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Research Article

Azilsartan Attenuates Cognitive Dysfunction in a Rat Model of Cisplatin-Induced Neurotoxicity: Effects on Hematological and CBC-Derived Inflammatory Indices

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Abstract

Background: Cisplatin (CIS) is one of the common anticancer agents; nevertheless, its utility is often associated with cognitive dysfunction as well as systemic toxicities. Current therapeutic options remain inadequate in its prevention or management. ***Objective***: To investigate the effect of AZIL to mitigate CIS-induced neurotoxicity and associated systemic alterations in rats. ***Methods***: Thirty-nine female rats were initially enrolled and divided into 4 groups: I: Normal control group (NC), II: Cisplatin group (CIS), III: azilsartan group (AZIL), and IV: CIS and AZIL combination group (CIS+AZIL). Neurotoxicity was induced using 2.5mg/kg CIS twice weekly for 4 weeks. Body weight was measured weekly. Cognitive performance was assessed via the Y-maze test at baseline and the end of the experiment. Blood was collected at the end of the study for hematology and inflammatory index assessments. ***Results***: Azilsartan mitigated CIS-induced neurotoxicity and enhanced cognitive function. By week 4 CIS markedly lowers body weight, which is not prevented by AZIL. In the combination group, AZIL significantly improved learning performance and spatial working memory (p=0.0009) at week 4 compared to the CIS group. Hematological alterations were minimal, while co-administration of AZIL showed non-significant effects in NLR and PNR (p>0.05). ***Conclusions***: Azilsartan mitigated CIS-induced neurotoxicity and improved cognitive performance and several hematological parameters, especially RBC and hemoglobin. Changes in CBC-derived inflammatory indices showed limited alterations.

Keywords: Azilsartan; Cognitive function; Cisplatin; Hematological parameter; Inflammatory marker; Neurotoxicity.

يخفف أزيلسارتان من ضعف الوظائف الإدراكية في نموذج الجرذان للسمية العصبية الناتجة عن السيسبلاتين: تأثيرات على مؤشرات الالتهاب والمرتبطة ب CBC

الخلاصة

الخلفية: السيسبلاتين (CIS) هو أحد العوامل المضادة للسرطان الشائعة؛ ومع ذلك، غالبا ما ترتبط فائدته بالخلل الإدراكي بالإضافة إلى السمية الجهازية. تظل الخيارات العلاجية الحالية غير كافية في الوقاية أو العلاج. **الهدف**: دراسة تأثير AZIL في التخفيف من السمية العصبية الناتجة عن CIS والتغيرات الجهازية المرتبطة بها في الجرذان. **الطرائق**: تم استخدام وتقسيم 39 فأرا أنثى في البداية إلى 4 مجموعات: الأولى: مجموعة الضابط الطبيعي (NC)، الثانية: مجموعة السيسبلاتين (CIS)، الثالثة مجموعة أزيلسارتان (AZIL)، والرابعة مجموعة CIS وAZIL المركبة (CIS+AZIL). تم تحفيز السمية العصبية باستخدام 2.5mg/لكل كغم CIS مرتين أسبوعيا لمدة 4 أسابيع. تم قياس وزن الجسم أسبوعيا. تم تقييم الأداء المعرفي عبر اختبار متاهة Y في البداية ونهاية التجربة. تم جمع الدم في نهاية الدراسة لتقييم أمراض الدم ومؤشرات الالتهاب. **النتائج**: خفف أزيلسارتان من السمية العصبية الناتجة عن السيسبلاتين وحسن الوظائف الإدراكية. بحلول الأسبوع الرابع، خفض نظام CIS وزن الجسم بشكل ملحوظ، وهو ما لم يمنع بواسطة AZIL. في المجموعة المركبة، حسن AZIL أداء التعلم والذاكرة العاملة المكانية بشكل ملحوظ (p=0.0009) في الأسبوع الرابع مقارنة بمجموعة CIS. كانت التغيرات الدموية طفيفة، بينما أظهر الإعطاء المشترك ل AZIL تأثيرات غير ذات دلالة في NLR وPNR (p>0.05). **الاستنتاجات**: خفف أزيلسارتان من السمية العصبية الناتجة عن السيسبلاتين وحسن الأداء المعرفي وتحسين العديد من المعايير الدموية، خاصة الكريات الحمر والهيموغلوبين. أظهرت التغيرات في مؤشرات الالتهاب المشتقة من CBC تغييرات محدودة.

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INTRODUCTION

Cisplatin (CIS), a platinum-derived chemotherapy, is widely used for the treatment of several tumors [1]. CIS as a platinum coordination complex can cause DNA damage via DNA adduct formation as well as DNA cross-links. It has been widely used since its approval in 1978 by the US Food and Drug Administration [2,3]. Nevertheless, adverse side effects during treatments as well as many iatrogenic toxicities limit the therapeutic benefits of CIS [4]. Among the most critical and serious problems correlated with CIS chemotherapy is neurotoxicity [4], which may manifest as peripheral sensory neuropathy and dysfunction of the central nervous system [5]. Experimental evidence and studies have further shown CIS can cause hippocampal dysfunction, synaptic integrity impairment, and neurotrophic signaling alteration and promote oxidative stress as well as neuroinflammation. These changes pathologically provide a biological base for chemotherapy-associated cognitive impairment or "chemobrain" [6–8]. Cognitive dysfunction caused by CIS is marked by impairment in learning, attention, memory, and recognition. In rodent studies, systemic CIS administration can lead to impairment of novel object recognition, as well as many other hippocampal-dependent behavioral abnormalities [7,9]. Toxicity of CIS is not restricted to the central nervous system;

systemic toxicity also can be caused by CIS and reflected by alterations in systemic health status and hematological parameters as well as inflammatory conditions [8,10]. Impairment of bone marrow function as well as alteration of circulating erythrocytes, neutrophils, lymphocytes, leukocytes, and platelets, and hemoglobin as well as hematocrit can be induced by CIS [11]. These hematological changes may also reflect alterations in systemic inflammatory as well as immune activity, because variations in circulating neutrophils and lymphocytes reflect alterations in adaptive and innate immunity [12,13]. Moreover, disturbances in the systemic inflammation can occur alongside the neuroinflammatory processes associated with CIS-induced cognitive dysfunction, providing a basis for evaluation of systemic inflammatory indices like neutrophil-to-lymphocyte ratio (NLR) alongside cognitive outcomes [7,8]. The classical renin-angiotensin system (RAS) is well acknowledged for its role in blood pressure regulation, inflammation, immune system regulation, and fluid homeostasis. Nonetheless, the RAS system has been proved to exist in the brain; this system's role and involvement in CNS functions has been recognized, involving alteration of neuroinflammation, apoptosis, and oxidative stress [14,15]. Azilsartan (AZIL), a unique angiotensin II receptor blocker (ARB) exhibits high lipophilicity and strong binding affinity to angiotensin II type 1 (AT1) receptors. The presence of an oxo-oxadiazole ring in AZIL that is absent from other clinically authorized ARBs made AZIL more lipophilic, less acidic, and more stable than other ARBs [18]. AZIL shows high selectivity for AT1 over AT2 receptors [16]. Current evidence proposes that ARBs, like AZIL, exert protective effects via reducing angiotensin-converting enzyme (ACE)/angiotensin (Ang II)/angiotensin II type 1 (AT1) receptor axis pathogenesis and promoting angiotensin converting enzyme 2 (ACE2)/angiotensin (Ang1-7)/mas receptor axis activity [17,18], However, whether AZIL can mitigate CIS–induced cognitive as well as systemic inflammatory disturbances is still insufficiently documented. This study evaluated AZIL in CIS-induced neurotoxicity via assessment of cognitive function, hematological alterations, and systemic inflammatory indices like NLR and PNR. We hypothesized that AZIL might mitigate CIS-induced cognitive and hematological alterations and potentially improve associated inflammatory indices.

METHODS

Drugs and chemicals

All the chemical reagents, drugs, and laboratory kits in the present experiment were obtained from an international standard supplier and manufacturer. Azilsartan Medoxomil potassium was obtained from Hikma Pharmaceuticals, United Kingdom; CIS from Accord, UK; and Carboxymethyl cellulose (CMC) was obtained from Celotech company, China.

Animals and ethical consideration

This study was carried out initially on 39 healthy adult female Wistar albino rats (only thirty-two rats completed and included in the analysis) weighing 200±40 g and aged 8-10 weeks, which were obtained from the animal house of the College of Pharmacy-University of Sulaimani. These rats were kept in a clean environment individually in polypropylene cages for one week to acclimatize to the standard laboratory conditions [temperature (25±2ºC), relative humidity (44-56%), light and dark cycles (12:12 hours)] of a well-ventilated animal house and free to drinking water and food. Animals that were not healthy at baseline and showing signs of illness, rats that were outside the predefined range of weight and age, and rats that died during the experimental period were excluded from final analysis. Behavioral assessment was not blinded, since the researcher was aware of treatment groups while laboratory assessors were blinded to the allocation of experimental groups. No specific humane endpoint criteria were predefined; however, animals were monitored throughout the study for general health and signs of distress, severe dehydration, and inability to eat or drink. At the end of the experimental period, the animals were humanely euthanized. All procedures were conducted in accordance with ARRIVE 2.0 guidelines [19], and the ethical standards for animal experimentation were approved by the Research Registration and Ethics Committee of the College of Medicine, Hawler Medical University (Certificate No: 6-53-10/5/2026) and carried out in strict accordance with the requirements of the Institutional Animal Ethics Committee.

Induction of neurotoxicity

Neurotoxicity in rats was induced through intraperitoneal injection of CIS (2.5 mg/kg) twice weekly for four consecutive weeks [20]. This study utilized a pre-established animal model of cisplatin-induced neurotoxicity that demonstrated neurological and cognitive impairment using that dosing schedule. CIS has been administered repeatedly over weeks instead of a single dose as a model of chronic CIS-induced neurotoxicity, which represents cumulative exposure rather than a single-dose effect [21].

Experimental design and treatment protocol

The study design is outlined in Figure 1. It illustrates the animal groups, treatment protocol, and outcome measurements; thirty-nine female rats were initially randomly allocated to four groups using the blocking randomization technique. Each group was comprised of

eight rats, except the CIS group, which consists of 15 rats.

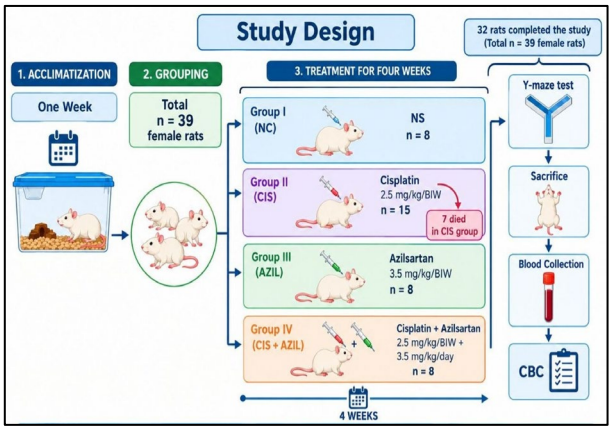


Figure 1: Experimental study design, groups, and treatment protocol. AZIL: azilsartan, BIW: twice weekly, CBC: complete blood count, CIS: cisplatin, NC: normal control, NS: normal saline, n: number.

Group I: Normal control (NC), kept as healthy control, received daily normal saline by gavage tube for four weeks, n=8. Group II: Cisplatin (CIS) received cisplatin 2.5 mg/kg intraperitoneally twice a week for four weeks, n=15. Group III: Azilsartan (AZIL) received azilsartan 3.5 mg/kg daily orally by gavage tube for four weeks (n=8). Group IV: Cisplatin+azilsartan (CIS+AZIL) received cisplatin 2.5 mg/kg intraperitoneally twice weekly in combination with daily azilsartan 3.5 mg/kg orally for four weeks, n=8. The doses were selected based on the literature [20,22]. Blood samples were collected on the 29th day. During the experimental period seven rats died from the CIS group; the overall mortality rate was calculated as % of mortality rate = no. of rats died/total number of enrolled rats * 100. The remaining thirty-two animals were completed in the study and enrolled in the analysis. AZIL was administered orally via gavage tube at a dose of 3.5/kg/day, based on previous studies that have shown protective and cognitive effects at this dose in rats [22,23]. AZIL was administered daily for four weeks, together with a CIS dosage of 2.5 mg/kg twice weekly, so this intervention was designed to evaluate the protective effect of AZIL in CIS-induced neurotoxicity. Azilsartan Medoxomil Potassium tablets were crushed and weighed to know the total weight of each tablet with the excipients, and then, according to the rats, different strengths of preparation were quantified by suspending in 0.5% CMC [22]. Each of them has been administered to animals orally via gavage tube once daily. AZIL were freshly prepared and suspended each day in 0.5% CMC just before administration. A carboxymethyl cellulose suspension was prepared via dissolving 0.5 g of CMC in 100 ml of distilled water and agitating until a homogeneous suspension was made. The body weight of each animal was measured at baseline and then once weekly throughout the experiment; body weight was recorded on the 29th day before scarification.

Evaluation of memory using the Y-maze test

A Y-maze test was conducted to evaluate spatial working memory and reference memory in 32 female Wistar albino rats that completed the study. The Y-maze test was performed 2 times at baseline and repeated at the end of the study in a clean, quiet room at room temperature and with the same light exposure. Rats were randomly selected for the tests at the baseline in each group, and the same selected rats were tested at the end of the study. The experimental apparatus is composed of three identical arms (A, B, and C) (each 50 cm long × 10 cm wide × 20 cm high) arranged at 120° angles to form a Y shape [24]. In this experiment two main types of Y-maze paradigms were conducted on the same day, which include: 1) A spontaneous alteration paradigm was conducted to assess working memory as well as spatial exploration. In this test animals were allowed to freely explore all 3 arms for 5 minutes, and all visits have been recorded. Visiting consecutive arms, such as (A-B-C) or (B-C-A), is counted as actual alterations, as well as the total number of entries recorded. The following equation has been used for measuring the percentage of consecutive entries into all three different arms [24]: Alteration %= n of consecutive entries into all three arms without repetition/possible total arm entries - 2 * 100. 2) A spatial reference memory paradigm was conducted to assess learning and memory of spatial location; this test is evaluated via a two-step protocol. The training phase in which rats were permitted to investigate two arms of the maze for 15 minutes, whereas the assigned “novel” arm (C) was blocked. The testing phase was in which, one hour later, rats were re-exposed to the maze for 5 minutes with the formerly blocked “novel” arm at this time open. The total number of entries into all arms and exploration of the novel arm, as well as time spent in the novel arm, were documented to assess memory retention and tendency for the unfamiliar environment [24].

Blood sampling and biochemical analysis

Blood samples were collected through caudal vena cava on day 29 following rats’ euthanasia. The samples of blood were then transferred into EDTA tubes for complete blood count (CBC) analysis and promptly transported to the laboratory for analysis using a Medonic™ M51 (Boule Medical AB, Sweden) hematology analyzer. Hematological parameters were reported in their respective units. The following inflammatory biomarkers, NLR and PNR (platelet to neutrophil ratio), are calculated from CBC-derived data [25,26] as follows: NLR= Neutrophil

count/Lymphocyte count. PNR= Platelet count/Neutrophil count.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 11.0.1 (GraphPad Prism Software LLC, CA, USA). Data is presented as mean ± standard deviation (S.D.). The Shapiro-Wilk test was utilized to evaluate the normality distribution of variables. Body weight and behavioral assessment were documented at different time points and analyzed using a two-way repeated measure ANOVA test with treatment group and time as factors involving treatment interaction. One-way ANOVA followed by Tukey's test is used for multiple group comparison, while the post hoc tests control the family-wise error rate. Where applicable, pairwise comparisons were articulated as mean differences accompanied by 95% confidence intervals (CIs) as well as effect size. A p-value of less than 0.05 was considered statistically significant.

RESULTS

As shown in Figure 2, thirty-nine female rats were initially allocated to four groups; throughout the experiment, 7 rats died from the CIS group, leaving thirty-two rats to compete in the study and be included in the statistical analysis, with 8 rats per group, so the overall mortality rate was 17.65% (7/39), while the mortality rate in the CIS group was 46.67% (7/15) during the experimental period. The effect of AZIL on body weight is shown in Figure 2, and measured weekly over the experimental period, at week 1 (baseline), the body weight among the groups was relatively comparable with no statistical difference between them (p> 0.05), while with some groups significant changes in body weights were observed from baseline compared to week 4.

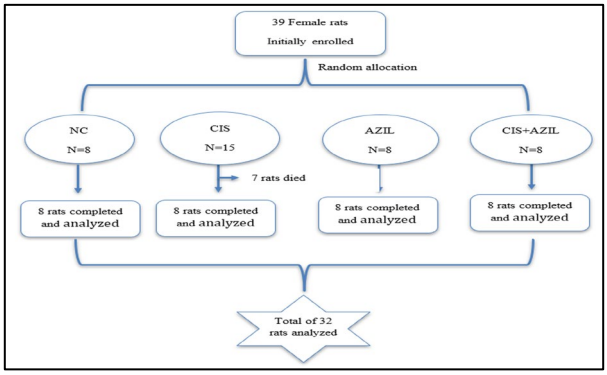


Figure 2: Animal flow diagram, showing allocation of groups, deaths, and animals completed the study and analyzed. NC: Normal control; CIS: Cisplatin; AZIL: Azilsartan.

In Figure 3, at week 2 there is a significant increase in body weight of the AZIL group compared to NC (mean difference= -37, CI: -66.7, -7.299, adjusted p< 0.008, Cohen's d= -1.624). No statistically significant difference was observed between different groups on week 3 (p>0.05).

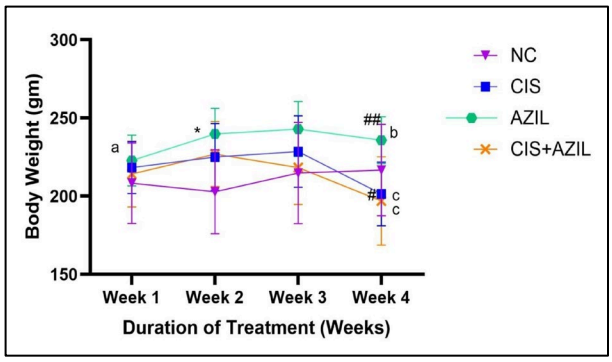


Figure 3: Effect of Azilsartan on Body Weight in Rats with Cisplatin-Induced Neurotoxicity. Data was presented as mean ± S.D, (n= 8 per group), data analyzed by two-way repeated measure ANOVA, with the treatment group and time (week 1 vs. week 4) as factors followed by Tukey's post hoc test. On Week 2: *p= 0.02 when comparing AZIL *vs*. NC, on Week 4: # p= 0.02 when comparing CIS vs. AZIL, and ## p= 0.005 when comparing AZIL vs. CIS+AZIL, non-identical letters "a,b,c," indicates significant difference with the baseline Week 1. NC: Normal Control; CIS: Cisplatin; AZIL: Azilsartan.

CIS showed a significant reduction in body weight on week 4 compared to baseline (mean difference=16.88, CI: 6.273, 27.48, adjusted p= 0.0004, d= 1.4), while the week 4 CIS group presented a reduction in body weight non-significantly compared to NC (mean difference=15.24, CI: -14.45, 95.5, adjusted p= 0.54, d= 0.699). The AZIL group maintained a higher body weight at week 4 compared to baseline significantly (mean difference= -13, CI: -23.6, -2.398, adjusted p= 0.0099, d= 1.14, p= 0.0099); in addition, AZIL at week 4 showed a statistically non-significant increase in body weight compared to the NC group (mean difference= 19.13, CI: -48.83, 10.58, adjusted p= 0.34, d= 0.699). (p= -0.840), while the combination of AZIL+CIS didn't significantly affect body weight (p= 0.98) compared to CIS at week 4, relative to baseline significantly lowering the body weight (mean difference= 17.25, CI: 6.648, 27.85, adjusted p= 0.0003, d= 1.51, p= 0.0003). The impact of azilsartan on learning (novel arm visit, exploration time to novel arm, time spent in novel arm) is presented in Figure 4 (A-C). At week 1 there were not significant differences between groups in the three parameters (p> 0.05), except for AZIL, which showed a significant increase in the time required to explore the novel arm compared to the CIS group (mean difference= -9.925, CI: -17.91, -1.94, adjusted p= 0.0091, d= -1.645). At week 4, there is a significant decline in the number of entries to the novel arm (mean difference=3.75, CI: 2.095, 5.405, adjusted p< 0.0001, d= 3) and time spent in the novel arm (mean difference=102.1, CI: 46.04, 158.2, adjusted p< 0.0001, d= 2.41) of the CIS group when compared to the NC group, but that decline is not significant for exploration time to the novel arm (mean difference= 6.8, CI: -1.185,

14.79, adjusted p= 0.121, d= 1.13). Moreover, the AZIL group at week 4 increased the number of novel arm visits and time spent in the novel arm significantly when compared to the NC group (mean difference=2.5, CI: 0.845, 4.15, adjusted p= 0.0011, d= 2) and (mean difference= -98.63, CI: -154.7, -42.54, adjusted p= 0.0001, d= -2.328, respectively), but the effect on exploration time to the novel arm is not significant compared to the NC (mean difference= 4.075, CI: -3.91, 12.06, adjusted p= 0.53, d= 0.676). Coadministration of AZIL with CIS at week 4 significantly increased the number of novel arm entries (mean difference= -2.75, CI: -4.405, -1.095, adjusted p= 0.0003, d= -2.2) and increased exploration time to that arm (mean difference= -13, CI: -20.99, -5.015, adjusted p= 0.0004, d= -2.155) as well as time spent in the novel arm (mean difference= -128.5, CI: -184.5, -72.41, adjusted (p< 0.0001), d= -3.034) when compared to the CIS group. Significant differences were found over time within groups; the CIS group showed a significant reduction in three parameters of the number of novel arm entries (mean difference= 2.25, CI: 0.857, 3.642, adjusted p= 0.0026, d= 1.17), exploration time to that arm (mean difference= 5.875, CI: 0.186, 11.56, adjusted p< 0.00001, d= 0.75), and time spent in it (mean difference= 52.25, CI: 8.868, 95.63, adjusted p= 0.002, d= 0.87) compared to baseline, while the AZIL group also significantly altered these three parameters when compared to its baseline (mean difference= 2, CI: 0.607, 3.392, adjusted p= 0.0065, d= 1.04) (mean difference= 13.08, CI: 7.386, 18.76, adjusted p< 0.0001, d= 1.67) (mean difference= -132.5, CI: -175.9, -89.12, adjusted p< 0.0001, d= 02.21) respectively, the combination group of CIS+AZIL significantly altered only time spent in the novel arm over time (mean difference= -44, CI: -87.38, -0.6177, adjusted p= 0047, d= 0.73).

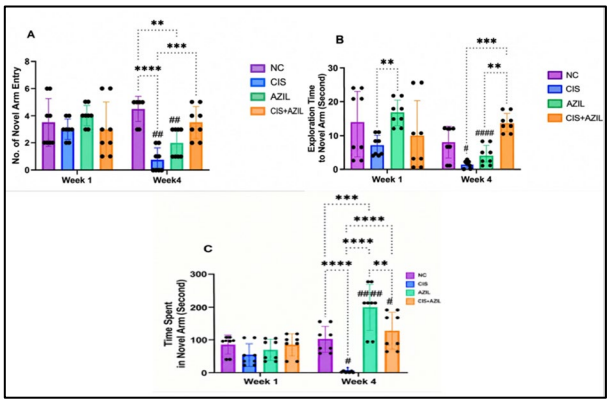


Figure 4: Effect of Azilsartan on Learning in Cisplatin-Induced Neurotoxicity in Rats (A). Novel Arm Visit (B). Exploration time to novel arm (C). Time spent in the novel arm. Data are presented as mean ± SD with individual data points shown (n= 8 per group); data are analyzed by two-way repeated measure ANOVA, with treatment group and time (week 1 *vs*. week 4) as factors followed by Tukey's *post hoc* test. **p< 0.01, ***p< 0.001, ****p< 0.0001 indicate significant differences between groups. #p< 0.05, ##p< 0.01, ###p< 0.001, and ####p< 0.0001 indicate significant differences compared at week 1 within the same group. NC: Normal Control; CIS: Cisplatin; AZIL: Azilsartan.

The impact of AZIL on the spatial working memory, as presented in Figure 5, does not show any significant differences among groups at week 1 in the % of spontaneous alteration (p> 0.05).

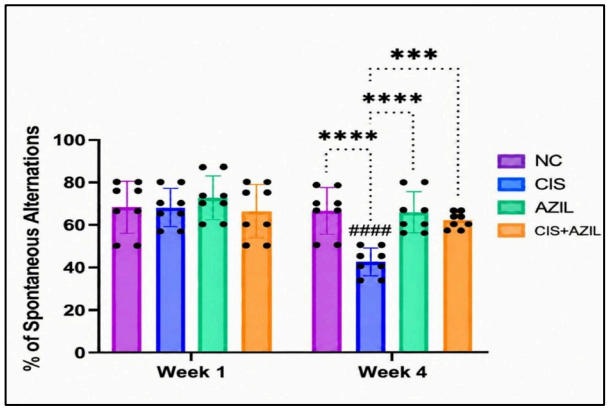


Figure 5: Impact of Azilsartan on learning in cisplatin-induced neurotoxicity in rats. Data represented as mean ± SD (n= 8 per group), with individual data points shown (n= 8 per group), data analyzed by two-way repeated measure ANOVA, with treatment group and time (week 1 *vs*. week 4) as factors followed by Tukey's post hoc test. (***p< 0.001, ****p< 0.0001) indicates significant differences between groups, and ####p < 0.0001 indicates significant differences compared with Week 1 within the same group." NC: Normal Control; CIS: Cisplatin; AZIL: Azilsartan.

However, at week 4, the CIS group experienced a significant reduction in % of spontaneous alteration when compared to the NC group (mean difference= 24.33, CI: 11.28, 37.37, adjusted p< 0.0001, d= 2.47); in contrast, the AZIL group as well as the CIS and AZIL combination group presented a significant elevation in % of spontaneous alteration when compared to the CIS group, even comparable to the NC (mean difference= -23.68, CI: -36.720, -10.63, adjusted p< 0.0001, d= -2.4) and (mean difference= -19.87, CI: -32.910, -6.831, adjusted p= 0.0009, d= -2.02, respectively). Over time, only the CIS group lowered the % of spontaneous alteration compared to its baseline significantly (mean difference= 25.93, CI: 15.32, 36.53, adjusted p< 0.0001, d= 1.77). The effect of AZIL on hematological parameters was demonstrated in Table 1. Analysis showed that CIS induced alterations in many blood parameters induced by CIS. The CIS group experienced a non-significant rise in total WBC count in comparison to the NC (p>0.05); meanwhile, the AZIL group resulted in values generally comparable to the NC, except for RBC values. The AZIL group showed significantly lower RBC values compared to the NC (p= 0.0008). For the combination group of CIS and AZIL, it is comparable to the NC group. Differential leukocyte counts, including neutrophils, lymphocytes, monocytes, eosinophils, and their percentages, did not reach a significant level. The effect of AZIL on inflammatory indices is presented in Figure 6 (A and B). The results for NLR as well as PNR showed exploratory differences between groups.

Table 1: The effect of azilsartan on hematological parameters in cisplatin-induced neurotoxicity in rats

Parameters	Parameters	NC	CIS	AZIL	CIS+AZIL
WBC (10^3/µL)	WBC	9.393±0.891	11.69±1.672	11.15±0.961	9.39±0.557
Neutrophil (10^3/µL)	Neutrophil	1.135±0.363	0.495±0.322	1.133±0.454	0.897±0.363
Lymphocyte (10^3/µL)	Lymphocyte	8.188±0.703	11.16±1.537	10.08±1.127	8.417±0.204
Monocyte (10^3/µL)	Monocyte	0.0±0.0	0.007±0.007	0.005±0.005	0.008±0.008
Eosinophil (10^3/µL)	Eosinophil	0.05±0.019	0.01±0.006	0.095±0.025	0.036±0.008
Basophil (10^3/µL)	Basophil	0.05±0.019	0.01±0.006[a]	0.103±0.024[b]	0.036±0.008
Neutrophil %	Neutrophil %	11.6±3.402	3.667±1.959	16.47±5.483	5.292±3.043
Lymphocyte %	Lymphocyte %	87.7±3.515	96.2±1.975	82.6±5.789	91.1±3.175
Monocyte %	Monocyte %	0.0±0.0	0.033±0.033	0.033±0.033	0.067±0.067
RBC (10^6/µL)	RBC (m/mm3)	7.192±0.109[a]	6.31±0.382	6.488±0.213[b]	5.597±0.1725
HGB (g/dL)	HGB	14.63±0.351[a]	12.73±0.994	13.12±0.5023	10.87±0.2242[b]
PLT (10^3µ/L)	PLT	619±36.48	578.5±64.07	803.3±59.11	560.7±101.7

Values are presented as mean±SEM (n=8 per group). One-way ANOVA was used to determine the differences between the groups, followed by Tukey's *post hoc* test. A non-identical superscript letter indicates significant differences between different groups. p= 0.004 (in basophil), p= 0.0008 (in RBC), p= 0.0012 (in HGB). NC: Normal Control; CIS: Cisplatin; AZIL: Azilsartan; WBC: white blood cell, RBC: red blood cell, HCT: hematocrit, PLT: platelet, PDW-CV: platelet distribution count.

The CIS group has a higher NLR value compared to NC and other groups, while the AZIL group showed numerically lower NLR and PNR values when compared to the CIS group, both when administered alone or given in combination with CIS, even though none of them reached the significant value (p> 0.05), indicating exploratory trends rather than confirmed treatment effects.

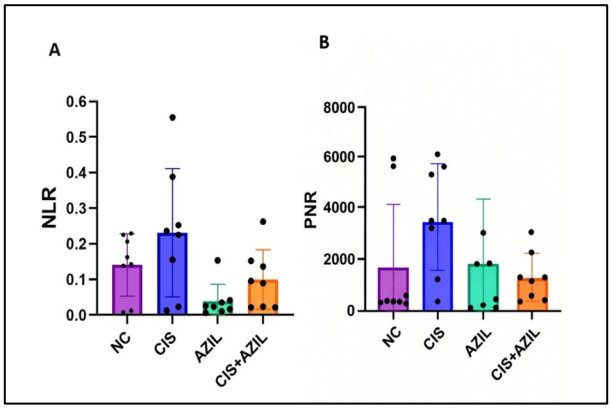


Figure 6: Effect of azilsartan on inflammatory indices in cisplatin-induced neurotoxicity in rats. (**A**): NLR, (**B**): PNR. Data represented as mean ± SD (n= 8 per group). Data were analyzed using one-way ANOVA. NC: Normal control, CIS: Cisplatin, AZIL: Azilsartan, NLR: Neutrophil to lymphocyte ratio, PNR: platelet to neutrophil ratio.

DISCUSSION

The cytotoxic effect of CIS can induce marked neurotoxicity, which might be present as memory impairment, learning deficit, and behavioral abnormality. Moreover, CIS-induced toxicity has been associated with dysregulation in inflammatory, oxidative, and apoptotic pathways [7,8]. Previous studies have drawn attention to RAS system involvement in many pathological conditions beyond its demonstrated role in blood pressure control and vascular effect, as well as this system's role and involvement in CNS functions, which has been recognized and may involve alteration of neuroinflammation, apoptosis, and oxidative stress [14,27]. Previous studies have highlighted that RAS-associated signaling, oxidative stress, and inflammatory pathways may contribute to CIS-induced neurotoxicity, particularly through excessive signaling via the Ang II/AT1 receptor, which is associated with neuroinflammatory processes. These mechanisms might contribute to systemic toxicity induced by CIS, including loss of body weight, although these mechanisms are not directly measured in the current study [14,15,28,29]. AZIL is one of the ARBs with potent AT-1 receptor binding properties and better antihypertensive activity in comparison to the older ARBs [30]. In this study, AZIL attenuated CIS-induced neurotoxicity, mainly by improving CIS-induced learning and memory impairment, and also improved several hematological parameters, especially RBC and hemoglobin changes. The increased mortality and reduction in body weight observed in CIS-treated rats reflect the overall systemic toxicity of CIS. Decreased food consumption, gastrointestinal irritation, dehydration, and general metabolic stress may be responsible for these effects. The study result revealed a mortality rate throughout the experiment in the CIS group, reaching 46.67% (7/15), so the overall mortality rate was 17.65%. This finding is consistent with previous reports showing that CIS exposure is suspected to be associated with a reduced survival rate and body weight in experimental rats [8, 31]. Research reported that the use of cytotoxic agents like CIS might lead to weight loss despite their efficacy against cancer. In this study, the CIS group maintained a notable reduction in body weight compared to both the NC group and the baseline, which is consistent with previous research indicating that weight loss and anorexia may be primary indicators of CIS toxicity [32,33]. AZIL alone increased body weight initially, suggesting good tolerability under experimental conditions, followed by a modest lowering in body weight at the end of the experiment, and previous data shows no weight loss following AZIL treatment [34]. Moreover, continued weight loss in combination groups of CIS and AZIL might indicate AZIL may have shown limited efficacy in resisting the weight-lowering effect of CIS. Nonetheless, weight loss induced by CIS might be associated with altered motivation or reduced activity;

previous studies on rats have documented reduction in weight, food intake, and change in locomotor activity following exposure to CIS [35, 36]; therefore, these systemic effects of CIS might have influenced Y-maze performance. In this study, CIS was administered as a chemo-brain model, and the neurotoxicity is evident by some behavioral parameters. In the Y-maze test, CIS administration resulted in impaired cognitive function, as evidenced by a reduction in the number of novel arm entries, the time spent in the novel arm, the exploration time to that arm, and the percentage of spontaneous alterations over time; in contrast, AZIL co-administration led to an increase in both the number of entries and the time spent in the novel arm, along with a rise in the percentage of spontaneous alterations that was even comparable to the normal control group. This improvement after the administration of AZIL may be partly due to the modulation of the RAS system in the brain. Former studies have proposed the blockade of the harmful pathway of AngII/AT1R, AZIL, might favor protective pathways like Ang IV/AT4R, and of the harmful pathway of AngII/AT1R, AZIL, might favor protective pathways like Ang IV/AT4R as well as Ang (1-7)/MasR. These pathways are related to improved memory consolidation, increased cerebral blood flow, neuroprotection, long-term potentiation, and cognition. This mechanism might explain the behavioral improvement seen in CIS-induced neurotoxic rats, even though these pathways have not been directly assessed in this study [37–39]. Peripheral blood cell counts are recognized and gained attention as a crucial indicator of systemic inflammation, whereas inflammatory indices calculated from lymphocytes, neutrophils, and platelets might give further information on the balance between adaptive and innate immune responses [12,13]. In the current study, CIS exposure for four weeks produced overall limited hematological disturbances alongside variable alterations of individual CBC parameters. This pattern is parallel with previous studies of CIS models that reported non-uniform hematological toxicity across cell lineages and may rely on dose, repeated exposure, and vulnerability of specific blood-cell populations [28]. Concurrent treatment of AZIL with CIS induced partial alterations of the hematological profile; the combination also demonstrated favorable trends in NLR as well as PNR. These alterations didn't reach a significant level; hence, they may be interpreted as exploratory findings rather than established anti-inflammatory effects. However, former experimental studies have reported that AZIL might modulate inflammatory signaling, such as TNF-alpha and NF-kB-related pathways [22,40]. Nevertheless, these mechanisms were not directly assessed in the current study.

Limitations

This study has many limitations that should be acknowledged when evaluating the findings. The relatively small sample size and the inclusion of only female animals in the study will limit the generalizability of the findings. Furthermore, the possible involvement of RAS was not verified directly via molecular analyses. Direct evaluation of hippocampal histopathology and oxidative stress, as well as pharmacokinetic parameters, was also not performed. The experimental duration was relatively short, and the lack of positive control neuroprotective agents further reduced the generatability of the results. Furthermore, CIS-induced systemic toxicity, body weight reduction, and potential activity alteration or motivation may have affected performance of the Y-maze test, since locomotor activity was not assessed separately; this potential confounding behavior cannot be neglected. Lastly, the largely nonsignificant alterations presented in inflammatory indices require cautious interpretation and should not be deemed as conclusive evidence of an anti-inflammatory effect. Further studies are required, incorporating both sexes, a larger size of sample, and a longer duration of treatment, as well as incorporating molecular, histological, and behavioral assessments to further explain the mechanisms and reproducibility of the finding.

Conclusion

Azilsartan attenuated CIS-associated deficits in selected cognitive behavioral parameters in rats. Nonetheless, its effects on hematological parameters as well as CBC-derived inflammatory indices were limited and predominantly nonsignificant. Further studies addressing molecular, histological, and mechanistic endpoints are necessary to establish whether RAS modulation contributes to the observed neuroprotective effects.

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Conflict of interests

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Data sharing statement

The data that supports the findings of this study are available from the corresponding author upon a reasonable request.

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