Galantamine mitigates neurotoxicity caused by doxorubicin *via* reduced neuroinflammation, oxidative stress, and apoptosis in rat model

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Abstract. – **OBJECTIVE:** Doxorubicin (DXR) is commonly used as a drug for cancer treatment. However, there have been reports of neurotoxicity associated with chemotherapy. Galantamine (GLN) is a medication that inhibits cholinesterase activity, providing relief from the neurotoxic effects commonly seen in individuals with Alzheimer's disease. This study explored the potential ameliorative effect of GLN on brain neurotoxicity induced by DXR.

MATERIALS AND METHODS: Forty rats were allocated into four separate groups for a study that lasted for a period of fourteen days. The control group was given normal saline, DXR group was given 5 mg/kg DXR every three days (cumulative dose of 20 mg/kg) through intraperitoneal injection. The GLN group was given 5 mg/kg GLN through oral gavage daily, while the DXR+GLN group was given DXR+GLN simultaneously. An analysis of brain proteins using ELISA to assess apoptosis through the concentration of inflammation and oxidative injury markers.

RESULTS: The DXR treatment led to increased neuroinflammation by elevation of nuclear factor kappa B (NF-κB), and cyclooxygenase-2 (COX-2), oxidative stress by rise of malondialdehyde (MDA), and decline of superoxide dismutase (SOD), and no changes in catalase and glutathione (GSH), cell death by elevation of Bax and caspase-3 and reduced Bcl-2, and increase lipid peroxidation, impaired mitochondrial function. When GLN is administered alongside DXR, it has been observed to positively impact various biological markers, including COX-2, NF-κB, MDA, SOD, Bax, Bcl-2, and caspase-3 levels. Additionally, GLN improves lipid peroxidation and mitochondrial activity.

CONCLUSIONS: DXR therapy in rats results in the development of neurotoxicity, and a combination of GLN can recover these toxicities, suggesting GLN promising evidence for mitigating the neurotoxic effects induced by DXR.

Introduction

The prevalence of cancer has increased significantly, making it a prominent cause of mortality worldwide[1]. Doxorubicin (DXR) is a commonly used drug for the treatment of various sorts of cancer, for example, breast, ovary, prostate, and thyroid cancer[2]. Some medications that can access the blood-brain barrier have the potential to cause neurotoxicity, even if they do not cross the barrier[3-5]. Although DXR has a restricted capability to penetrate the blood-brain barrier, it can still cause substantial neurotoxicity and intellectual problems in patients of various age groups[6]. DXR disrupts the function of mitochondria, leading to an overproduction of reactive oxygen species (ROS), which results in oxidative stress elevation and neuronal damage[6,7]. Additionally, the concentration of Bax is increased, and Bcl-2 is reduced by DXR, resulting in mitochondrial dysfunction and apoptosis[8]. The effects of DXR include the activation of neuroinflammatory pathways, leading to increased levels of peripheral interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and IL-6. These inflammatory mediators have the capability to pass through the blood-brain barrier (BBB) and trigger neuroinflammation within the central nervous system[9].

Elevated concentrations of systemic inflammatory cytokines, which are also connected to aging and neurodegeneration, contribute to the development of neurotoxicity[10,11]. It has been observed

that inflammation can negatively affect the BBB, yielding heightened oxidative stress and neuronal damage[12]. Changes in neuronal function that can affect cognitive function have been linked to elevated concentrations of IL-1β, TNF-α, and IL-6, nuclear factor kappa B (NF-κB), and cyclooxygenase-2 (COX-2)[13,14]. Furthermore, there is an elevation in neuronal oxidative stress characterized by the heightened production of ROS, dropped concentrations of antioxidants like glutathione (GSH) and superoxide dismutase (SOD), and an elevation in malondialdehyde (MDA), an indicator of lipid peroxidation[14-16]. These factors contribute to the deterioration of cellular structure and function[16]. It has been observed that oxidative stress can have detrimental effects on the body, including speeding up the aging process, harming neurons, and making the brain more vulnerable to metabolic and synaptic disruptions associated with conditions like Alzheimer's and Parkinson's illnesses[17]. Uncontrolled oxidative stress can lead to a decrease in the levels of endogenous antioxidants, for example, SOD and GSH, and an increase in MDA levels, which is reported in diseases associated with oxidative stress[18].

Apoptosis is a fascinating biological phenomenon in various processes such as development, aging, and injury[19]. External agents can also trigger apoptosis through the activation of death receptors[20]. During apoptosis, the cytoplasmic protein Bax becomes activated and binds to the outer mitochondrial membrane, which helps in the formation of pores[21]. These openings act as channels for the liberation of cytochrome C, which then activates caspase-3, initiating the process of apoptosis[22]. On the other hand, Bcl-2 is a protein that works against apoptosis by counteracting the actions of Bax, thereby reducing the evacuation of cytochrome c[22]. Thus, the balance between Bax and Bcl-2 expression is crucial for controlling apoptosis[23]. Furthermore, uncontrolled oxidative stress can lead to apoptosis, which is the primary mechanism behind neurotoxicity and subsequent neurodegeneration[24].

Scientific research[25] has demonstrated that the use of GLN in treatment has shown significant improvements in diminishing inflammation, oxidative stress, and apoptosis in patients and experimental models with Alzheimer's disease, leading to a decrease in neurotoxicity. Based on our previous investigation[9], it was found that co-administering GLN can help alleviate the cognitive impairment caused by DXR. This is achieved by improving the performance of rats

in behavioral tasks and reducing neuroinflammation through the decrease in IL-1β, TNF-α, and IL-6 concentrations and their gene encoding[9,26]. These findings are steady with another study[27] that demonstrated the potential of the acetylcholinesterase inhibitor donepezil to mitigate the neurotoxic effects of DXR therapy.

Despite the prevalence of neurotoxicity in cancer patients undergoing breast cancer treatment, there is still limited knowledge regarding the prevention of DXR therapy-induced side effects. This study examined the influence of DXR-induced neurotoxicity in female rodents, which can have a detrimental effect on the value of life for cancer survivors. Hence, it is quite fascinating to explore the potential effects of neurotoxicity on various aspects of brain health, such as neuroinflammation, oxidative stress, lipid peroxidation, impaired mitochondrial function, and apoptosis. This includes examining the concentrations of COX-2, MDA, SOD, GSH, Catalase, Bax, Bcl-2, and caspase-3, which are all factors that play an indispensable role in the degeneration of neurons[28,29]. The findings could provide valuable insights for clinicians and researchers to enhance their understanding of the impact of DXR on the brain of cancer survivors. This includes its effects on neuroinflammation, oxidative stress, lipid peroxidation, impaired mitochondrial function, and apoptosis. Additionally, the study highlights the potential preventive benefits of GLN in rats undergoing DXR therapy.

Materials and Methods

Drugs

DXR was obtained from the EBEWE Pharma Corporation, (Attersee, Austria). GLN was obtained from the Sigma-Aldrich Company (St. Louis, MO, USA).

Animals and Drug Administration

The animal house facilities at the well-known Pharmacy College, Qassim University, provided access to a group of forty female Wistar rats. These rats ranged in weight from 220-250 g and had reached the age of 12 weeks. The rats were kept in their own unique cages made of plastic and maintained in a laboratory environment that was carefully monitored and controlled. The environment had a light and dark cycle that lasted for 12 hours, and the temperature was adjusted to 25 degrees Celsius. The rodents were fed a regular meal and allowed

unrestricted access to a solution that was based on water. The rats were distributed into four batches: a control group, which received saline, and three treatment groups, GLN, DXR, and DXR+GLN, each consisting of ten rats. The rats in the treated groups received an intraperitoneal injection of a total dose of 20 mg/kg of DXR, which was administered at a dosage of 5 mg/kg every three days for a period of 12 days (the total dose). The GLN group and the DXR+GLN group both received a daily oral gavage dose of 5 mg/kg for the rats. GLN treatment began on the same day as DXR administration and continued every day thereafter.

Enzyme-Linked Immunosorbent Assays

After the last dose of medicine was given to the animals on day 14, they were euthanized via asphyxiation with carbon dioxide. The brains were removed from the animals in the control group as well as the treated groups (GLN, DXR, and DXR+GLN). Using a Q-Sonica Homogenizer (Qsonica L.L.C, Newtown, CT, USA), the brain was sonicated in Lysing Buffer known as Neuronal Lysing Buffer (N-PER), and then it was centrifuged for ten minutes at a speed of 12,000 x g. The supernatant was transferred with extreme care into a container with a capacity of 200 uL for later usage. The bicinchoninic acid assay (Pierce, Waltham, MA, USA) was used to determine the amount of protein present in each of the samples. Utilizing enzyme-linked immunosorbent assay kits (ELISA) procured from a reputable supplier (MyBioSource), we measured the concentrations of a variety of components present in the samples, adhering to the instructions provided by the manufacturer[30].

Complex I Activity of Mitochondria

The quantification of brain samples was performed using the Bradford method. The spectrophotometric assay at 340 nm was used to measure the mitochondrial capacity to oxidize NADH. The result was calculated by measuring the amount of NADH oxidized per milligram of protein and comparing the treatment to the control (saline) as a percentage[31].

Assessment of Lipid Peroxidation

In accordance with the standard operating protocols for laboratories, the brain tissues were cleaned and then homogenized with lysis buffer (N-PERTM). After that, the bicinchoninic acid assay was utilized to determine the total content of protein in each and every sample, and this was done before the lipid peroxidation assay was carried out. A spectrophotometric technique that included the utilization of thiobarbituric acid was applied in the course of an analysis of the levels of lipid peroxidation that were present in the sample. At a wavelength of 532 nm, the origination of thiobarbituric acid-reactive substances (TBARS) was calculated, and this provided the basis for the calculation of the lipid peroxidation index. The measurement of TBARS was normalized to the total protein content. This led to the estimate of the amount of TBARS that were generated per milligram of protein[31].

Statistical Analysis

The findings were run *via* the GraphPad Prism version 10.153 (Boston, MA, USA) so that a one-way analysis of variance could be achieved. The variables that were observed across all of the treatment groups were connected to the variables that were seen in the control group, and the results of that relationship were then reported as means and standard errors of the means. A p-value < 0.05 was considered statistically significant.

Results

Effect of GLN on DXR-Induced Oxidative Stress

After DXR treatment, the ELISA analysis exhibited a substantial rise in MDA concentrations and a decrease in SOD in the rat brain in contrast to the saline group ($p=0.05$), but there were no substantial changes in the levels of catalase or GSH. However, administration of GLN in conjunction with DXR was successful in mitigating the increase in MDA levels and bringing about an increase in SOD that was observed in the rats that had been treated with DXR (Figure 1A-D).

Effect of GLN Treatment on DXR-Induced Neuroinflammation

ELISA testing revealed a considerable increase in the COX-2 and NF-κB concentrations in rat tissues extracted from the brain that had been treated with DXR in comparison to those in the control group; this influence was prevented by the concurrent administration of GLN (Figure 2A-B).

Effect of GLN Treatment on DXR-Induced Neuronal Apoptosis

According to the results of an ELISA test, there was a discernible rise in Bax and caspase-3 concentrations and a drop in Bcl-2 concentrations in DXR-treated rat brain tissues in comparison to that in the saline group; however,

the influence was mitigated by the concurrent administration of GLN (Figure 3A-C).

Effect of GLN Treatment on DXR-Induced Lipid Peroxidation and Mitochondrial Complex I Activity

ELISA testing revealed a significant increase in the amount of lipid peroxidation as well as a decline in the amount of mitochondrial complex I activity in DXR-treated rat brain tissues in comparison to those treated with saline; however, this impact was prevented by the concurrent administration of GLN (Figures 4 and 5).

Discussion

Extensive clinical and experimental studies[32] have demonstrated the potential for neurotoxicity associated with DXR. This study investigated the effects of DXR-induced neurotoxicity and neuronal apoptosis, as well as the potential impact of GLN. This study has effectively demonstrated that the treatment with DXR leads to neuronal apoptosis by causing an increase in neuroinflammation, oxidative stress, lipid peroxidation, and mitochondrial impairment. Nevertheless, the concurrent use of GLN demonstrated a mitigating effect against DXR therapy.

The use of chemotherapy has been found to trigger an inflammatory reaction, as indicated by former data showing augmented levels of TNF-α, IL-6, and IL-1β after DXR therapy[30]. These mediators can penetrate the BBB, prompting central inflammation, oxidative stress, and neurotoxicity[33]. The increase in proinflammatory cytokines in the brain can disturb the permeability and structural stability of the BBB[34]. The existing exploration reveals an observed increase in MDA concentration and no significant changes in catalase and GSH concentration in the brain tissues of rats exposed to DXR treatment correlated to the saline group. This indicates the occurrence of oxidative stress, which can potentially harm cellular structures, lead to neurotoxicity, and contribute to the development of neurodegeneration. Thus, the MDA, catalase, SOD, and GSH levels were measured to evaluate oxidative stress in the rat brain after DXR therapy. The concentrations of MDA showed a statistically substantial increase and
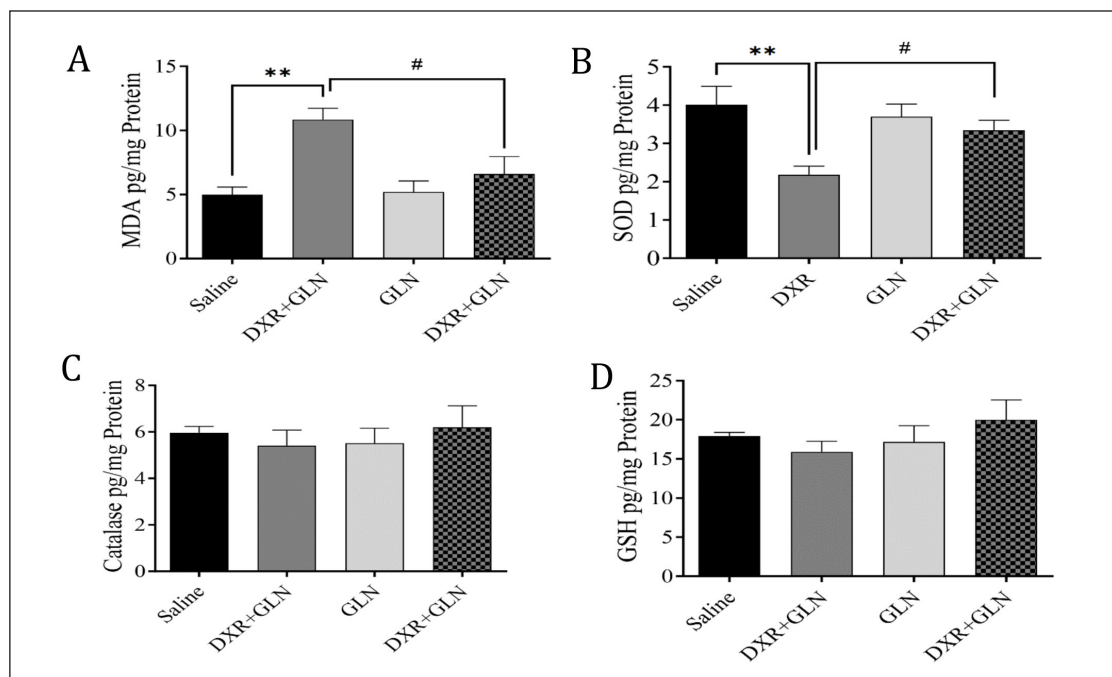

Figure 1. Investigating the impact of DXR and GLN on MDA, SOD, catalase, and GSH concentrations (**A-D**). The DXR administration at a dosage of 20 mg/kg through i.p. resulted in an elevation of MDA levels in the rat brain. Additionally, it led to a decrease in SOD levels, while having no impact on catalase and GSH levels. The concentrations of MDA, SOD, catalase, and GSH concentrations comparable to that in the saline were not altered by GLN treatment. The co-administration of GLN effectively prevented the increase in MDA levels and promoted the increase in SOD levels induced by DXR treatment in a group of 7 rats. (* $p<0.05$, ** $p<0.01$, and # $p<0.05$ were detected in comparison to the rat saline group and DXR, respectively (utilizing one-way ANOVA test).

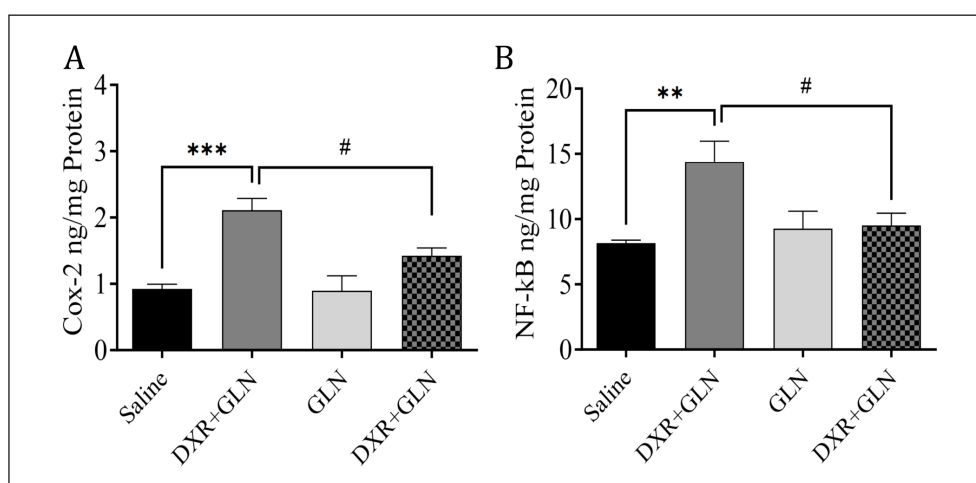**Figure 2.** DXR and GLN impact on the COX-2 and NF-κB concentrations (**A-B**). When compared to the rats given saline instead of DXR, the rats given DXR had higher levels of COX-2 and NF-κB expression after receiving an intraperitoneal injection of 20 mg/kg of DXR. However, treatment with GLN on its own did not provide the same results. Co-administration of GLN reduced an increase in COX-2 and NF-κB levels that would have been caused by treatment with DXR (n = 7). ** $p<0.01$ and *** $p<0.01$ comparable with the saline utilizing (a one-way ANOVA test). (# $p<0.05$) DXR+ GLN related to the rats in DXR.

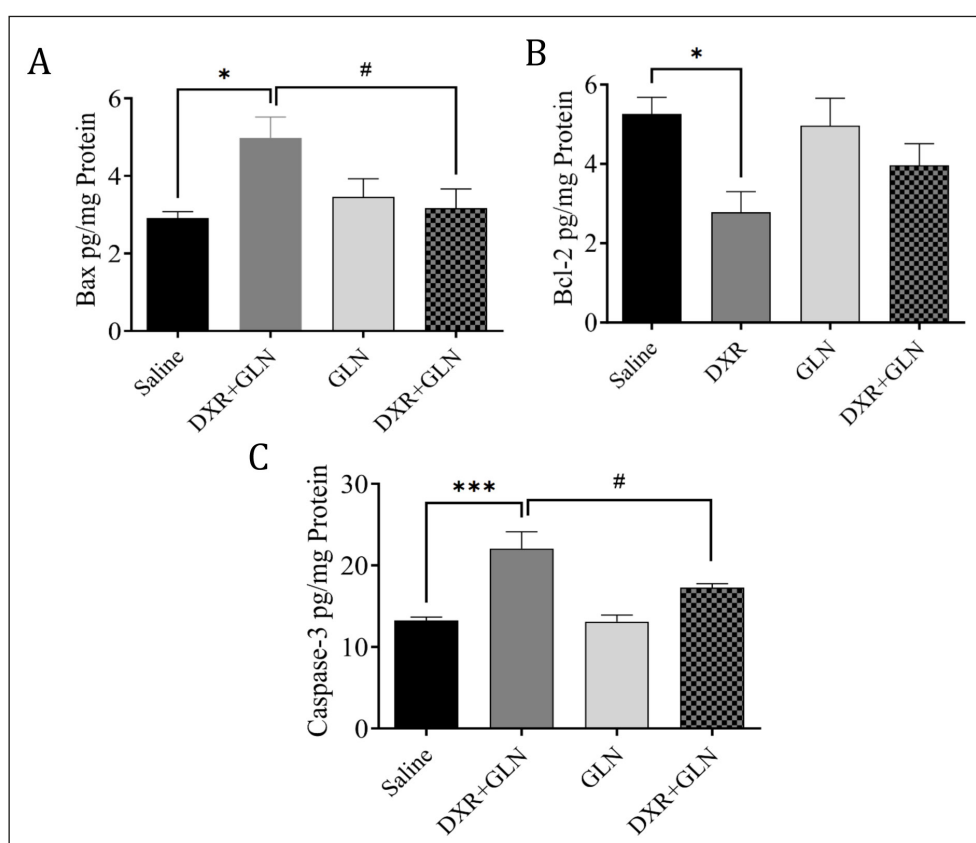**Figure 3.** DXR and GLN impact on the Bax, Bcl-2, and caspase-3 concentrations (**A-C**) The Bax and caspase-3 concentrations were augmented, and Bcl-2 was dropped in rats comparable with DXR (20 mg/kg, intraperitoneal injection), related to the saline group. However, GLN treatment did not have the same effect. The GLN co-administration was found to prevent the increase in Bax, Bcl-2, and caspase-3 concentrations, which were induced by DXR treatment alone. (n=7). * $p<0.05$ comparable with the saline utilizing (a one-way ANOVA test). (# $p<0.05$) DXR+ GLN related to the rats in DXR.
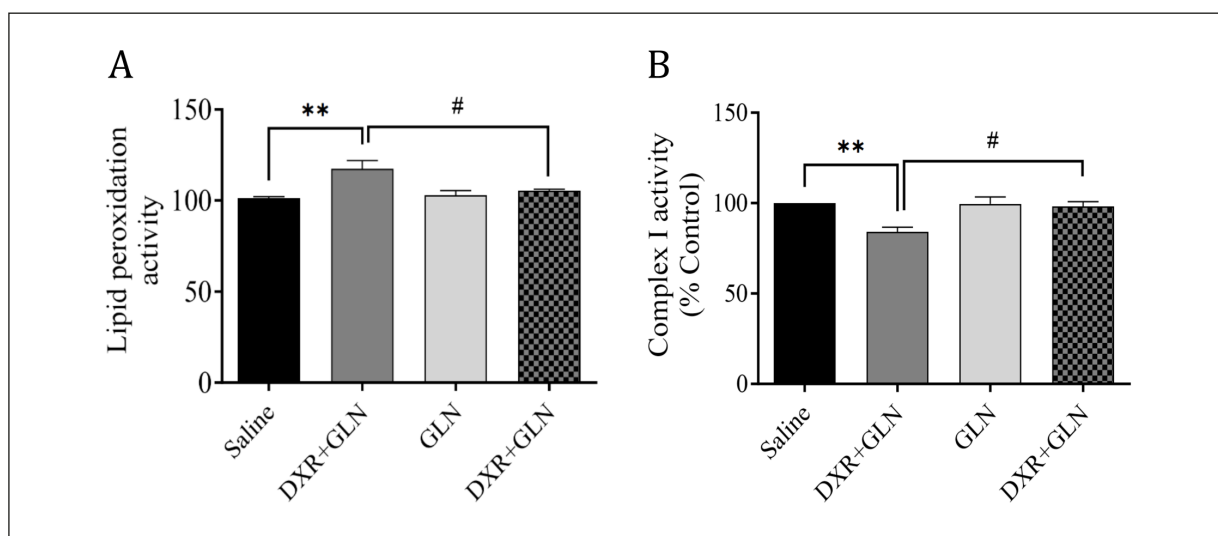
Figure 4. DXR and GLN impact on lipid peroxidation and complex I of mitochondrial function (**A-B**) The concentration of lipid peroxidation was elevated, and complex I activity was dropped by DXR curative (20 mg/kg, i.p.) compared to the saline group. However, GLN administration did not have any effect. The co-administration of GLN prevented the increase in lipid peroxidation and increased mitochondrial function triggered by DXR treatment (n=7). * $p<0.05$ comparable with the saline utilizing (a one-way ANOVA test). (# $p<0.05$) DXR+GLN comparable to the rats in DXR.
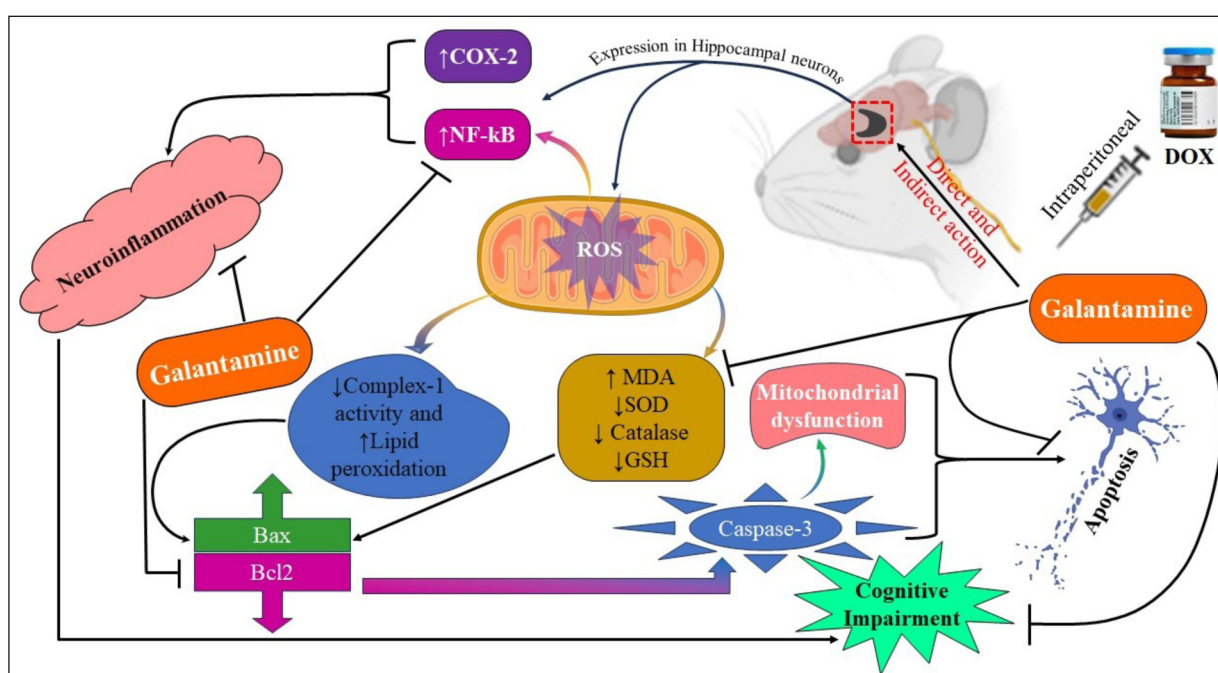

Figure 5. Diagrammatic representation of the process by which GLN mitigates DXR-induced neurotoxicity.

substantial decrease in SOD compared to the saline group. At the same time, the DXR treatment did not cause any noticeable changes in the catalase and GSH concentrations. Thus, these findings indicate that the rise in oxidative stress detected in rats undergoing DXR treatment may contribute

to the development of neurotoxicity. According to the information found in various sources, it has been observed that GLN displays antioxidant properties. The results of this study suggest that when GLN and DXR are given together, they can help reduce the harmful effects on the central ner-

vous system. The observed enhancement can be credited to the successful refurbishment of MDA and SOD concentrations in the brains of rats. As observed in this study, the protective effects of GLN align with previously documented findings.

To evaluate the effects of DXR on oxidative stress in the brain of rats, the quantities of MDA, SOD, catalase, and GSH were measured after the drug was administered[35,36]. The result indicated that there was a substantial increase in the amount of MDA and reduced in SOD concentration found in the brain samples when correlated to the saline group. Despite this, the DXR therapy did not produce any discernible changes in the amounts of catalase or GSH that were present. The rise mentioned above in oxidative stress, which was seen in rats that were given the DXR treatment, appears to have been related to a contemporaneous rise in neuroinflammation by a rise in the countenance concentrations of NF-κB and COX-2, which precipitated neuronal degradation. Accordingly, these data suggest that this association may cause neurotoxicity. According to the available literature, there is evidence to recommend that GLN possesses antioxidant and anti-inflammatory properties. According to the results of this study, the neurotoxicity was prevented when GLN and DXR were given concurrently by reducing oxidative stress and neuroinflammation. This attenuating effect is thought to be caused by the refurbishment of MDA, SOD, NF-κB, and COX-2 concentrations in the brains of rats. The findings of this investigation, which demonstrated the protective benefits of GLN, are consistent with results that have been described in earlier studies[27,37].

Apoptosis can be triggered by oxidative injury and neuroinflammation[38]. The levels and activity of Bax, caspase-3, and Bcl-2 serve a vital role in maintaining cellular function and triggering apoptosis processes[21]. Excessive Bax expression leads to harm to the mitochondrial membrane, leading to the liberation of cytochrome C from the mitochondria and subsequent triggering of caspase-3[22]. Indeed, it is noteworthy that neurodegenerative illnesses, including Alzheimer's and Parkinson's, have been linked to elevated Bax and caspase-3 protein expression[39]. In addition, studies[40,41] have shown that DXR treatment exerts its effect by modifying mitochondrial function, advancing to an overproduction of Bax and the activation of caspase-3, ultimately triggering apoptosis. The current study revealed an increase in the concentrations of Bax and caspase-3 expression after DXR administration. These findings indicate that the treatment with DXR can lead to neurotoxicity, resulting in neurodegeneration. Nevertheless, the simultaneous use of GLN showed a suppressive impact on the increased levels of Bax and caspase-3, and an improvement in Bcl-2 levels caused by DXR. These findings indicate that GLN protects against brain neurotoxicity caused by DXR in rats.

This study has both notable strengths and limitations. This study represents an innovative contribution to our understanding of the impact of DXR on neurotoxicity. To reduce the potential for bias, all of the animals utilized in this probe were of equivalent strain and age, and all of the experiments were performed at the same time. The rodents selected for the study were cancer-free, allowing for a focused assessment of the direct effects of DXR therapy while minimizing any potential confounding factors related to malignancy. Multiple doses are given to animal subjects to mimic the dosing regimen seen in human subjects. Further investigation may delve into different amounts of DXR and GLN, as well as other mechanisms pertaining to neurotoxicity. This could offer valuable insights for practitioners and researchers seeking effective treatments for chemotherapy-induced neurotoxicity.

Conclusions

The results confirmed the theory that DXR triggers neurotoxicity through the activation of neuroinflammation, oxidative damage, and neuronal apoptosis. In addition, the study examined markers related to neuroinflammation, oxidative stress, and apoptosis in order to well comprehend the fundamental mechanism of neurotoxicity. The findings indicated that DXR triggers oxidative stress and inflammatory mediators, leading to a rise in MDA, COX-2, and the concentrations of Bax and caspase-3 biomarkers of pro-apoptotic and inhibition in Bcl-2 anti-apoptotic protein. Additionally, it causes mitochondrial injury and lipid peroxidation, resulting in the development of neurotoxicity. The observed effects of DXR were successfully suppressed when GLN was administered at the same time, suggesting that GLN shows promise as a potential therapeutic option for reducing neurotoxicity caused by DXR therapy.

Conflict of Interest
No conflict of interest needs to be reported.

Authors' Contributions
M.A.A, A.S.A, S.K.A, and A.H.A. contributed equally regarding all aspects of the research, including conceptualization, research design, experiment conduct, data analysis, and writing and revising the manuscript.

Funding
None.

Ethics Approval
The study was authorized by the Institutional Committee on Animal Protection and Use of the Scientific Research Deanship at Qassim University (approval number 23-20-16).

Informed Consent
Not applicable.

Data Availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Acknowledgements
Researchers would like to thank the Deanship of Scientific Research, Qassim University for funding publication of this project.

References

1) Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis 2017; 9: 448-451.

2) Peter S, Alven S, Maseko RB, Aderibigbe BA. Doxorubicin-Based Hybrid Compounds as Potential Anticancer Agents: A Review. Molecules 2022; 27: 4478.

3) Alhowail AH, Pinky PD, Eggert M, Bloemer J, Woodie LN, Buabeid MA, Bhattacharya S, Jasper SL, Bhattacharya D, Dhanasekaran M, Escobar M, Arnold RD, Suppiramaniam V. Doxorubicin induces dysregulation of AMPA receptor and impairs hippocampal synaptic plasticity leading to learning and memory deficits. Heliyon 2021; 7: e07456.

4) Borchelt DR, Zhou W, Kavelaars A, Heijnen CJ. Metformin Prevents Cisplatin-Induced Cognitive Impairment and Brain Damage in Mice. PLoS One 2016; 11: e0151890.

5) Alhowail AH, Chigurupati S, Sajid S, Mani V. Ameliorative effect of metformin on cyclophosphamide-induced memory impairment in mice. Eur Rev Med Pharmacol Sci 2019; 23: 9660-9666.

6) Alhowail AH, Bloemer J, Majrashi M, Pinky PD, Bhattacharya S, Yongli Z, Bhattacharya D, Eggert M, Woodie L, Buabeid MA, Johnson N, Broadwater A, Smith B, Dhanasekaran M, Arnold RD, Suppiramaniam V. Doxorubicin-induced neurotoxicity is associated with acute alterations in synaptic plasticity, apoptosis, and lipid peroxidation. Toxicol Mech Methods 2019; 29: 457-466.

7) Gao Y, Dong J, Chen M, Wang T, Yang Z, He K, Li Y, Wang K, Jiang J, Zhang S. Protective effect of low-dose radiation on doxorubicin-induced brain injury in mice. Arch Biochem Biophys 2022; 729: 109390.

8) Du J, Zhang A, Li J, Liu X, Wu S, Wang B, Wang Y, Jia H. Doxorubicin-Induced Cognitive Impairment: The Mechanistic Insights. Front Oncol 2021; 11: 971.

9) Alsikhan RS, Aldubayan MA, Almami IS, Alhowail AH. Protective Effect of Galantamine against Doxorubicin-Induced Neurotoxicity. Brain Sci 2023; 13: 971.

10) Li X, Li C, Zhang W, Wang Y, Qian P, Huang H. Inflammation and aging: signaling pathways and intervention therapies. Signal Transduct Target Ther 2023; 8: 239.

11) Liu C, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: From Clinical Significance to Quantification. Adv Sci 2021; 8: e2004433.

12) Galea I. The blood–brain barrier in systemic infection and inflammation. Cell Mol Immunol 2021; 18: 2489-2501.

13) Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, Ho RC. IL-1β, IL-6, TNF- α and CRP in Elderly Patients with Depression or Alzheimer's disease: Systematic Review and Meta-Analysis. Sci Rep 2018; 8: 12050.

14) Alhowail AH. Pioglitazone ameliorates DOX-induced cognitive impairment by mitigating inflammation, oxidative stress, and apoptosis of hippocampal neurons in rats. Behav Brain Research 2024; 457: 114714.

15) Juszczyk G, Mikulska J, Kasperek K, Pietrzak D, Mrozek W, Herbet M. Chronic Stress and Oxidative Stress as Common Factors of the Pathogenesis of Depression and Alzheimer's Disease: The Role of Antioxidants in Prevention and Treatment. Antioxidants 2021; 10: 1439.

16) Singh A, Kukreti R, Saso L, Kukreti S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Molecules 2019; 24: 1583.

17) Liguori I, Russo G, Curcio F, Bulli G, Aran L, Del-la-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging 2018; 13: 757-772.

18) Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov 2021; 20: 689-709.

19) Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicologic Pathology 2016; 35: 495-516.

20) Zhou X, Jiang W, Liu Z, Liu S, Liang X. Virus Infection and Death Receptor-Mediated Apoptosis. Viruses 2017; 9: 316.

21) Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Front Oncol 2022; 12: 985363.

22) Zhang M, Zheng J, Nussinov R, Ma B. Release of Cytochrome C from Bax Pores at the Mitochondrial Membrane. Sci Rep 2017; 7: 2635.

23) Renault TT, Floros KV, Chipuk JE. BAK/BAX activation and cytochrome c release assays using isolated mitochondria. Methods 2013; 61: 146-155.

24) Kim GH, Kim JE, Rhie SJ, Yoon S. The Role of Oxidative Stress in Neurodegenerative Diseases. Experimental Neurobiology 2015; 24: 325-340.

25) Liu Y, Zhang Y, Zheng X, Fang T, Yang X, Luo X, Guo A, Newell KA, Huang XF, Yu Y. Galantamine improves cognition, hippocampal inflammation, and synaptic plasticity impairments induced by lipopolysaccharide in mice. J Neuroinflammation 2018; 15: 112.

26) Alhowail AH, Almogbel YS, Abdellatif AAH, Alsalehi NF, Alghenaim FA, Aldubayan MA, Felemban SG. CMF and MET treatment induce cognitive impairment through upregulation of IL-1alpha in rat brain. Eur Rev Med Pharmacol Sci 2021; 25: 4385-4393.

27) Ongnok B, Khuanjing T, Chunchai T, Pantiya P, Kerdphoo S, Arunsak B, Nawara W, Jaiwongkam T, Apaijai N, Chattipakorn N, Chattipakorn SC. Donepezil Protects Against Doxorubicin-Induced Chemobrain in Rats via Attenuation of Inflammation and Oxidative Stress Without Interfering With Doxorubicin Efficacy. Neurotherapeutics 2021; 18: 2107-2125.

28) Silvestro S, Sindona C, Bramanti P, Mazzon E. A State of the Art of Antioxidant Properties of Curcuminoids in Neurodegenerative Diseases. Int J Mol Sci 2021; 22: 3168.

29) Guo YL, Wei XJ, Zhang T, Sun T. Molecular mechanisms of melatonin-induced alleviation of synaptic dysfunction and neuroinflammation in Parkinson's disease: a review. Eur Rev Med Pharmacol Sci 2023; 27: 5070-5082.

30) Alsaud MM, Alhowail AH, Aldubayan MA, Almami IS. The Ameliorative Effect of Pioglitazone against Neuroinflammation Caused by Doxorubicin in Rats. Molecules 2023; 28: 4775.

31) Alhowail AH. Pioglitazone ameliorates DOX-induced cognitive impairment by mitigating inflammation, oxidative stress, and apoptosis of hippocampal neurons in rats. Behav Brain Res 2024; 457: 114714.

32) Śliwa-Tytko P, Kaczmarska A, Lejman M, Zawitkowska J. Neurotoxicity Associated with Treatment of Acute Lymphoblastic Leukemia Chemotherapy and Immunotherapy. Int J Mol Sci 2022; 23: 5515.

33) Versele R, Sevin E, Gosselet F, Fenart L, Candela P. TNF-α and IL-1β Modulate Blood-Brain Barrier Permeability and Decrease Amyloid-β Peptide Efflux in a Human Blood-Brain Barrier Model. Int J Mol Sci 2022; 23: 10235.

34) Yang J, Ran M, Li H, Lin Y, Ma K, Yang Y, Fu X, Yang S. New insight into neurological degeneration: Inflammatory cytokines and blood–brain barrier. Front Mol Neurosci 2022; 15: 1013933.

35) Simeonova R, Vitcheva V, Kondeva-Burdina M, Popov G, Shkondrov A, Manov V, Krasteva I. Alcesefoliside protects against oxidative brain injury in rats. Rev Bras Farmacogn 2019; 29: 221-227.

36) Guo J, Liu H, Zhao D, Pan C, Jin X, Hu Y, Gao X, Rao P, Liu S. Glucose-lowering effects of orally administered superoxide dismutase in type 2 diabetic model rats. NP J Sci Food 2022; 6: 36.

37) Alsikhan RS, Aldubayan MA, Almami IS, Alhowail AH. Protective Effect of Galantamine against Doxorubicin-Induced Neurotoxicity. Brain Sci 2023; 13: 971.

38) Jian Z, Guo H, Liu H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Oxidative stress, apoptosis and inflammatory responses involved in copper-induced pulmonary toxicity in mice. Aging 2020; 12: 16867-16886.

39) Kamal H, Tan GC, Ibrahim SF, Shaikh MF, Mohamed IN, Mohamed RMP, Hamid AA, Ugusman A, Kumar J. Alcohol Use Disorder, Neurodegeneration, Alzheimer's and Parkinson's Disease: Interplay Between Oxidative Stress, Neuroimmune Response and Excitotoxicity. Front Cell Neurosci 2020; 14: 282.

40) Kciuk M, Gielecińska A, Mujwar S, Kołat D, Kałuzińska-Kołat Ż, Celik I, Kontek R. Doxorubicin—An Agent with Multiple Mechanisms of Anticancer Activity. Cells 2023; 12: 659.

41) Zhang Y, Ahmad KA, Khan FU, Yan S, Ihsan AU, Ding Q. Chitosan oligosaccharides prevent doxorubicin-induced oxidative stress and cardiac apoptosis through activating p38 and JNK MAPK mediated Nrf2/ARE pathway. Chem Biol Interact 2019; 305: 54-65.