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Research Article

Comparison of Pioglitazone and Galantamine in Preventing Doxorubicin-Induced Glutamate Receptors mRNA Overexpression

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Abstract

Background and Objective: Doxorubicin (DXR) is a widely employed pharmaceutical agent in the therapeutic management of various malignancies. Nevertheless, it is important to note that DXR has been associated with various chemotherapy-related complications, such as neurotoxicity and glutamate overexpression. This investigation compared the protective properties of pioglitazone (PI) and galantamine (GLN) in mitigating mRNA glutamate AMPA and NMDA receptor overexpression caused by DXR in the brain. **Materials and Methods:** Sixty rats were divided into six distinct groups for this study (saline, PI, GLN, DXR, DXR+PI and DXR+GLN). The rats underwent RT-PCR analyses to evaluate the expression of glutamate receptors (AMPA and NMDA) subunits in relation to neurotoxicity. Results were analyzed using One-way ANOVA in GraphPad Prism version 10. **Results:** The administration of DXR treatment resulted in a notable upregulation in the expression of mRNA of the GluA1-AMPA receptor and mRNA expression of NR2A and NR2B-NMDA receptors. However, this effect was restored when co-administered with PI and GLN. **Conclusion:** The DXR therapy has been observed to elicit neurotoxic effects in rats through the upregulation of mRNA of glutamate receptors (AMPA and NMDA). While PI and GLN exhibits a reduction in AMPA and NMDA.

Key words: Rats, doxorubicin, pioglitazone, galantamine, glutamate receptors, neurotoxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The global incidence of cancer has been recognized as a significant cause of death¹. Doxorubicin (DXR) is a widely utilized chemotherapeutic agent that is commonly prescribed for the management of diverse malignancies, encompassing, but not restricted to, breast and prostate cancer^{2,3}. Chemotherapeutic agents possessing the capacity to pierce the blood-brain barrier (BBB) have been noted to prompt cognitive impairment, commonly known as chemobrain, even in cases where the BBB remains unimpaired⁴. The potential induction of notable neurotoxic effects and cognitive dysfunction has been observed in individuals of different age groups when exposed to doxorubicin, despite its limited capacity to cross the blood-brain barrier⁵. The DXR elicits mitochondrial dysfunction, leading to the generation of excessive reactive oxygen species (ROS), consequently initiating oxidative stress and neurotoxicity⁶. Doxorubicin, also known as DXR, has been found to exert a stimulatory effect on the expression of Bax, a pro-apoptotic protein, while simultaneously reducing the levels of Bcl-2, an anti-apoptotic protein⁷. The aforementioned molecular modification subsequently leads to impairment of mitochondrial function and the induction of programmed cell death, also known as apoptosis⁸. The DXR has been shown to prompt neuroinflammation via the augmentation of interleukin-6, tumor necrosis factor-alpha and peripheral interleukin-1 beta⁹. Pro-inflammatory cytokines are capable of inducing an inflammatory response within the central nervous system by piercing the blood-brain barrier (BBB)¹⁰.

Glutamate, a widely distributed excitatory neurotransmitter in the brain, can also be produced within the brain¹¹. There are two main types of glutamate receptors: ionotropic and metabotropic receptors¹². There is a general consensus that ionotropic receptors, such as AMPARs and NMDARs, have a significant impact on the regulation of synaptic function and cognitive encoding in neurons¹³. When glutamatergic neurons become active, they release glutamate from the presynaptic terminal¹⁴. This glutamate then binds to postsynaptic receptors, such as AMPARs and NMDARs, causing these receptors to undergo conformational changes^{14,15}. The AMPARs enable the entry of Na⁺ ions, resulting in the depolarization of the membranes of postsynaptic neurons¹⁴. This depolarization causes the expulsion of Mg²⁺ ions from NMDARs, leading to the influx of Ca²⁺ ions, which plays a crucial role in the formation of memories¹⁶. As a result, changes in the expression of these receptors can have a significant impact on the brain¹⁷.

The PI therapy has been scientifically proven to improve cognitive function in individuals with diabetes and Alzheimer's disease¹⁸. Furthermore, PI therapy demonstrates enhanced effects on inflammation, oxidative stress and neuronal survival in neurodegenerative diseases¹⁹. Thus, previous study found that the combination of PI and DXR can help alleviate cognitive decline in rats, specifically in tasks involving exploring new arms in the Y-maze, recognizing novel objects in the NOR test and reducing transfer latency in the elevated plus maze (EPM)⁹. In addition, the research revealed the potential of PI to alleviate neuroinflammation by decreasing the levels of IL-1 β , TNF- α and IL-6, which were found to increase in response to DXR⁹.

Galantamine (GLN) is an acetylcholinesterase inhibitor commonly used in the management of Alzheimer's disease²⁰. It works by inhibiting the enzymatic degradation of acetylcholine, which helps increase its levels and improve cognitive performance²¹. This has been demonstrated in both clinical and preclinical research studies²¹. In addition, certain medications such as donepezil and galantamine have been found to have a positive impact on neuroinflammation²². These medications work by reducing the levels of inflammatory mediators such as IL-1B, IL-6 and TNF- α , which can increase due to DXR therapy⁴.

Despite the frequent occurrence of neurotoxicity in cancer patients undergoing DXR therapy, studies have shown that DXR increases the expression of certain subunits that are associated with neurotoxicity. Increased expression of AMPAR and NMDAR leads to an influx of Ca²⁺, which can result in damage and toxicity to neurons⁶. The PI and GLN have been found to enhance cognitive function and alleviate neuroinflammation resulting from DXR^{4,9}. Thus, this study proposed that the simultaneous administration of PI and GLN alongside DXR therapy may potentially mitigate the overexpression of AMPARs and NMDARs subunits mRNA, thereby potentially reducing neurotoxicity. These findings have the potential to enhance practitioners' and researchers' understanding of the additional protective mechanisms of certain substances after DXR therapy.

MATERIALS AND METHODS

Investigation region: This research was carried out during March 2023 at the Pharmacology laboratory of the College of Pharmacy, Qassim University, Buraydah, Saudi Arabia.

Drugs: The DXR was acquired from EBEWE Pharma Company, (Attersee, Austria), GLN was purchased from Sigma-Aldrich, St. Louis, Missouri, USA and PI was purchased from Tabuk Pharma. Company (Tabuk, Saudi Arabia).

Experimental animals and drug treatment: Sixty 12-week old female Wistar rats weighing 210-230 g were obtained from the College of Pharmacy, Qassim University's Animal House. Individual plastic cages housed these rats in regulated laboratory conditions. The environment was a 12 hrs light-dark cycle at $25 \pm 2^\circ\text{C}$. Water and a standard food were freely available to the rodents. The DXR, PI, GLN, DXR+GLN and DXR+PI rats ($n = 10$) were assigned to control and five treatment groups. The DXR-/DXR+PI-treated rats received 5 mg kg^{-1} DXR intraperitoneally every three days for 12 days (total dosing: 20 mg kg^{-1}). The drinking water of PI and DXR combined PI rats was 2 mg kg^{-1} PI. The GLN and DXR mixed GLN rats received 5 mg kg^{-1} oral GLN daily. Daily PI and GLN were provided starting with DXR.

Reverse Transcription Polymerase Chain Reaction (RT-PCR):

The RNA was isolated from the brain for each individual group saline, DXR, PI, GLN, DXR+PI and DXR+GLN utilizing TRIzol reagent protocol as product manufactured instructions

(Sigma-Aldrich, St., Louis, Missouri, USA). This method was consistent with our previous study, where the mRNA expression concentrations of *GluA1*, *NR2A* and *NR2B* in comparison to *GAPDH* was measured (Table 1).

Statistics: Results were analyzed using One-way ANOVA in GraphPad Prism version 10 (GraphPad, Boston, Massachusetts, USA). The variables in all treated groups were compared to the saline group and provided as Means $\pm$ Standard error. The p-values below 0.05 were considered statistically significant.

RESULTS

PI or GLN prevents DXR elevate mRNA expression of *GluA1*

of AMPARs: The evaluation of mRNA expression of AMPARs-containing *GluA1* was carried out following a two-week treatment of DXR, PI, GLN, or a combination of these drugs. As shown in Fig. 1, the rats that underwent DXR treatment exhibited a significant increase in the mRNA concentrations of

Table 1: Primers utilized in the study

Gene	Sequence (5'-3')	Length (bp)
<i>GluA1</i>	Forward: GCCAGATCGTGAAGCTAGAAA	80
<i>GluA1</i>	Reverse: CTCGCTCTCCTTGAACCTTATT	
<i>NR2A</i>	Forward: GGAGGAGGTTGGGTCAATTTAT	86
<i>NR2A</i>	Reverse: AGTAGGCACTTGGGACTTTAC	
<i>NR2B</i>	Forward: GAGGAACCAGGCTACATCAAA	83
<i>NR2B</i>	Reverse: GGTCAACAGGTAAGGTCATAG	
<i>GAPDH</i>	Forward: ACTCCATTCTCCACCTTTG	104
<i>GAPDH</i>	Reverse: CCCTGTTGCTGTAGCCATATT	

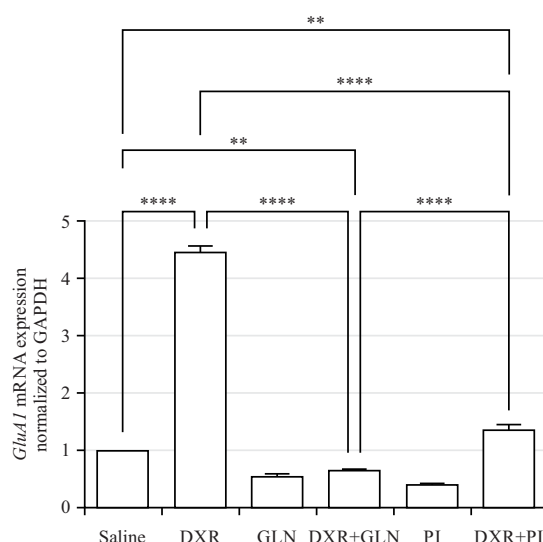


Fig. 1: DXR, PI, GLN and combination of PI or GLN with DXR effects on the expression of AMPAR containing-*GluA1*

The study examined the impact of DXR on the mRNA expression of the *GluA1* subunit of AMPARs in comparison to saline rats. The results revealed a notable increase in *GluA1* mRNA in the saline group, which was significantly enhanced by the co-administration of PI or GLN with DXR. The bars represent the Mean $\pm$ SEM, with different levels of significance indicated by asterisks (** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$)

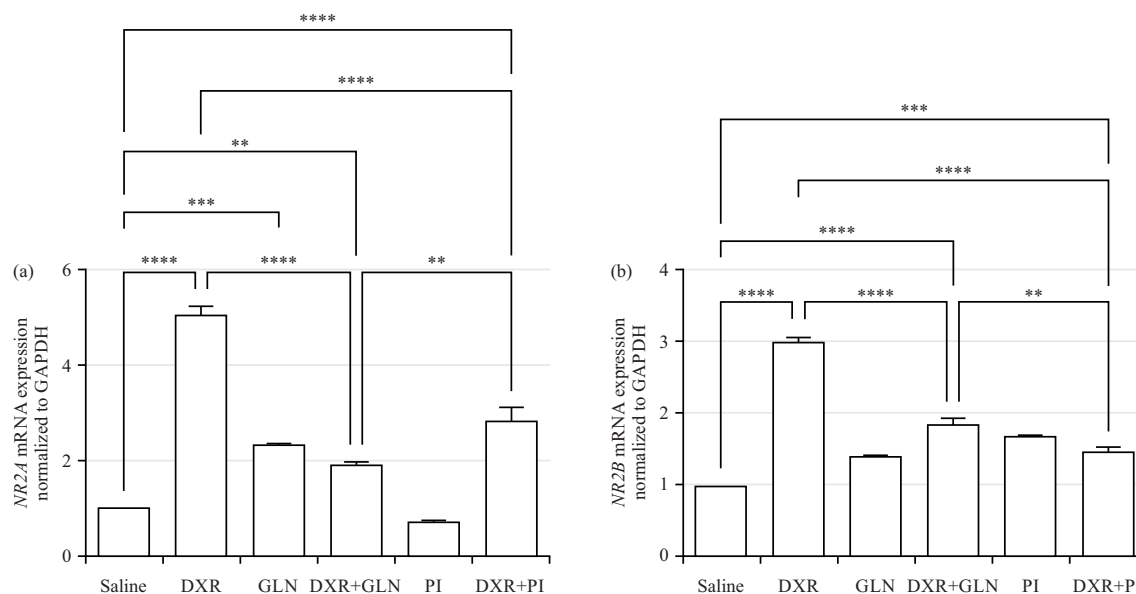


Fig. 2(a-b): Effects of different DXR, PI, GLN and a combination of PI or GLN with DXR on the mRNA expression of NMDAR-containing-*NR2A* and *NR2B*

The levels were compared to those observed in rats treated with saline. There was a notable increase in the expression of *NR2A* and *NR2B* mRNA in the group that received DXR, compared to the saline group. However, the mRNA concentrations of *NR2A* and *NR2B* showed a significant improvement with the co-administration of PI or GLN. The use of bars in this context demonstrates the depiction of the average value along with the standard error of the mean (** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$)

GluA1 expression compared to the levels observed in the saline rats. However, this increase was reversed when PI and GLN were administered together.

PI or GLN prevents DXR-induced elevation in mRNA expression of NMDA receptor subunits *NR2A* and *NR2B*. The mRNA expression concentrations of *NR2A* and *NR2B* were evaluated following a two-week treatment with DXR, PI, GLN and a combination of PI or GLN with DXR. The rats treated with DXR showed a notable increase in the mRNA expression of NMDAR-containing-*NR2A* and *NR2B* subunits compared to the rats treated with saline. This indicates the toxic effects of DXR. However, the elevation was reversed when PI or GLN were administered alongside DXR (Fig. 2a, b).

DISCUSSION

This study highlights the potential adverse effects of DXR, which can lead to neurotoxicity by elevating the expression of glutamate receptor mRNA in rats. The majority of the findings indicated that PI and GLN have neuroprotective effects by reducing the expression of glutamate receptors mRNA, thus improving toxicity levels. Extensive research has shown that DXR can have negative effects on cognitive function and can

be toxic to the nervous system^{23,24}. In addition, a recent study has found that DXR treatment can lead to cognitive impairment²⁵. This is believed to be caused by an increase in the expression of certain mRNA molecules related to glutamate receptors, specifically *GluA1* subunits of AMPARs and glutamate subunits of NMDRs. These changes in gene expression are thought to contribute to neuroinflammation and neurotoxicity⁹. Nevertheless, the combined use of PI and GLN showed their ability to prevent the increase in effects caused by DXR in the rat model.

The AMPA receptor is made up of four subunits, known as *GluA1-4*, that come together to form a tetramer²⁶. The subunits are expressed in varying ways within a single receptor composition. In the adult brain, the Ca^{2+} permeability of *GluA1* differs from that of *GluA2* due to the presence of arginine in its receptor^{26,27}. This arginine effectively blocks Ca^{2+} permeability through AMPARs²⁷. Thus, the inclusion of AMPAR with *GluA1* in its composition leads to the entry of Ca^{2+} at the cellular level, resulting in neuronal toxicity²⁸. In the brain of rats treated with DXR, this study found that the *GluA1* subunit mRNA was upregulated. This upregulation may lead to an increase in Ca^{2+} influx, which in turn can cause neuronal toxicity. Thus, these findings provide further evidence for the existing hypothesis that DXR leads to neuronal toxicity.

The NMDA receptor is made up of several subunits, such as *NR1*, *NR2A*, *NR2B* and *NR2C*²⁹. These subunits play a crucial role in synaptic plasticity and memory function²⁹. Impairment of memory function and long-term potentiation can occur when NMDA receptors are blocked or their expression is reduced due to prenatal exposure to nicotine or alcohol³⁰⁻³². This can be observed through electrophysiological techniques³². On the other hand, in Alzheimer's disease, there is an excessive activation of NMDA receptors due to an abundance of glutamate released from dying neurons³³. This results in an increased entry of Ca^{2+} into neurons, leading to neuronal toxicity and the start of apoptosis³⁴. This finding was consistent with previously reported research that shows DXR therapy decreases the removal of glutamate in the brains of rodents, while also increasing the mRNA expression of both AMPA and NMDA receptors³⁵. Yet, the results of this study provide additional support for the idea that PI and GLN can help restore the levels of *NR2A* and *NR2B* mRNA expression that have been increased as a result of DXR treatment.

This study has certain strengths and limitations. This study is the first, to the best of the authors' knowledge, to demonstrate the protective effect of certain substances against neurotoxicity induced by DXR. Specifically, these substances were found to elevate the expression of mRNA subunits of glutamate receptors. Furthermore, the animals used in this study were carefully selected to ensure they were of the same strain, age and condition. This included using cancer-free rats and conducting the experimental time simultaneously across all study groups. This approach helped to minimize any potential factors that could have affected the results. The doses were administered repeatedly to replicate the treatment regimen used in human patients can be ascribed to the inherent limitations imposed by the existing laboratory infrastructure.

CONCLUSION

The results corroborated the theory that DXR triggers neurotoxicity by increasing the expression of glutamate receptors. In addition, the study examined the molecular mechanism responsible for neurotoxicity. The findings indicated that DXR enhanced the expression of the mRNA of *GluA1* component of AMPARs, as well as the mRNA of *NR2A* and *NR2B* subunits of NMDARs. The observed effects of DXR were successfully inhibited when PI or GLN were administered simultaneously, leading to the restoration of both AMPA and NMDA receptor subunits. This suggests that PI and GLN have promising plausible as a viable therapeutic option for reducing neurotoxicity caused by DXR therapy.

SIGNIFICANCE STATEMENT

The current study aims to assess the potential protective effects of pioglitazone and galantamine against the elevation of glutamatergic receptors (specifically AMPARs and NMDARs) induced by doxorubicin. It has been found in a previous study that doxorubicin can lead to a significant increase in the expression of AMPARs and NMDARs, resulting in neurotoxicity. The study showed that rats treated with pioglitazone and galantamine experienced significant improvements in AMPARs and NMDARs subunits, which helped reduce neurotoxicity. These findings indicate that pioglitazone has a slight advantage in reducing toxicity in neurons.

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