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Evaluation of Glucosamine Effect Against Heart and Brain Damage Induced by Y-radiation or Aluminium Chloride in Female Rats

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HIGHLIGHTS

- High dose of gamma irradiation induced heart and brain injures.
- The harmful effect of aluminium chloride on heart and brain tissues.
- The beneficial effect of glucosamine.
- Histopathological examination of heart and brain tissues in rats.

Abstract: Glucosamine is known as anti-inflammatory, antioxidant and as neuroprotective as well as using to treat many of diseases. This work aimed to investigate the remedial effect of glucosamine (20mg/kg b.wt) against the damage induced by a single dose of γ-radiation (8Gy) or aluminium chloride ($AlCl_3$) (100mg/kg b.wt) in the heart and brain tissues of female rats. Serum aspartate aminotransferase (AST), cholesterol, triglycerides (TGs), LDH and creatine kinase (CPK) were measured. Moreover, gene expression of amyloid protein precursor (APP) and seladin-1 were estimated in the brain tissue. Also, acetylcholinesterase activity (AChE) and p-tau protein expression were estimated in brain homogenate. Metallothioneine (MT) was estimated in the heart and brain tissues. Heart and brain histopathological examination was performed. Irradiation significantly decreased serum AST, CPK and LDH, as well as MT levels in heart and brain tissues. Also, gene expression of seladin-1 decreased. On the other hand, irradiation significantly increased serum TGs level and brain AchE activity, tau protein, and β-amyloid percursor (APP). $AlCl_3$ administration (21 days) induced disturbance in most of the estimated parameters, especially AST, TGs, and MT. Glucosamine treatment with irradiation or $AlCl_3$ improved most of the measured parameters. In addition, histopathological examination confirmed the biochemical results. In conclusion: Glucosamine could be used to improve the heart and brain damages induced by γ-radiation exposure or $AlCl_3$.

Keywords: Seladin-1; heart; γ-radiation; glucosamine; $AlCl_3$.

INTRODUCTION

Aluminium (Al) neurotoxicity in animals has been clearly recognized and presented to be involved in the etiology of neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis, Parkinson's disease [1]. Major sources of Al exposure include certain foods, especially corn, yellow cheese, flour, salt and spices, vegetables and tea leaves, as well as cookware and containers. Also, it is used in the medical field as a major constituent of drugs such as antacids, phosphate binders, buffered aspirins, vaccines, injectable allergens [2], creams and food additives which allowed easy entry into the body [1].

Important evidence proposes that exposure to ionizing radiation can lead to the progress of AD. Although radiation therapy is an essential tool in the management of primary [3] and metastatic [4] brain tumors, it is also responsible for different adverse neurological effects, for example, cognitive dysfunction or dementia, which might happen in >20% of brain tumor patients old 50 years or over and cured by radiotherapy [5]. On the other hand, many studies have described the presence of several physiological and cognitive effects of ionizing radiation at different doses [6]. Such cognitive alterations are often exhibited as shortages in hippocampal-dependent tasks of learning, memory and spatial information processing [7]. Adverse effects of ionizing radiation on the cardiovascular system have the potential for a large impact on public health. High doses of radiation applied to the heart during radiotherapy used in breast cancer [8], Hodgkin's disease [9] or childhood cancers [10] increase the cardiovascular incidence and mortality. Both cardiac dysfunction and progressive loss of cognitive functioning are prominent features of aging [11]. Yet, it is time for a more integrated view of the heart-brain connection as recent data indicate that cardiovascular conditions contribute to cognitive impairment [12].

D-glucosamine (GlcN) and *N*-acetyl-d-glucosamine (GLcNAc) are naturally occurring amino sugars and essential carbohydrate components of biologically important glycoproteins, glycolipids, and glycosaminoglycans. GlcN has therapeutic potential in the treatments of various diseases including osteoarthritis, inflammatory bowel disease and gastritis [13]. GlcN has excellent antioxidant activities, as manifested by a strong chelating effect on ferrous ions and protection of macromolecules such as protein, lipid, and deoxyribose from oxidative damage induced by hydroxyl radicals [14]. Fülöp and coauthors [15] reported that, in the perfused heart, GlcN improved functional recovery following ischemia and this appeared to be mediated via an increase in O-linked N-acetylglucosamine (O-GlcNAc) levels of nucleocytoplasmic proteins. Moreover, Hwang and coauthors [16] investigated the neuroprotective effect of GlcN in a rat middle cerebral artery occlusion model. Moreover, du Souich and coauthors [17] reported that there is primary evidence suggesting that chondroitin sulphate (natural glycosaminoglycan) may also recover other inflammatory disorders such as atherosclerosis.

In view of that, the present study was planned to evaluate whether GlcN have any improvement influence on the heart and brain recovery after irradiation or $AlCl_3$ toxicities. To realize this aim, cardiac and brain biomarkers were examined. Histopathological examination was assessed to confirm the biochemical results.

MATERIAL AND METHODS

Animals

Wistar female albino rats, weighing 180-200 g, were obtained from the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt. Animals were left one week for acclimatization on lab environment before starting the onset of the experiment. All animal procedures were performed according to the Ethics Committee of the National Research Center for Radiation Research and Technology (NCRRT),(No: 7A/17), Atomic Energy Authority.

Irradiation facilities

Irradiation was performed through the use of a Canadian Gamma Cell-40 (^{137}Cs) at NCRRT, Cairo, Egypt. The dose rate was 0.675 Gy/minute.

Treatment

Aluminium chloride (El Gomhoureya For Drugs Trade & Medical Supplies Co., Cairo, Egypt), was dissolved in distilled water at a dose of 100 mg/kg b.wt [18]. Glucosamine sulfate "Joflex capsules" 500 mg (pharaonia pharmaceuticals Co., Cairo, Egypt), the capsule was evacuated to dissolve in distilled water at a dose of 20 mg/kg b.wt [19].

Experimental design

Rats were randomly divided into six groups (n=8) as follows: Group (1): untreated control, Group (2): Irradiated group (IRR): rats exposed to 8Gy of γ-radiation as a single dose then stayed for three weeks, Group (3): $AlCl_3$ treated group: rats orally received 100 mg/kg b.wt $AlCl_3$ daily for three weeks, Group (4): GlcN treated group (Glu): rats orally received 20 mg/kg b.wt glucosamine daily for three weeks, Group (5): IRR+ Glu group: rats exposed to γ-radiation (8Gy) then orally received GlcN daily for three weeks, Group (6): $AlCl_3$ + Glu group: rats were orally received $AlCl_3$ and GlcN daily for 21 days.

Animals were fasted for 12 h before sacrificed. Whole blood was collected via decapitation, then centrifuged at 3000 rpm for 15 min. The resulting serum was separated and used for biochemical determinations. Heart and brain tissues were removed, washed in ice-cold saline, then the final heart and brain weights were recorded. The heart was separated into two portions, the first one was immediately fixed in 10% formalin for histopathological analysis and the other was homogenized (1:5 w/v) in 0.25M sucrose to be used for biochemical assays. The brain was separated into three parts, one for histopathological analysis, the second part for biochemical assays and the other part was snap-frozen at -80 °C for gene expression and protein determination.

Biochemical parameters estimated in serum

Serum cholesterol (C), triglycerides (TG) and aspartate transaminase (AST) were estimated according to Naito and Kaplan [20], Fossati and Prencipe [21] and Murray [22], respectively. Lactate dehydrogenase (LDH) was determined according to Pesce [23], using Helios γ UV/VIS Spectrophotometer and creatine kinase (CPK) was measured according to Dawson and coauthors [24], using commercial kits of ELITECH (Biomed, Egypt).

Biochemical parameters investigated in heart and brain homogenate

In the heart and brain tissues, metallothionine (MT) was evaluated by Ag-saturation hemolysate method according to Scheuhammer and Cherian [25] and Bienengräber and coauthors [26] using Thermo Scientific iCE 3000 series Atomic Absorption Spectrometry. In brain tissues, acetylcholinesterase activity (AchE) was estimated according to the method of Ellman and coauthors [27] kits (CUSABIO, China). While amyloid protein precursor (APP) and seladin-1 were determined as RT-PCR analysis according to the method of Pfaffl [28]. Moreover, p-Tau protein expression level was determined by Western blot method.

Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from each frozen sample using a Qiagene kit (USA) according to a standard protocol. The isolated total RNA was converted into complementary DNA (cDNA) using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, USA). Real-time PCR was performed using Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and an SYBR® Green PCR Master Mix (Applied Biosystems) in a final volume of 10 µl with the following thermal cycling conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The sequences of PCR primer pairs used for each gene are shown in table 1. Data were analyzed with the ABI Prism sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). The relative expression of studying genes was calculated using the comparative threshold cycle method. All values were normalized to the beta-actin genes as an invariant endogenous control (reference gene). The relative quantification was then calculated by the expression $2^{-\Delta\Delta Ct}$ [28].

Table 1. The sequences of PCR primer pairs

Gene name	Primer sequence
Amyloid protein precursor (APP) gene	Forward primer: 5-CCTTACCGGTGCCTAGTTGGT-3 Reverse primer: 5-GTCCATCCGCTCCTGGTGTA-3
Seladin-1 gene	Forward primer : 5-ATCGCAGCTTTGTGCGATG-3 Reverse primer: 5-CACCAGGAAACCCAGCGT-3
β-actin gene	Forward primer : 5-CCAGGCTGGATTGCAGTT- 3 Reverse primer: 5-GATCACGAGGTCAGGAGATG-3

Detection of p-tau by Western blot analysis

Tissue proteins were extracted using TRIzol reagent and protein concentrations were estimated by the Bradford method. Twenty micrograms of protein per lane were separated with 10% SDS polyacrylamide gel electrophoresis gels and electrophoretically transferred to poly vinylidene fluoride (PVDF) membranes. Membranes were then incubated at room temperature for 2 h with blocking solution comprised of 5% nonfat dried milk in 10 mM Tris-Cl, pH 7.5, 100 mM NaCl, and 0.1% Tween 20. Membranes were incubated overnight at 4 °C with the indicated primary antibodies (p-Tau and beta-actin) and then incubated with a mouse anti-rabbit secondary monoclonal antibody conjugated to horseradish peroxidase at room temperature for 2 h. After each incubation, the membranes were washed four times with 10 mM Tris-Cl, pH 7.5, 100 mM NaCl and 0.1% Tween 20 at room temperature Chemiluminescence detection was performed with the Amersham detection kit according to the manufacturer's protocols (Amersham. Life Science Inc., USA). The amount of study protein was quantified by densitometric analysis (Biomed Instrument Inc., USA) using BioRad software, USA. Results were expressed as arbitrary units after normalization for β-actin protein expression.

Histopathological examination

Brain and cardiac muscle tissue specimens were fixed in 10% formalin, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, embedded in paraffin blocks and sectioned at 4-6 µm thick. The obtained tissue sections were deparaffinized using xylol and stained using hematoxylin and eosin (H and E) for histopathological examination through the electric light microscope according to Bancroft and coauthors [29].

Statistical analysis

Comparisons between different groups were carried out by using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison tests, using 'Instant software, v. 5 (GraphPad Inc., San Diego, California, USA)'. The p- value was set at < 0.05. Data were presented as a mean ± standard error (SE).

RESULTS

Estimation of relative heart and brain weights

There were non-significant changes in the relative heart and brain weights in all groups of the experiment, except the relative heart weight of the irradiated and the irradiated treated with glucosamine groups which showed a significant elevation compared to the control group (Table 2).

Table 2. Effect of glucosamine on absolute and relative weights of heart and brain in rats exposed to γ-radiation and $AlCl_3$.

Groups	Heart		Brain	
	Abs. wt. (g)	Relative wt. (%)	Abs. wt. (g)	Relative wt. (%)
Control	0.543±0.020	0.335±0.009	1.47±0.044	0.910±0.023
IRR	0.825±0.087*	0.449±0.033*	1.48±0.049	0.851±0.027
$AlCl_3$	0.517±0.013	0.332±0.005	1.37±0.054	0.883±0.034
Glu	0.621±0.031	0.360±0.013	1.39±0.096	0.811±0.054
IRR+Glu	0.718±0.043*	0.394±0.020*	1.49±0.063	0.841±0.082
$AlCl_3$+Glu	0.574±0.029	0.343±0.010	1.46±0.038	0.876±0.018

*,#,$Significant different from the control, IRR, and $AlCl_3$ groups, respectively. Data represented as a mean ± SE., n =6.

Assessment of serum cardiac biomarkers

Irradiated rats recorded significant elevation in cholesterol and TGs levels as compared to the normal control. While $AlCl_3$ group showed a significant increase in cholesterol level only when compared to the control level. On the other hand, glucosamine treatment post irradiation or $AlCl_3$ caused amelioration in cholesterol and TGs levels (Figure 1).

Rats treated with $AlCl_3$ resulted in a significant increase in AST activity, but other groups exhibited a significant decrease in AST activity when compared to the control group. Glucosamine administration post IRR or $AlCl_3$ exposure attenuated their effects on the AST activity (Figure 1).

Figure 2 reflects a significant reduction in CPK and LDH activities in the irradiated group, while $AlCl_3$ group had no significant change in CPK and LDH activities when compared to the control group. Glucosamine treatment post- irradiation had a slight amelioration in CPK activity and restored LDH activity as compared to the control values.

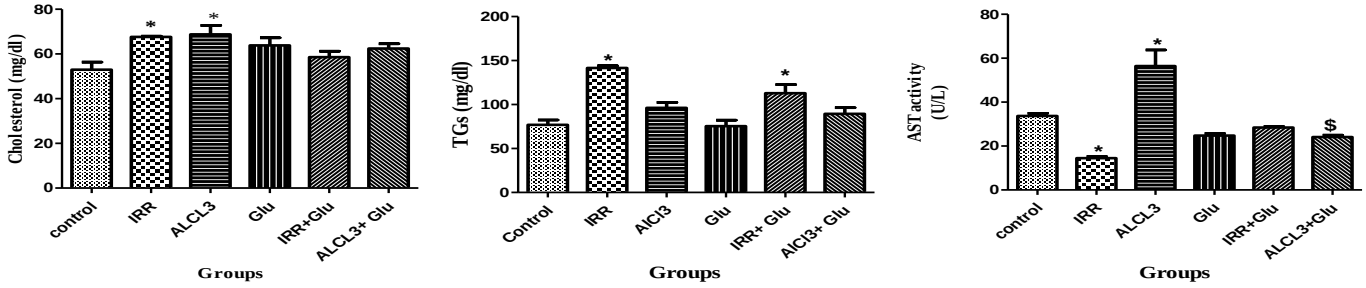

Figure 1. Effect of glucosamine on serum cholesterol, TGs and AST in rats exposed to γ-radiation and $AlCl_3$.
*,#,$Significant different from the control, IRR, and $AlCl_3$ groups, respectively. Data represented as a mean ± SE, n =6.

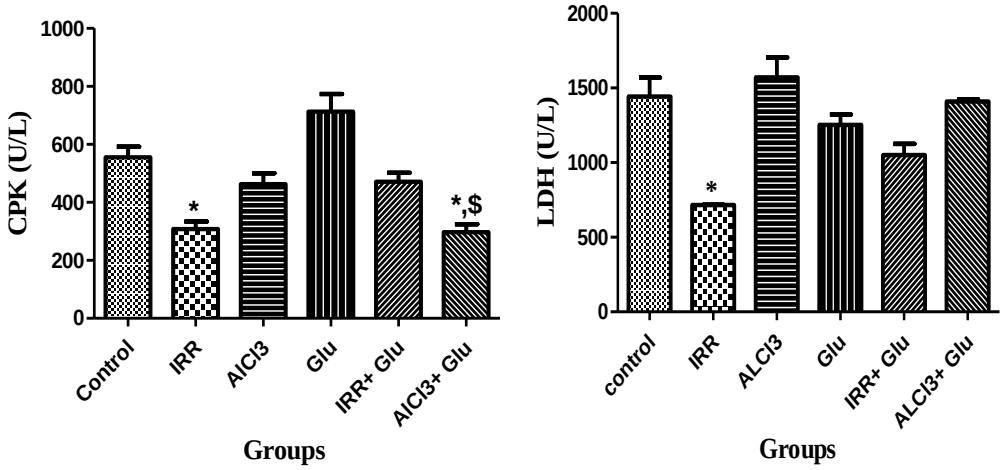

Figure 2. Effect of glucosamine on serum LDH and CPK activities in rats exposed to γ-radiation and $AlCl_3$.
*,#,$Significant different from the control, IRR, and $AlCl_3$ groups, respectively. Data represented as a mean ± SE, n =6.

Assessment of metallothionine (MT) in the heart and brain tissues

Cardiac MT level displayed a significant decrease in the IRR, $AlCl_3$, Glu, IRR+Glu and $AlCl_3$+Glu by 89.12, 90.52%, 89.18%, 57.73%, and 84.16% from the control, respectively. Whereas, glucosamine administration post- irradiation led to improvement in the MT level as compared to the irradiated group.

As well as, brain MT level showed a significant reduction in the IRR, $AlCl_3$, Glu, IRR+Glu and $AlCl_3$+Glu by 75.87%, 64.83, 76.14%, 47.29%, and 51.53% from the control, respectively. Whereas, glucosamine administration post- irradiation/ $AlCl_3$ led to improvement in the MT concentration as compared to the irradiated group and $AlCl_3$, respectively (Figure 3).

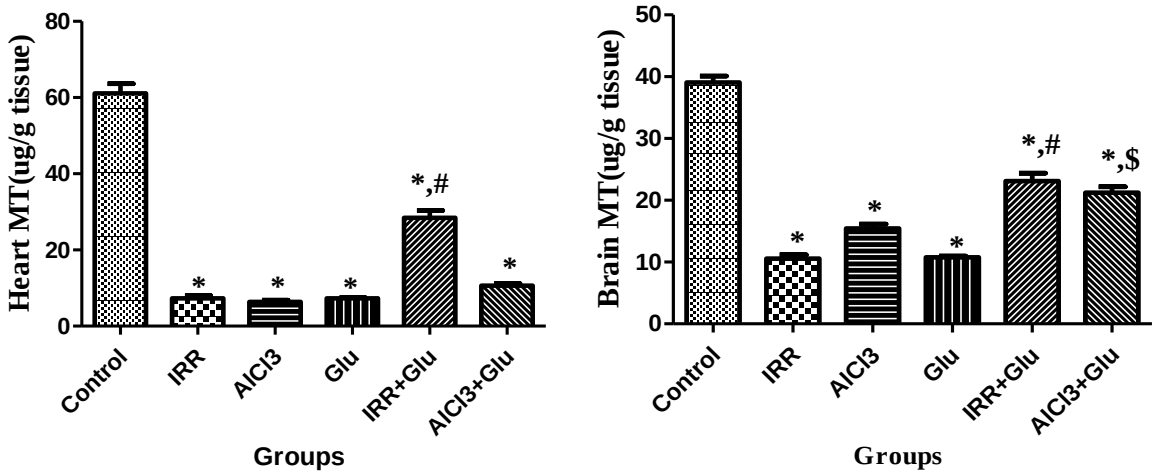

Figure 3. Effect of glucosamine on MT concentration in heart and brain tissues of rats exposed to γ-radiation and $AlCl_3$. *,#,$Significant different from the control, IRR, and $AlCl_3$ groups, respectively. Data represented as a mean ± SE., n =6.

Estimation of APP and seladin-1 gene expression and p-Tau protein expression in brain tissue

Results indicated that exposure of rats to either γ-irradiation or $AlCl_3$ recorded a significant up-regulation in APP mRNA and p-Tau protein expression levels and a significant down-regulation of expression of seladin-1 as compared to the control group. On the other hand, glucosamine administration alone showed non-significant changes in the levels of APP and seladin-1 mRNA and p-Tau protein expression levels when compared to the control group. Moreover, administration of glucosamine post irradiation / $AlCl_3$ alleviated these effects on levels of APP and seladin-1 mRNA expression, it still showed a significant increase in APP mRNA and p-Tau protein levels and a significant decrease in seladin-1 mRNA levels as compared to the control group (Table 3, Figure 4).

Table 3. Effect of glucosamine on relative gene expression of β-amyloid Precursor and Seladin-1 in brain tissues of rats exposed to γ-radiation and $AlCl_3$

Groups	APP	Seladin-1
Control	1.030 ± 0.017	1.003 ± 0.009
IRR	15.30 ± 1.305*	0.207 ± 0.015*
$AlCl_3$	10.60 ± 0.839*	0.513 ± 0.026*
Glu	1.037 ± 0.020	1.017 ± 0.012
IRR +Glu	7.047 ± 0.536*,#	0.637 ± 0.026*,#
$AlCl_3$+Glu	6.389 ± 0.462*,$	0.747 ± 0.038*,$

*,#,$Significant different from the control, IRR, and $AlCl_3$ groups, respectively. Data represented as a mean ± SE, n =6.

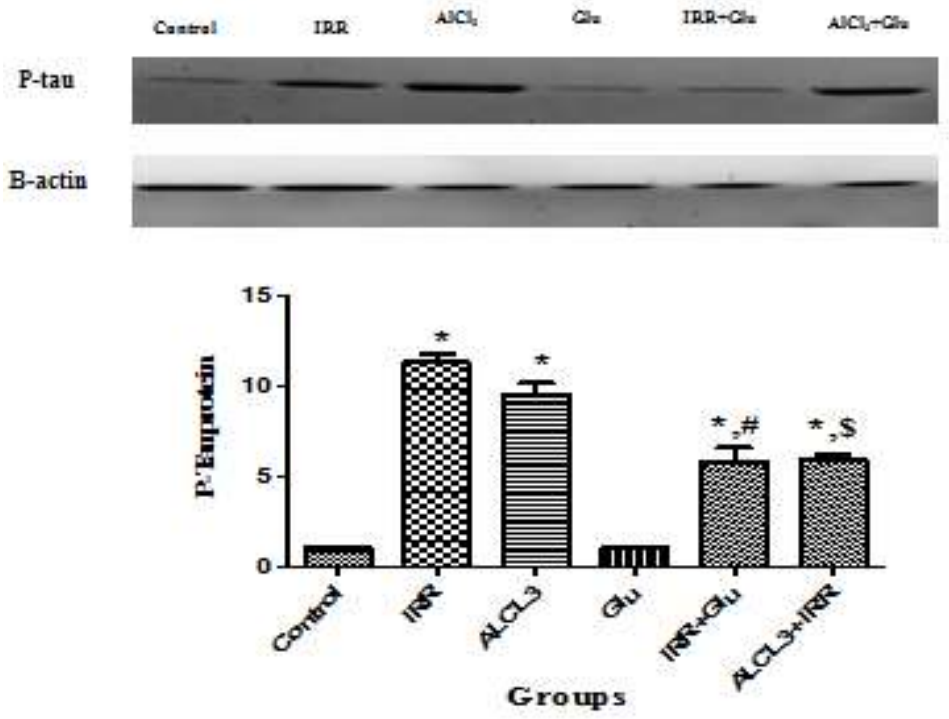

Figure 4. Effect of glucosamine on p-tau protein expression in irradiated or AlCl3 rats. Results were quantified by densitometry and corrected by reference to B-actin. Data represented as a mean± SE, Significance at p<0.05. *Significant different from the control group, #Significant different from IRR group and $Significant different from AlCl3 group.

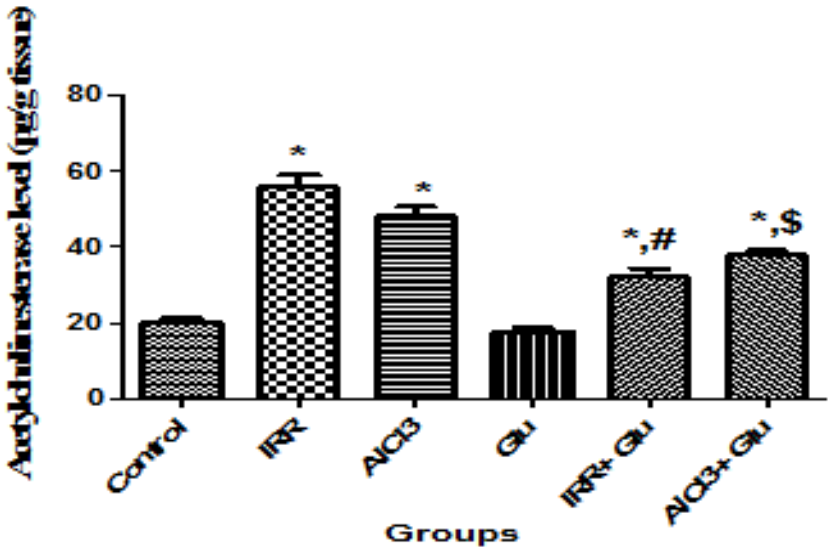

Figure 5. Effect of glucosamine on acetylchloinesterase concentration in braintissue of irradiated or $AlCl_3$ rats. Results were quantified by densitometry and corrected by reference to B-actin. Data represented as a mean± SE, Significance at p<0.05.
*Significant different from the control group, #Significant different from IRR group and $Significant different from $AlCl_3$ group.

Determination of acetylcholinesterase concentration in brain tissue

Acetylcholinesterase level increased significantly post irradiation / $AlCl_3$ as compared to the control group. Moreover, glucosamine administration after radiation / $AlCl_3$ exposure reduced this elevation when compared to the irradiated and $AlCl_3$ groups, respectively (Figure 5).

Histopathological examination of heart and brain

Cardiac muscle of control and glucosamine groups showed normal histological structure characterized Figure 6 (a). While, irradiated rats revealed muscular degeneration, also, inter-muscular congestion of blood capillaries and oedema were seen Figure6 (b). Whereas, the irradiated rats treated with glucosamine displayed improvement of cardiac muscle with minimal pathological alterations Figure 6 (c). Alternatively, aluminium chloride group showed swelling and granularity of myocytes, disorganization of myofibrils with the destruction of muscular striation Figure 6 (d). Cardiac muscle of aluminium chlorid and glucosamine group revealed an improvement of myofibrils with mild swelling of myocytes Figure 6 (e).

However, cerebral cortex tissue section of control and glucosamine groups showed normal histological structure Figure 7 (a). Moreover, the irradiated group revealed mild to moderate degree of neuronal degeneration that accompanied with peri-vascular and peri-neuronal oedema Figure7 (b). Cerebral cortex of irradiated rats treated with glucosamine showed mild neuronal swelling and neuronophagia with improvement of cerebral cortex as compared with irradiated group Figure7 (c). In addition, cerebral cortex tissue section of aluminium chloride group revealed abnormal cellular morphology accompanied by neuronal degeneration with neuronophagia, gliosis and pericellular edema Figure7 (d). Cerebral cortex of aluminium chloride rats treated with glucosamine showed normal cellular morphology and also exhibiting significantly reduced morphologic abnormalities in all cerebral regions with better recovery in degenerated neuronal Figure 7 (e).

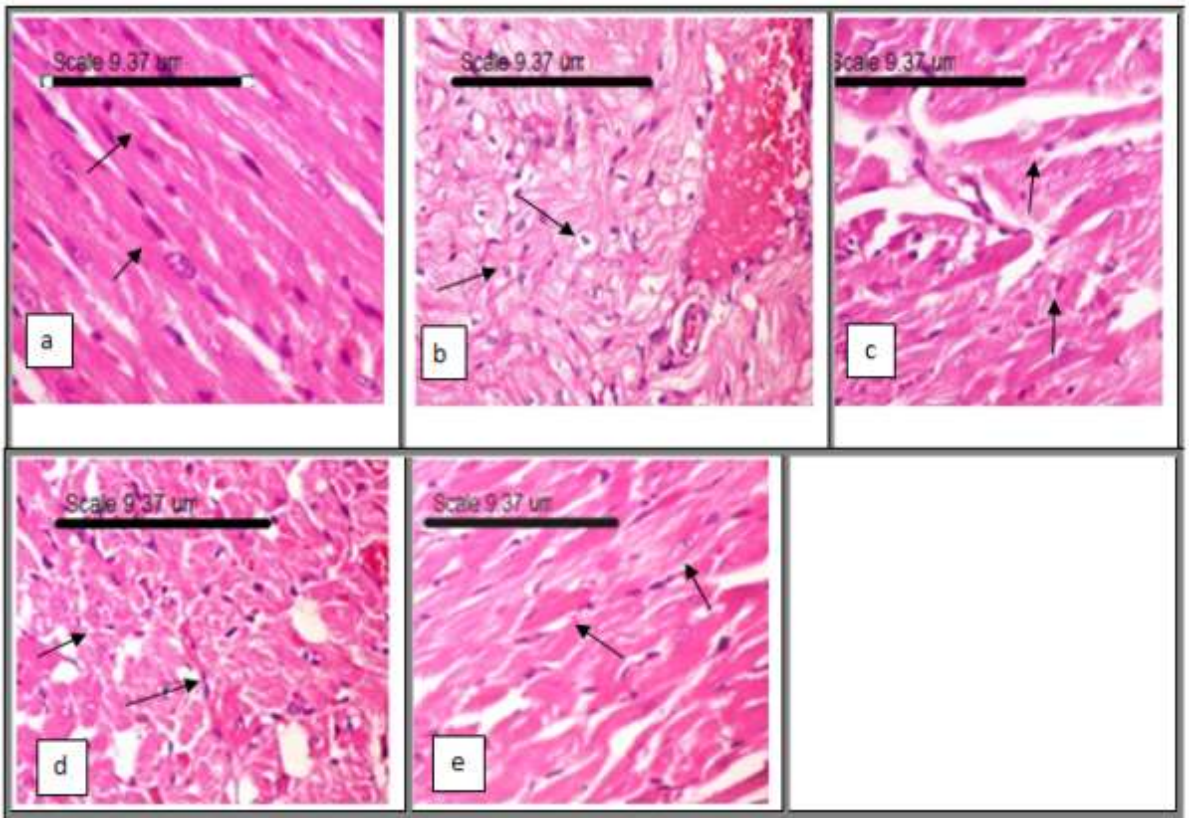

Figure 6. Cardiac muscle micrograph showing (a) Normal histological structure characterized by branching of the striated muscle arrow (b) Muscular degeneration with marked granularity and vacuolations of myocytes and congestion of blood capillaries arrow (c) Mild inter-muscular oedema and degeneration of myocytes arrow (d) Disorganization of myofibrils with destruction of muscular striation arrow (e) Mild swelling of myocytes arrow (H&Ex400).

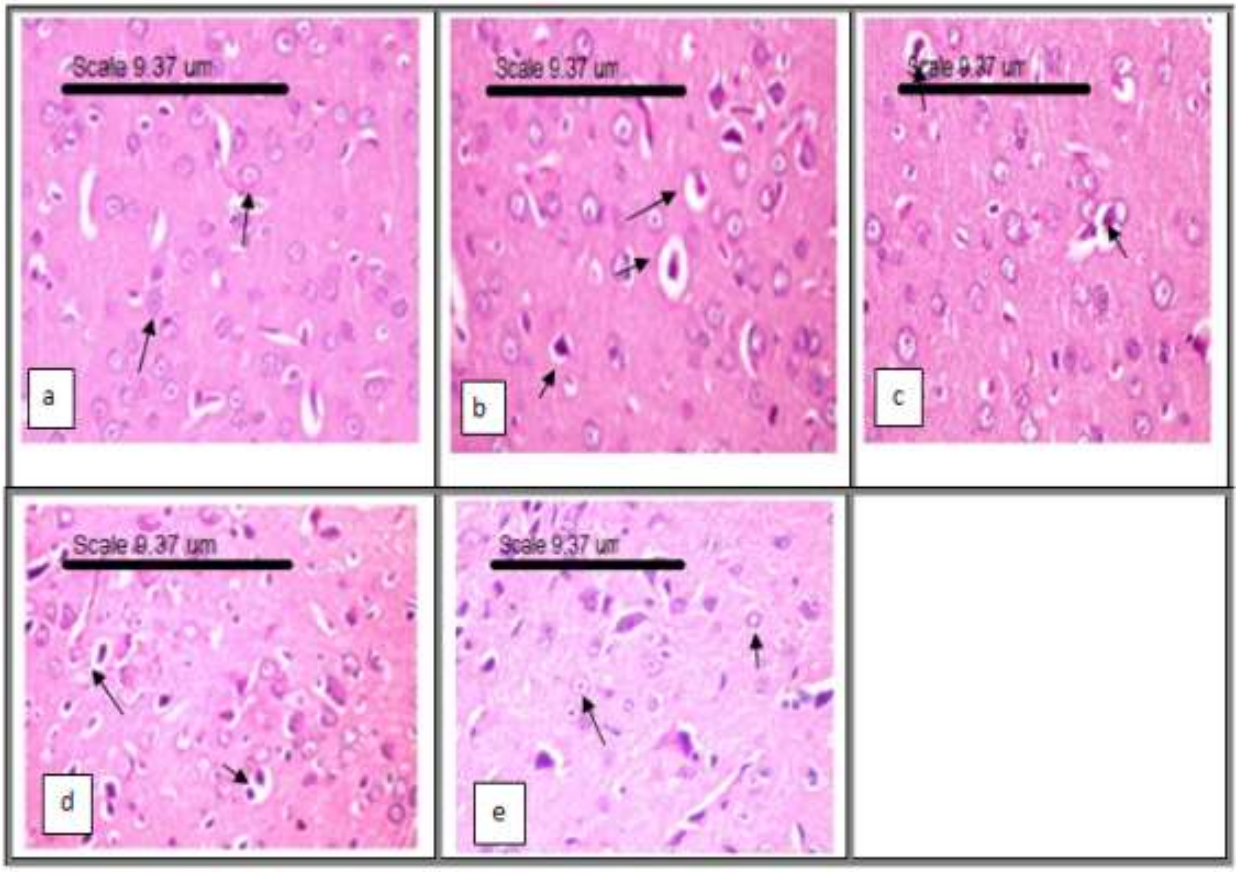

Figure 7. Cerebral cortex micrograph showing (a) normal histological structure which consisted of several layers of neuronal cells arrow (b) Neuronal degeneration, per-vascular and peri-neuronal oedema arrow (c) mild neuronal swelling and neuronophagia arrow (d) Neuronal degeneration with neuronophagia, gliosis and pericellular edema arrow (e) a large number of intact neuronal cells arrow (H&Ex400).

DISCUSSION

Alterations in the organ/body weight ratio have often been used as guide's toxicity [30]. The present study recorded an increase in heart weight and this may be explained by the study of Chello and coauthors [31], they reported that ionizing radiation caused an increase in the concentration of collagen. Moreover, Hasslacher and coauthors [32] stated that there was a positive correlation between the increase in collagen content and heart weight.

While the increase in cholesterol and TGs post irradiation in the current study is accordance with Onody and coauthors [33], who stated that there is a correlation between radiation-induced oxidative stress and elevated levels of lipid fractions. On the other hand, $AlCl_3$ administration caused an increase in cholesterol levels. This result is in accordance with Sarin and coauthors [34] and Newairy and coauthors [35], who referred dyslipidemia to aluminium accumulation in the liver. Our results showed a decline in LDH and CPK activities post irradiation and this data is in agreement with Sherif and coauthors [36], but it is disagreed with Mansour and Tawfik [37] who reported that γ-radiation produced significant elevations in CPK and LDH. So, the great production of free radicals and lipid peroxides might cause the leakage of the cytosolic enzymes, including the aminotransferases, creatine kinase and phosphatase enzymes. This disagreement may be due to the difference in the time period of the two experiments or due to the death of the heart cells.

Moreover, the decline in brain acetylcholine is the most notable biochemical change in AD patients [38]. AChE–terminates nerve impulse transmission by rapid hydrolysis of acetylcholine; thus, AChE inhibition serves as a mechanism for the treatment of neurological disorders such as AD and senile dementia [39]. Fishman and coauthors [40] found that the brains from the patient suffering from AD have presented reduced AchE activity in the hippocampus and cortex. AchE is decreased following long-term postnatal exposure of $AlCl_3$ [41]. Where Sunanda Rao and Raju [42] study showed that administration of $AlCl_3$ (4.2 mg/kg per day i.p. for 28 d) is concurrent with decreased AchE activity. Further, AD is characteristic by the accumulation of neuritic plaques and neurofibrillary tangles (NFT). The main component of the plaques is amyloid-beta (Aβ), a peptide produced from the cleavage of the amyloid precursor protein. Ample evidence proposes that the

increase of Aβ in the brain of AD patients is related to the increased production or reduced clearance, accompanied by inflammation [43], oxidative stress [44], NFT formation [45], neuronal loss [46] and ultimately results in AD-related cognitive impairment [47]. Concerning the main role of oxidative stress in the AD, the supplementation of anti-oxidant vitamins and products decreased AD occurrence in patients [48]. In earlier stages of AD pathogenesis, oxidative stress might result in Aβ deposition in order to shield neurons from oxidative damage [49]. Consequently, accumulated Aβ induces Ca^{2+} dependent oxidative stress by stimulating NADPH oxidase in astrocytes, resulting in depleted glutathione (GSH) concentrations in astrocytes and nearby neurons, which might be adequate to get neuronal death [50]. In addition, exposure of mice to 2–10 Gy X-rays showed determined changes in neurogenesis, which were accompanied by spatial memory preservation deficits [51].

There is emerging evidence supporting a relationship between cerebral hemodynamic impairment and cognitive function. Cardiac failure, atherosclerosis, steno occlusive and small artery diseases affect the blood supply to the brain, most likely affecting the function of the neurovascular unit, and blood brain barrier. A disrupted blood brain barrier produces inflammation, oxidative stress and exposes neurons to neurotoxic proteins [52]. On the same line, Valenti and coauthors [53] showed evidence suggesting that the vascular risk factors play an important role in the pathogenesis of AD. Arterial hypertension represents an important risk factor for dementia, and it has been noted that certain antihypertensive medications, such as angiotensin-converting enzyme inhibitors (ACE-Is), independently from blood pressure regulation, might be protective against dementia, and thus, could lead to improve cognitive outcomes [54]. The neurotoxicity of Al and its relation to initiation and progress of neurodegenerative disease containing Parkinson's disease and Alzheimer's disease has been defined [55]. Whereas, the exact mechanism of Al-induced neurotoxicity is not clearly defined, however, some possible mechanisms have been proposed. Induction of oxidative stress [56], disorder in the intracellular hemostasis of calcium ion [57], rise in intracellular accumulation and structural modification of beta-amyloid peptide [58], promotion of apoptosis is among the most cited mechanisms [59]. Moreover, the incidence of vascular pathology leads to chronic cerebral hypoperfusion, blood- brain barrier breakdown, and inflammation that most likely precede neuronal death and neurodegeneration [60].

Seladin-1 (Selective AD indicator-1) is a neuroprotective gene that was identified and found to be downregulated in AD-vulnerable brain regions [61]. In the present study, there was a significant decrease in the Seladin-1 gene expression in irradiated rats or $AlCl_3$ administrated group. These changes in Seladin-1 gene expression could be attributed to the oxidative stress elucidated in severe inhibition of MT induction and the disturbance in lipid profile TGs. Decreased levels of Seladin-1 seem to disturb normal lipid raft formation as a result of low membrane cholesterol levels, leading to altered APP-BACE (amyloid precursor protein-β-secretase) compartmentalization [62]. Treatment of γ-radiation damage and toxic effect of $AlCl_3$ using glucosamine led to an improvement in seladin-1, the protective gene against oxidative stress [63], in turn, inhibition in Aβ accumulation and tau protein occurred. On the other hand, inhibition of Seladin-1/DHCR24 (Dehydroxycholesterol reductase 24) has been shown to increase Aβ accumulation accompanied by the imbalance of cytosolic Ca^{2+} [64].

The protective effect of glucosamine is related to that: Proteomic studies suggest that there were more than 1500 proteins in the cell are modified by O-linked β-N-acetylglucosamine (O-GlcNAc) and that these proteins have diverse functions including cytoskeletal proteins, nuclear pore proteins, RNA polymerase II, transcription factors, proto-oncogene products, tumor suppressors, hormone receptors, phosphatases, and kinases [65]. O-GlcNAc levels have been shown to be elevated in response to different forms of cellular injury [66]. Yuzwa and coauthors [67] suggest O-GlcNAcase as a potential therapeutic target could hinder the progression of Alzheimer's disease. In addition, Nöt and coauthors [68] exhibit that glucosamine increases the survival rate post trauma-hemorrhage without resuscitation; this effect may be GlcNAc associated with the glucosamine-induced increase in protein O-glycosylation.

Perturbations in the metabolism of UDP-GlcNAc (Uridine diphosphate N-acetylglucosamine), which alter the regulation of many O-GlcNAc modified proteins, have been implicated in Alzheimer's disease, diabetes and cancer [69]. According to Dalirfardouei and coauthors [70] , GlcN protects cardiomyocytes not only through an increase of -modified proteins, but also through attenuation of NF-κB activation, blocking the NF-κB signaling causes reduction of some pro-inflammatory mediator such as IL-6 and TNF-α cytokine as well as ICAM-1 in heart tissue, resulting in decreasing inflammatory responses in heart tissue.

In addition, Jamialahmadi and coauthors [71] reported that the glucosamine can inhibit scopolamine-induced impairments of spatial learning and memory in rats. Who referred this effect due to the antioxidant effect of GlcN and the anti neuroinflammatory effects of GlcN (which is conceivable as another explanation for its beneficial effects on memory impairments in scopolamine model).

Exposure to gamma radiation could be changed the biochemical markers responsible for the heart and brain functions which led to retard these functions. Glucosamine as a cardioprotective and neuroprotective drug can improve the damaging effect of gamma radiation, especially Seladin 1, APP, acetylcholinesterase, MTs, heart markers as well as the changes in the histopathological examination.

CONCLUSION

We concluded that glucosamine could be used to ameliorate the damage effects of the radiotherapy in cancer patients.

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