.G., Wheeler, K.T., Chan, M.D. (2012) Radiation-induced brain injury: A review. *Frontiers in Oncology,* **19**(2), 73. doi: 10.3389/fonc.2012.00073. PMID: 22833841; PMCID: PMC3400082.

Huo, K., Sun, Y., Li, H., Du, X., Wang, X., Karlsson, N., Zhu, C., Blomgren, K. (2012) Lithium reduced

neural progenitor apoptosis in the hippocampus and ameliorated functional deficits after irradiation to the immature mouse brain. *Molecular and Cellular Neuroscience*, **51**, 32–42,

Hwang, S., Kim, J.K. (2022) N-acetylcysteine induces apoptotic, oxidative and excitotoxic neuronal death in mouse cortical cultures. *Chonnam Medical Journal,* **58**(1), 18-23.

Jenrow, K.A., Brown, S.L., Lapanowski, K., Naei, H., Kolozsvary, A., Kim, J.H. (2013) Selective inhibition of microglia-mediated neuroinflammation mitigates radiation-induced cognitive impairment. *Radiation Research,* **179**(5), 549-556.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275 (PMID: 14907713)

Makale, M.T., McDonald, C.R., Hattangadi-Gluth, J.A., Kesari S (2017) Mechanisms of radiotherapy-associated cognitive disability in patients with brain tumours. *Nature Reviews Neurology*, **13**(1), 52-64.

McTyre, E., Scott, J., Chinnaiyan, P. (2013) Whole brain radiotherapy for brain metastasis. *Surgical Neurology International,* **4**(Suppl 4), S236–S244. https://doi.org/10.4103/2152- 7806.111301.

Miranda, K.M., Espey, M.G., Wink, D.A. (2001) A rapid, simple spectrophotometric method for simulatenous detection of nitrate nitrite. *Nitric Oxide,* **5**(1), 62–71. .

Monica, E.K., Engblom, D., Sipra Saha, S., Anders Blomqvist, A., Jakobsson, P.J., Ericsson-Dahlstrand, A. (2001) Inflammatory response: Pathway across the blood–brain barrier. *Nature*, **410**, 430–431.

Paglia, D.E., Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, **70**, 158–169 (PMID: 6066618).

Park, J.H., Kang, S.S., Kim, J.Y., Tchah, H. (2015) The antioxidant N-acetylcysteine inhibits inflammatory and apoptotic processes in human conjunctival epithelial cells in a high-glucose environment. *Investigative Ophthalmology & Visual Science,* **56**, 5614-5621.

Pipova Kokoˇsov´a, N., Kiskov´a, T., Vilhanov´a, K., ˇStafurikov´a, A., Jendˇzelovský, R., Raˇcekov´a, E., ˇSmajda, B. (2020) Melatonin mitigates hippocampal and cognitive impairments caused by prenatal irradiation. *European Journal of Neuroscience,* **52**, 3575–3594.

Prakash, A., Jaspreet Kaur Kalra, J.K., Kumar, A. (2014) Neuroprotective effect of N-acetyl cysteine against streptozotocin- induced memory dysfunction and oxidative damage in rats. *Journal of Basic and Clinical Physiology and Pharmacology,* **26**(1), 0792–6855.

Prakash, A., Kalra, J.K., Kumar, A. (2015) Neuroprotective effect of N-acetylcysteine against streptozotocin-induced memory dysfunction and oxidative damage in rats. *Journal of Basic and Clinical Physiology and Pharmacology,* **26**, 13–23.

Riazi, K., Galic, M.A., Pittman, Q.J. (2010) Contributions of peripheral inflammation to seizure susceptibility: cytokines and brain excitability. *Epilepsy Research,* **89**, 34–42.

Sahin, S., Alatas, O., (2013) The protective effects of n-acetyl cysteine against acute hepatotoxicity. *Indian Journal of Gastroenterology,* **32**(5), 311–315.

Saleh, AAS. (2015) Anti-neuroinflammatory and antioxidant effects of N-acetyl cysteine in long-term consumption of artificial sweetener aspartame in the rat cerebral cortex. *The Journal of Basic & Applied Zoology*, **72**, 73–80.

Samuni, Y., Goldstein, S., Dean, O., Berk, M., (2013) The chemistry and biological activities of N-acetylcysteine. *Biochimica et Biophysica Acta,* **1830**(8), 4117–4129.

Sandhir, R., Sood, A., Mehrotra, A., Kamboj, S.S. (2012) N-Acetyl cysteine reverses mitochondrial dysfunctions and behavioral abnormalities in 3-nitropropionic acid-induced Huntington's disease. *Neurodegenerative Diseases*, **9**, 145–157.

Shungu, D.C. (2012) N-acetylcysteine for the treatment of glutathione deficiency and oxidative stress in schizophrenia. *Biological Psychiatry,* **71**(11), 937–938.

Thabet, N.M., Rashed, E.R., Abdel-Rafei, M.K., Moustafa, E.M. (2021) Modulation of the Nitric Oxide/BH4 Pathway Protects Against Irradiation-Induced Neuronal Damage. *Neurochemical Research,* **46**(7), 1641–1658.

Uchiyama M., Mihara, M. (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, **86**(1), 271–278.

Warrington, S.J., Strutt, H., Strutt, D. (2013) The Frizzled-dependent planar polarity pathway locally promotes E-cadherin turnover via recruitment of RhoGEF2. *Development*, **140**(5), 1045-1054.

Wilke, C., Grosshans, D., Duman, J., Brown, P., Li, J. (2018) Radiation-induced cognitive toxicity: pathophysiology and interventions to reduce toxicity in adults. *Neuro-oncology,* **20**(5), 597–607.

Yuceli, S., Yazici, G.N., Mammadov, R., Suleyman, H., Kaya, M., Ozdogan, S. (2020) The effect of rutin on experimental traumatic brain injury and edema in rats. *In Vivo*, **34**(5), 2453-2460.

Zhan, C., Yang, J. (2006) Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Pharmacological Research*, **53**, 303–309.

Zhang, Y., Gao, L., Cheng, Z., Cai, J., Niu, Y., Meng, W., Zhao, Q. (2017) Kukoamine A prevents radiation-induced neuroinflammation and preserves hippocampal-neurogenesis in rats by inhibiting activation of NF-κB and AP-1. *Neurotoxicity Research,* **31**, 259–268.