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Impact of Cyclosporine A on Cognitive Functions and Neuronal Oxidative Stress, Apoptosis and Inflammatory Markers in Rats

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Abstract: Cyclosporine A (CYP-A), a potent immunosuppressive agent, significantly impacts organ transplantation and the treatment of autoimmune disorders, and its ability to control autoimmune reactions and prevent organ rejection has made it indispensable in contemporary medicine. However, its negative effects, such as nephrotoxicity, neurotoxicity, and hepatotoxicity, limit its usefulness. The study investigated the effects of CYP-A on cognitive and neurological functions in rats over a fifteen-day period. Four groups of six rats each were treated with normal saline (control) or varying doses of CYP-A (20, 50, and 100 mg/kg). Behavioural assessments included the elevated plus maze (EPM) and novel object recognition (NOR) tests used to evaluate memory capabilities and recognition memory respectively, and the Y-maze assessed the exploration of a new environment and navigational skills. Post-experiment, brain tissues were analyzed for apoptosis, neuroinflammation, and oxidative stress markers to assess potential neurotoxicity. Histopathological analysis was also performed to provide further insights into CYP-A's effects on brain tissue. Compared to the control rats, there were significant changes in the memory parameters with two higher doses (50 and 100 mg/kg). The findings indicated that MDA, TNF-α, NF-κB, PGE2, and Caspase-3 levels increased significantly in the CYP-A 100 treatment compared to the control animals. However, a significant drop was observed in CAT, and GSH when comparing the CYP-A 100 group to the control rats. Conversely, comparable alterations were also noted in MDA,

TNF-α, PGE2, Bcl-2, Bax, and Caspase-3 levels between the CYP-A 100 and other treatment groups (20 or 50 mg/kg). Moreover, the histopathological analysis revealed that as compared to the control rats, CYP-A at three different dose levels (20, 50, or 100 mg/kg, p.o.) exhibited various changes. It was noticed that as the dose levels of CYP-A increased, there was a corresponding increase in brain tissue architectural changes. Precisely, the group treated with 100 mg/kg exhibited severe haemorrhages, inflammation, necrosis, and congestion compared to the control animals. In conclusion, CYP-A induces neurotoxicity, leading to oxidative stress, neuroinflammation, apoptosis, and cognitive deficits. Understanding the underlying mechanisms of this neurotoxicity is crucial to mitigating the adverse effects of CYP-A and improving patient outcomes. Further research is needed to develop targeted therapies and enhance the safe use of CYP-A.

Keywords: Cyclosporine-A; neurotoxicity; cognitive functions; inflammation; apoptosis; oxidative stress; SDG 3 Good health and well-being

1. Introduction

The field of organ transplantation and the treatment of autoimmune disorders have been significantly advanced by powerful immunosuppressive agents such as cyclosporine A (CYP-A). Due to its remarkable ability to control autoimmune reactions and prevent organ rejection, CYP-A has become an essential component of medical treatment in organ transplants [1]. CYP-A exerts its effects by binding to the cytosolic receptor cyclophilin, forming the CYP/cyclophilin complex[1,2]. This complex inhibits calcineurin, also known as Ca2+/calmodulin-regulated protein phosphatase 2B. Inhibition of calcineurin prevents the dephosphorylation of the nuclear factor of activated T cells (NFAT), thereby inhibiting T-lymphocytes from producing crucial cytokines necessary for immune responses, such as interleukin-2 (IL-2)[1,2].

Despite its benefits, the negative effects of CYP-A, including nephrotoxicity, neurotoxicity, and hepatotoxicity, limit its usefulness as an immunosuppressive drug[3,4,5]. Neurotoxicity, which was previously considered relatively uncommon, is now recognized more frequently and is documented in up to 40% of patients. Tremors are the most common neurological problem, occurring in 20–30% of patients on CYP-A therapy. Severe and occasionally fatal toxicity has been reported in a small percentage of transplant patients[6]. Recent studies indicate that around 10% of patients receiving CYP-A therapy experience neurological side effects, often necessitating dosage adjustments[7]. Neurotoxicity, defined as the harmful effects of a substance on the nervous system, can result in various neurological symptoms, including tremors, seizures, headaches, disorientation, altered vision, changes in consciousness, and behavioural or emotional disturbances[6].

The exact processes underlying CYP-A-induced neurotoxicity are still not fully understood. However, several factors are believed to contribute to its occurrence. One possible explanation is that CYP-A directly induces neurotoxicity by crossing the blood-brain

barrier (BBB) and entering the CNS, where it may interfere with neuronal function[8]. Previous research has indicated that inhibition of the AC/cAMP/PKA signaling pathways mediates the BBB dysfunction caused by CYP-A[9]. Additionally, repeated administration of CYP-A reduces brain-derived neurotrophic factor (BDNF) protein synthesis in the midbrain and hippocampus and downregulates its receptor, tyrosine kinase B[10]. Furthermore, administration of oral CYP-A at 50 mg/kg for 14 days significantly increased levels of neuronal cholinesterase, arginase, and TBARS[11]. In bicuculline-induced convulsion rats, a single subcutaneous injection of CYP-A (200 mg/kg) enhanced nitric oxide (NO) generation in the dorsal hippocampus[12]. Another study found that peripheral CYP-A suppresses the IFN-γ-Shh-BDNF pathway, negatively impacting neurogenesis and cognitive function during brain development. Moreover, oxidative stress plays a major role in CYP-A-induced neurotoxicity. CYP-A increases the generation of reactive oxygen species (ROS) in the mitochondria and initiates endoplasmic reticulum stress[13]. Neuroinflammation and increased oxidative stress can contribute to cognitive deficits, such as difficulties with learning and memory[14]. Elevated levels of ROS, NO, malondialdehyde (MDA), and inducible nitric oxide synthase (iNOS) have been linked to oxidative damage in the body[15].

Apoptosis, evidenced by markers such as cell body shrinkage, positive TUNEL staining, and internucleosomal DNA breakage, is the most likely cause of CYP-A-induced neuronal failure[16,17]. Several studies have reported that CYP-A induces apoptosis through mechanisms such as PARP cleavage, increased expression of pro-apoptotic components like Bax and p53, and decreased levels of the anti-apoptotic factor Bcl-2. This has been observed in cultured rat mesangial cells and a rat model of chronic nephrotoxicity[18,19]. Additionally, Caspase-3 activation has been noted following CYP-A treatment. Caspases play a crucial role in apoptotic events, particularly those mediated by the mitochondria. The cleavage of ProCaspase-3 into its active form is significantly enhanced by CYP-A in a time-dependent manner. This research also indicates that Caspase-9 and Caspase-3, but not Caspase-8, are activated in response to CYP-A-induced apoptosis[20].

Maintaining the pharmacokinetic parameters of CYP-A has been contentious due to its complex pharmacokinetics. In studies with adult male immunosuppressed mice at a dosage of 15 mg/kg/d of CYP-A, enhanced neurogenesis was observed in the dentate gyrus of the hippocampal region[21]. Conversely, in C57BL/6 mice, CYP-A at 40 mg/kg inhibited the formation of neuroblast dendrites in the dentate gyrus's subgranular zone[22]. Human studies across various organ-transplant groups, including bone marrow, kidney, and liver, have been conducted both before and after transplants. CYP-A therapy affects physiological elements of drug pharmacokinetics, such as lipoprotein concentrations. Both specific and non-specific methods have been used to evaluate drug concentration in plasma or blood[23]. These factors complicate comparisons of studies aimed at determining variables affecting drug availability in the human body. Due to the challenges of using CYP-A clinically, extensive research has identified potential causes of pharmacokinetic heterogeneity and toxicity in animal models.

According to previous literature, CYP-A may induce neurotoxicity through various mechanisms dependent on dosage, including oxidative stress, neuroinflammation, and cellular death. We plan to test this by closely examining the rats' apoptotic processes, neuronal inflammation, oxidative stress tolerance, and cognitive capacities (Figure 1).

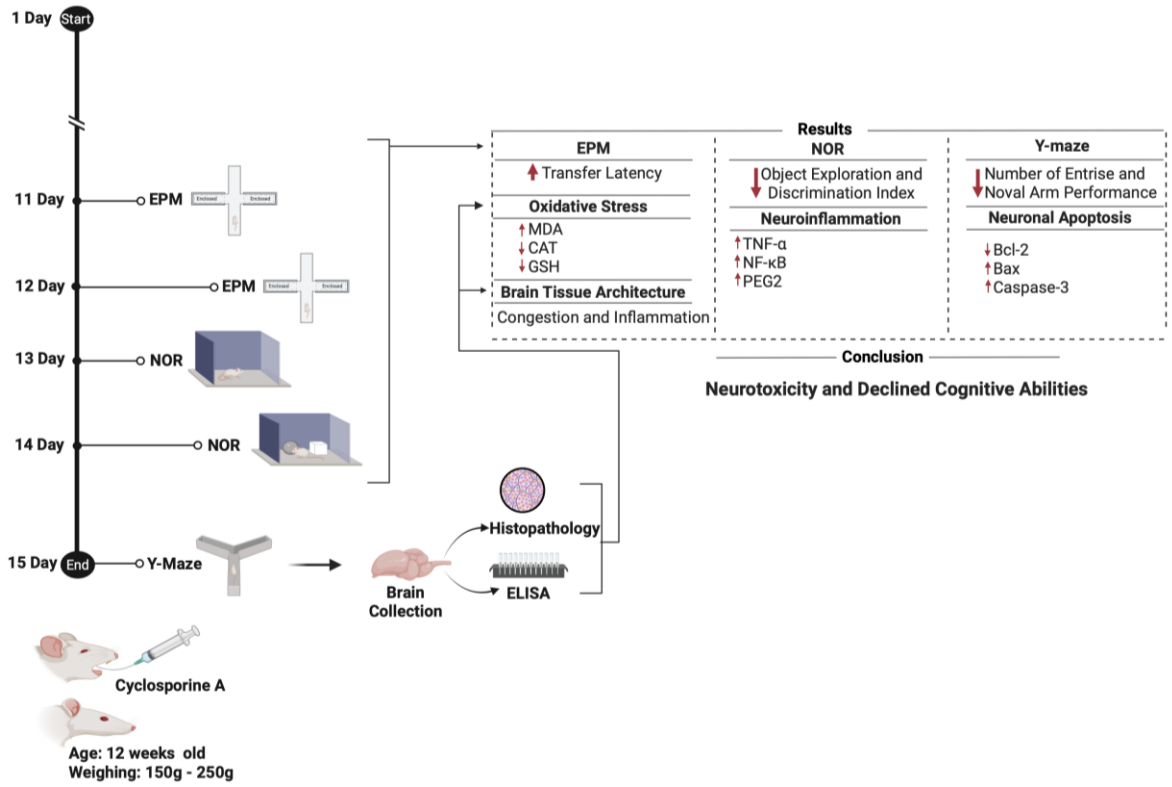

Figure 1. A graphical abstract illustrating the effects of CYP-A on cognitive functions, oxidative stress, neuroinflammation, neuronal apoptosis, and brain architecture in rats.

2. Materials and Methods

2.1. Animals

A total of twenty-four Sprague Dawley male rats, each weighing between 150 and 250 grams at the start of the experiment, were used. These rats, approximately twelve weeks old, were obtained from the College of Pharmacy at Qassim University in Saudi Arabia. The study's procedures were ethically reviewed and approved by Qassim University's Deanship of Scientific Research's Committee on Research Ethics (Approval Number: 23-69-09; 20/12/2023). The rats were randomly divided into four groups (n = 6) and housed in cages of three. They were provided with a standard pellet diet (First Milling Company, Jeddah, Saudi Arabia) and had unrestricted access to water. The housing environment was maintained at a room temperature of 22 ± 1°C with a humidity range of 60–70%.

2.2. Treatment Groups and Schedule

Figure 1 shows the duration of the experimental program, which spanned fifteen days for both medication administration and maze operations. The animals were divided into four

groups. The control group received normal saline. The other three groups were treated orally with different doses of CYP-A (Sandimmun, SN: 20012115289926, NOVARTIS): a low dose (CYP-A 20; 20 mg/kg), a middle dose (CYP-A 50; 50 mg/kg), and a high dose (CYP-A 100; 100 mg/kg) for fifteen days. The dosage choices were based on previous studies[11,24]. The elevated plus maze (EPM) was used for acquisition and retention trials on days 11 and 12. The novel object recognition (NOR) task included habituation, training, and testing phases on days 13 and 14. The Y-Maze was used for training and testing phases on day 15. Finally, brain tissues from each rat were collected for ELISA analysis and histopathological examination.

2.3. Cognitive Assessments

2.3.1. Elevated plus maze (EPM)

Animals are tested on their TL performance to determine their learning acquisition and memory retention capacities using the EPM test[25], in which a wooden maze measuring 50 cm (L) by 10 cm (B) with two open arms and two enclosed arms, measuring 50 (L) cm by 10 cm (B) by 30 cm (H) is used to assess these metrics. A 50 cm height above the floor is maintained during the studies. In this context, TL is defined as "the time it takes an animal to enter any enclosed arm from an open arm's starting point". The EPM operations were conducted over two days, on days 11 and 12 of the timeline. On the first testing day, TL values were recorded in seconds as the rats explored the maze from a consistent starting position at the end of each open arm. On day 2, the process was repeated, and TL values were recorded again. TL values from day 1 were indicative of acquisition performance, while TL values from day 2 were used for retention assessment[25,26].

2.3.2. Novel object recognition (NOR)

Animals are tested for recognition memory with two different items in the NOR task[25]. A wooden box that measured 60 cm x 60 cm x 40 cm was used for this test in conjunction with two cylindrical items that were identical and labelled as familiar objects (FO1 and FO2) and a rectangular object that was used as a novel object (NO). Three phases of the operations were performed over two days (days 13 and 14). Day 13 marked the start of the habituation phase, in which the rats were allowed to freely explore the wooden box for five minutes in the absence of any items. Following a 24-hour break (on day 14), the second segment took the form of a training session. In this stage, each animal had five minutes to investigate two known items (FO1 and FO2) that were positioned at two different corners as well as 10 cm from the wooden box's wall to allow for unrestrained exploration. The final part started as a test session four hours later. Rats were given five minutes to investigate one new item (NO) and one familiar object (FO) in this session. "The time consumed by each animal pointing its nose to an object within a distance ≤ 2 cm or touching or sniffing the object" is the definition of exploration time, which was noted for FO and NO during the test session. The DI% was used to evaluate the rats' capacity to distinguish between NO and FO[25,26].

2.3.3. Y-maze

An animal's tendency to spend time in a new setting and its predisposition to investigate a fresh arm may both be inferred from the results of the Y-maze. Rats were allowed to investigate a wooden Y-shaped environment with three arms that were 120 degrees apart throughout this test, with a height of 30 cm, a breadth of 10 cm, and a length of 50 cm for each arm. There were two stages to the process: on day 15, training and exam sessions were held. Out of the three arms, arm 'A' was identified as the novel arm and arm 'B' as the beginning point. Each animal was given five minutes to become acquainted with arms 'B' and 'C' during the training session, while the novel arm remained closed. After a four-hour break, each animal was given five minutes to explore further all three arms during the test session. The animals' time and number of entries in each arm were noted during the test period. In conclusion, each animal's proportion of time in the new arm was computed using previously developed methods [25,26].

2.4. Biochemical Analysis—Enzyme-Linked Immunosorbent (ELISA) Assay

2.4.1. Preparation of brain homogenate

At the end of the behavioural trials on day 15, all rats were humanely euthanized by cervical decapitation following the administration of mild anaesthesia (ketamine — 100 mg/kg and xylazine — 10 mg/kg). The brains were then carefully extracted and homogenized in cold phosphate-buffered saline (pH 7.4). The homogenized brain tissues were immediately centrifuged at 4000 rpm for 10 minutes. The resulting homogenates were stored at -80°C until further analysis. The total protein content in each brain tissue homogenate sample was measured using the Biuret colourimetric method (Crescent Diagnostics, Saudi Arabia).

2.4.2. Oxidative stress

This study evaluated oxidative indicators, such as glutathione (GSH) and catalase (CAT) as antioxidant markers and malondialdehyde (MDA) as an oxidative marker. MyBioSources Inc., San Diego, CA, USA, provided the rat-specific double antibody sandwich ELISA kits for MDA (MBS 738685), GSH (MBS 2540412), and CAT (MBS 2704433). All tests were performed in compliance with the guidelines included in the corresponding assay kits.

2.4.3. Neuroinflammation

The levels of three specific inflammatory markers were examined in brain homogenates in order to examine the effect of CYP-A on neuroinflammation. MyBioSources (San Diego, CA, USA) provided rat-specific ELISA kits for prostaglandin E2 (PGE2; MBS 7606497), nuclear factor kappa B (NF-κB; MBS 453975), and tumour necrosis factor-alpha (TNF-α; MBS 2507393). The tests were carried out in accordance with the manufacturer's instructions.

2.4.4. Apoptosis

The levels of two pro-apoptotic proteins (Bax and Caspase-3) and one anti-apoptotic protein (Bcl2) were specifically evaluated for apoptosis in the brain homogenate. The following rat-specific ELISA kits were purchased from MyBioSources Inc., San Diego, CA, USA: B-cell lymphoma 2 (Bcl2; MBS 452319), Bcl2 associated X protein (Bax; MBS 2703209), and cysteinyl aspartate specific proteinase 3 (Caspase-3; MBS 729893). The instructions in the manufacturer's handbook were followed for performing the test procedures.

2.5. Histopathological Analysis of Brain Tissue Architecture

After completing the treatment plan, brain tissues from all experimental groups were collected and immediately fixed in a 10% neutral formalin buffer for 48 hours. Then, tissues were properly processed using a Leica automated tissue processor, Leica TP1020, followed by dehydration through a series of graded alcohols. Samples were embedded in paraffin wax using a Leica embedding unit (Leica EG1160). Subsequently, a rotatory microtome (Leica RM2125) was used to cut thin sections (5 μm thick), which were then stained with hematoxylin and eosin staining. The stained slides were analyzed by independent pathologists in a blinded manner under a light microscope at 100× magnification. Photographs were taken, and the histopathological changes were analyzed.

2.6. Statistical Analysis

For the presentation of the results, the mean and standard deviation were used. GraphPad Prism version 9.5.0 (GraphPad Software Inc., San Diego, CA, USA) was used to conduct the statistical analysis. A Tukey-Kramer post-hoc test was used to discover the significant levels between two chosen groups after a one-way ANOVA analysis was conducted to find the levels of significance across all groups. Student's unpaired t-test was specifically used to compare the matching groups of FO and NO in the NOR test. It was determined that a p-value of < 0.05 represented the statistical significance level, confirming the statistical significance of the test.

3. Results

3.1. CYP-A Extended Transfer Latency (TL) of Rats in the EPM Test

The treatment rats' TL time was evaluated using the EPM test. Shorter TL values indicate facilitated memory and learning. Figures 2a and 2b illustrate how CYP-A therapies affect the TL values on days 1 and 2. The purpose of examining TL on the first day was to assess learning, while the objective of the second day's examinations was to evaluate memory. A one-way ANOVA revealed differences in the groups' TL performance [$F(3,20) = 8.689$, $p<0.001$ for TL on the first day and $F(3,20) = 11.23$, $p<0.001$ for TL on the second day], highlighting the impact of CYP-A on memory functions. Additionally, rats treated with CYP-A 100 exhibited a longer TL time (75.67±5.451 S; $p<0.001$) on day 1 compared to the control group (38.33±4.536 S). This difference indicates a decrease in the rats' ability to learn

following CYP-A 100 treatment. When CYP-A 20 mg/kg was administered, the TL time on day 1 was shorter (50.00±4.033 S; $p<0.05$) than in comparison with the CYP-A 100 group. Furthermore, the TL duration of the CYP-A 50 group was significantly greater than ($p<0.01$) that of the control group, measuring 66.83±7.901 S.

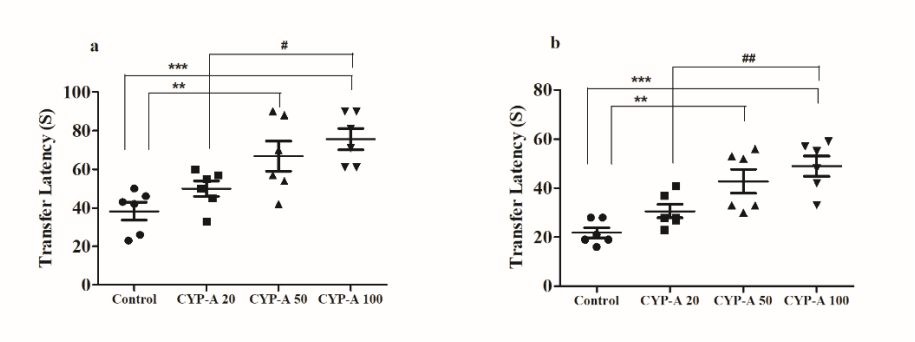

Figure 2. Impact of CYP-A on rats' first-day TL and second-day TL utilizing an EPM test (a) and (b) respectively. The mean ± SEM (n = 6) is used to express the results. Tukey-Kramer multiple comparisons test was conducted after the one-way ANOVA [$F(3,20)=8.689$, $p<0.001$ for first day TL and $F(3,20)=11.23$, $p<0.001$ for second day TL]. **$p<0.01$ and ***$p<0.001$ vs control; ##$p<0.01$ vs CYP-A 20.

On day 2, the TL time for the CYP-A 20 group (30.67±2.789 S) was significantly less ($p<0.01$) than that of the CYP-A 100 group (49.00±4.107 S), indicating CYP-A's impact on memory during higher dose. Additionally, TL increased from 21.83±2.056 S in the control group to 42.83±4.895 S ($p<0.01$) in the CYP-A 50 group. Furthermore, the TL differences between the CYP-A 100 group and the control group were highly significant ($p<0.001$), with the CYP-A 100 group showing more than twice the duration, demonstrating that CYP-A administration significantly affected memory function.

3.2. CYP-A Alters the Cognitive Performance of Rats in the NOR Test

The rats' memory and recognition were evaluated using the NOR test after receiving CYP-A. For the memory evaluation, the study investigated how CYP-A at three different dose levels (20, 50, or 100 mg/kg, p.o.) affected the exploration time of the familiar object (EFO) and the novel object (ENO), as well as the discrimination index % (DI%) between these items during the test session, as shown in Figures 3a–c, respectively.

One-way ANOVA analysis revealed significant differences in EFO length across groups [$F(3,20)=2.711$, $p<0.05$]. The group of CYP-100 rats explored for a considerably shorter period of time (14.00±1.949 S; $p<0.05$) than the control group (23.00±2.543 S) (Figure 3a). However, there was no significant difference between the CYP-20 (18.83±1.759 S) and CYP-A 50 (18.00±2.595 S) groups compared to the control group.

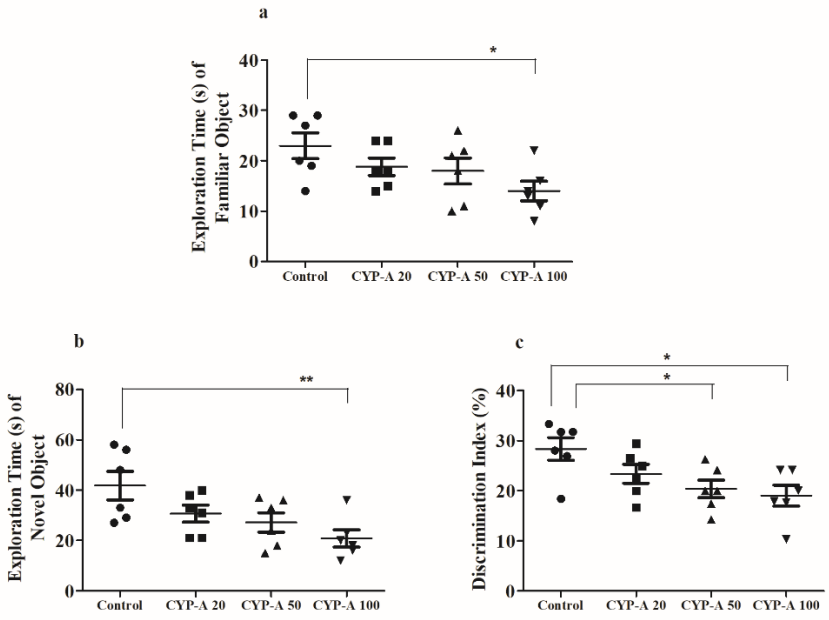

Figure 3. The effects of CYP-A on rats' (a) EFO, (b) ENO, as well as (c) DI% during the test session using the NOR test. The data is presented as mean ± SEM for the six subjects. One-way ANOVA Tukey-Kramer multiple comparisons test was used to compare data among the groups [$F(3,20)=2.711$, $p<0.05$ for EFO; $F(3,20)=4.445$, $p<0.05$ for ENO; and $F(3,20)=4.242$, $p<0.05$ for DI %]. *$p<0.05$ and **$p<0.01$ vs control.

Furthermore, CYP-A changed the ENO [$F(3,20) = 4.445$, $p<0.05$] among the treated groups when considering the exploration duration of the NO (Figure 3b). Additionally, there was a notable difference in the ENO between the control group (41.83±5.665 S) and the CYP-A 100 group (20.83±3.390 S; $p<0.01$). On the other hand, there were no significant differences between CYP-20 (30.67±3.333 S) and CYP-A 50 (27.17±3.877 S) compared to the control group, and also other treated groups.

The results of the one-way ANOVA analysis, as presented in Figure 3c, indicated that treatment with CYP-A led to significant changes in DI% [$F(3,20)=4.242$; $p<0.05$]. The duration of exploration for both FO and NO was considerably impacted by CYP-A, with a reduction in exploration time for each type of object. Furthermore, following CYP-A 100 treatment, the rats' DI% significantly decreased (19.04±2.096%; $p<0.05$) compared to the control group (28.35±2.232%). Additionally, there was a decline in the DI% of the CYP-A 50 group (20.37±1.783%, $p<0.05$). It is worth noting that while there was a slight decrease among the CYP-A 20 group (23.37±1.880%), it was not a significant change compared to the control.

3.3. CYP-A Alters the Cognitive Performance of Rats in Y-Maze

Figure 4 illustrates how CYP-A alters rat behaviour, namely novel arm exploration and environment adaption, both of which are tested in the Y-maze test. An analysis of variance in one way ($F(3,20)=7.488$, $p<0.01$ for the number of entries in the known arm (NEKA); $F(3,20)=5.529$, $p<0.01$ for the number of entries in novel arms (NENA);

F(3,20)=10.73, $p<0.001$ for the percentage of time spent in novel arm (TSNA%) demonstrated a statistically significant effect of CYP-A administered with different doses.

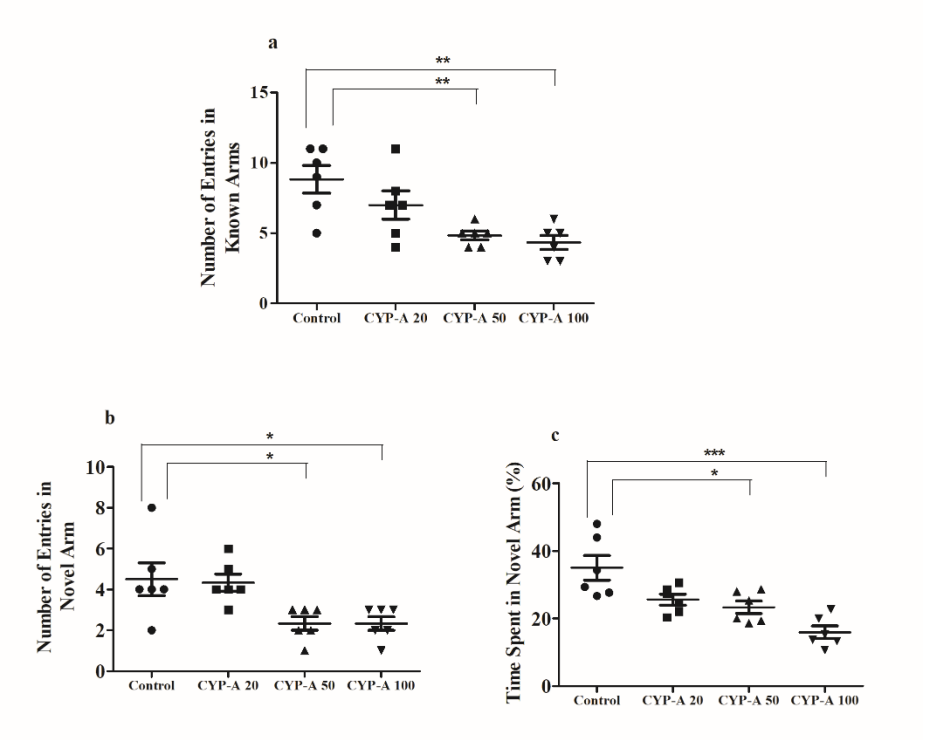

Figure 4. Impact of CYP-A on the following: (a) NEKA; (b) NENA; and (c) TSNA% during the rats' Y-maze test session. The mean ± SEM (n = 6) is used to express the results. ANOVA in one direction Tukey-Kramer multiple comparisons test was performed after the data were analyzed (F(3,20)=7.488, $p<0.01$ for NEKA; F(3,20)=5.529, $p<0.01$ for NENA; F(3,20)=10.73, $p<0.001$ for TSNA%]. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs control.

In particular, the administration of CYP-A 100 markedly demonstrated cognitive deficits by lowering NEKA (4.333±0.4944, $p<0.01$) in comparison to the control groups (8.833±0.9804, $p<0.01$) (Figure 4a). Similarly, when CYP-A 50 was administered, fewer NEKA were seen (4.833±0.3073; $p<0.01$) in comparison to the control group. However, there was no significant change in low-dose CYP-A 20 (7.000±1.000) compared with the control group.

Additionally, the administration of CYP-A 50 and CYP-A 100 had a similar impact on NENA (2.333±0.3333; $p<0.05$) compared to the control group's performance (4.500±0.8062) (Figure 4b). No significant difference was observed in CYP-A 20 (4.333±0.4216) when compared to the control group.

The TSNA% decreased when CYP-A was administered at 50 mg/kg (23.33±1.854%, $p<0.05$) and 100 mg/kg (15.95±1.843%, $p<0.001$). However, CYP-A 20 was calculated at 25.61±1.63 % compared to the control group, indicating no significant difference (Figure 4c).

3.4. CYP-A Induces the Oxidative Stress in Rats' Brain

The effects of CYP-A on certain antioxidant and oxidative indices in the brain are shown in Figure 5a–c. By assessing oxidative and antioxidant markers such as MDA, GSH, and CAT in brain homogenates, the effects of CYP-A-induced oxidative damage were further investigated. Among these markers, MDA is one of the important by-products of lipid peroxidation and a sign of oxidative stress. CYP-A administration substantially altered MDA levels in the brain (ng/mg protein) among treatment groups [$F(3,20) = 8.573$, $p<0.01$] (Figure 5a). CYP-A 100 caused significant oxidative damage to the brain, shown by the increase in MDA levels to 338.0±48.95 ($p<0.05$), compared to the control levels of 149.8±25.14. Notably, the doses of CYP-A 20 (164.0±11.24; $p<0.01$) and CYP-A 50 (200.7±17.16; $p<0.05$) resulted in a significantly lower level of brain MDA in comparison to the CYP-A 100 group.

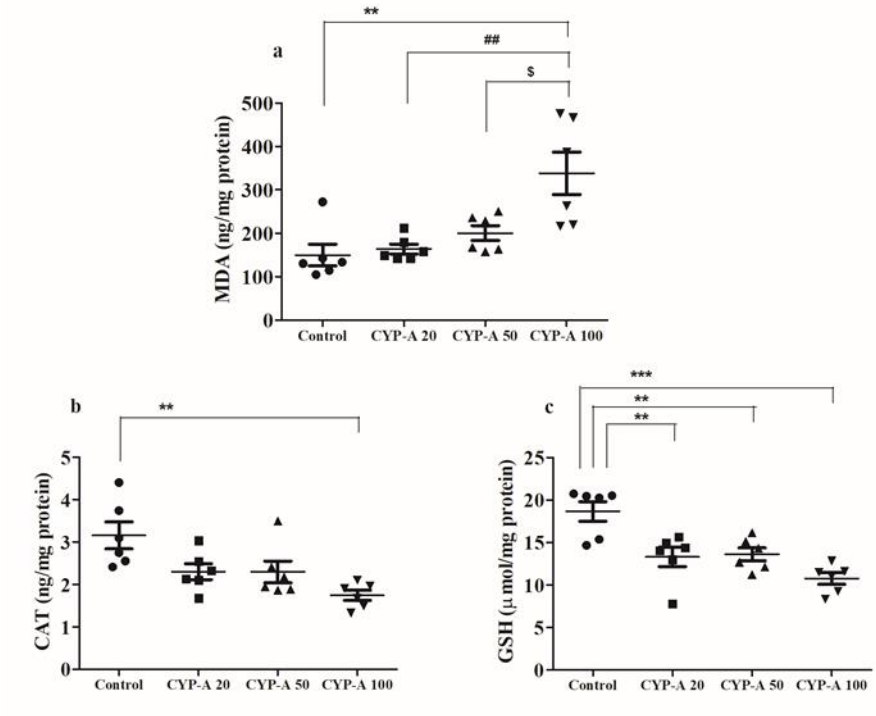

Figure 5. Effects of CYP-A on oxidative indices in the rat brain: (a) MDA, (b) CAT, and (c) GSH. The mean ± SEM (n = 6) is used to express the results. Tukey-Kramer multiple comparisons test was conducted after the one-way ANOVA [$F(3,20)=8.573$, $p<0.001$ for MDA; $F(3,20)=6.349$, $p<0.01$ for CAT; $F(3,20)=11.69$, $p<0.001$ for GSH]. **$p<0.01$ and ***$p<0.001$ vs control; ##$p<0.01$ vs CYP-A 20; \$$p<0.05$ vs CYP-A 50.

A statistically significant change ($F(3,20)=6.349$, $p<0.01$) was noted in brain catalase levels (ng/mg protein) after CYP-A therapy (Figure 5b). In particular, CYP-A 100 treatment decreased antioxidant capabilities in brain tissues, as evidenced by a significant drop in catalase levels (1.749±0.1219; $p<0.01$) compared to the control group (3.160±0.3157). The level of CAT was slightly decreased in both CYP-A 20 (2.304±0.1872) and CYP-A 50 (2.299±0.2543), although there was no significant change compared with the control group.

Concurrently, a one-way ANOVA comparison of all treatment groups revealed a substantial modification [F(3,20)=11.69, p<0.001] in GSH levels (µmol/mg protein) following CYP-A delivery (Figure 5c). Furthermore, it was observed that CYP-A treatment led to a significant drop in brain GSH levels (10.78±0.6800, p<0.001) in comparisons between the CYP-A 100 group and the control group (18.67±1.157). Additionally, the brain GSH levels in the CYP-A 20 and CYP-A 50 groups decreased by 13.31±1.159 (p<0.01) and 13.61±0.7719 (p<0.01) respectively, suggesting that the oxidative parameters in the rats are significantly affected by the administration of CYP-A at these levels.

3.5. CYP-A Induces the Neuroinflammation in Rats' Brain

To ascertain if CYP-A may result in neuronal inflammation, three specific inflammatory markers, including TNF-α, nuclear factor kappa B (NF-κB), and prostaglandin E2 (PGE2), were evaluated in brain tissues (Figure 6).

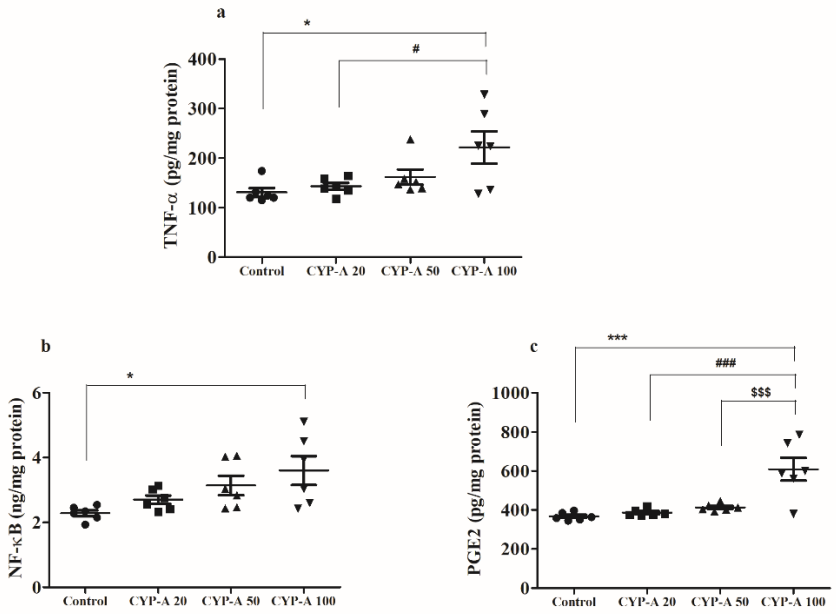

Figure 6. Effect of CYP-A on inflammatory parameters like (a) TNF-α, (b) NF-κB, and (c) PGE2 in rats. The results are expressed by mean ± SEM (n=6). One-way ANOVA [F(3,20)=4.524, p<0.05 for TNF-α; F(3,20)=4.141, p<0.05 for NF-κB; and F(3,20)=13.57, p<0.001 for PGE2] followed by Tukey-Kramer multiple comparisons test. *p<0.05 and ***p<0.001 vs control; #p<0.05 and ###p<0.001 vs CYP-A 20; $$$$p$<0.001 vs CYP-A 50.

Following targeted treatment, the brain homogenates were examined for the presence of TNF-α, a major proinflammatory cytokine. TNF-α levels (pg/mg protein) were altered after receiving CYP-A medication [F(3,20)=4.524, p<0.05] (Figure 6a). As anticipated, CYP-A 100 greatly increased TNF-α levels (221.8±32.66, p<0.05) in comparison to the control rats (131.0±8.937). Furthermore, a significant difference (p<0.05) in TNF-α levels between CYP-A 20 and CYP-A 100 was noted, with a change from (143.1±6.905) to (221.8±32.66) recorded. However, the CYP-A 50 group (162.1±15.54) showed no significant variation with CYP-A 100.

The CYP-A treatments had a significant influence on the parameter NF-κB (ng/mg protein) ($F(3,20)=4.141$, $p<0.05$) among the treatment groups, as shown in Figure 6b. In particular, compared to the control treatment (2.284±0.090), CYP-A 100 therapy resulted in a significant rise (3.602±0.4428, $p<0.05$) in NF-κB levels in the brain. Additionally, there were marginal increases in both CYP-A 20 and CYP-A 50 (2.702±0.1331 and 3.141±0.2985, respectively).

The impact of CYP-A therapies on brain homogenates' PGE-2 levels (pg/mg protein) is depicted in Figure 6c. PGE-2 levels were significantly influenced by these therapies ($F(3,20)=13.57$, $p<0.001$). Furthermore, PGE-2 levels increased significantly ($p<0.001$) after administering CYP-A 100 (609.1±59.12) compared to the control group (366.8±8.293), suggesting that inflammatory responses were upregulated in the brain. Additionally, when comparing CYP-A 50 with CYP-A 100, there was a significant rise in PGE-2 levels from (413.0±7.863) to (609.1±59.12; $p<0.001$). Similarly, an increase was observed after the administration of CYP-A 100 when compared to CYP-A 20 (386.5±7.549; $p<0.001$).

3.6. CYP-A Results in Apoptosis in the Rats' Brain

To determine the effect of CYP-A on rat brains, three crucial parameters were investigated: an anti-apoptotic marker, Bcl-2, and two pro-apoptotic markers, Bax and Caspase-3 (Figure 7).

A significant difference ($F(3,20)=5.532$, $p<0.01$) was observed in the brain's Bcl-2 levels (pg/mg protein) across the various treatment groups. For instance, CYP-A 100 treatment significantly ($p<0.01$) lowered brain Bcl-2 levels (488.0±49.64) when compared to CYP-A 20 (798.2±68.79). Notably, these reduced Bcl-2 levels were induced by a high dosage of CYP-A 100 (Figure 7a). However, there were no significant differences in Bcl-2 levels between the control group (663.4±54.75) and the other groups: CYP-A 20, CYP-A 50 (599.6±43.88), and CYP-A 100.

Figure 7b indicates that CYP-A dosages had a significant impact ($F(3,20)=3.423$, $p<0.05$) on brain Bax levels (ng/mg protein). It is noteworthy to emphasize a significant increase ($p<0.05$) in Bax levels (7.008±0.7528) in the brains of rats induced with CYP-A 100 compared to those of rats induced with CYP-A 20 (4.988±0.3882). By contrast, other groups did not exhibit a significant impact (CYP-A 20, CYP-A 50 (5.288±0.3595), and CYP-A 100) when compared to the control group (5.429±0.3330).

After the rats received CYP-A treatments, there were noticeable changes ($F(3,20)=8.309$, $p<0.001$) in levels of the pro-apoptosis marker Caspase-3 (ng/mg protein). As expected, brain Caspase-3 levels increased significantly (3.353±0.3978; $p<0.01$) following CYP-A 100 therapy in comparison to the control group (1.609±0.2711). CYP-A 100's brain Caspase-3 levels were significantly higher compared to CYP-A 20 mg/kg (1.610±0.2572; $p<0.01$) as well as CYP-A 50 mg/kg (1.950±0.1849; $p<0.05$) (Figure 7c).

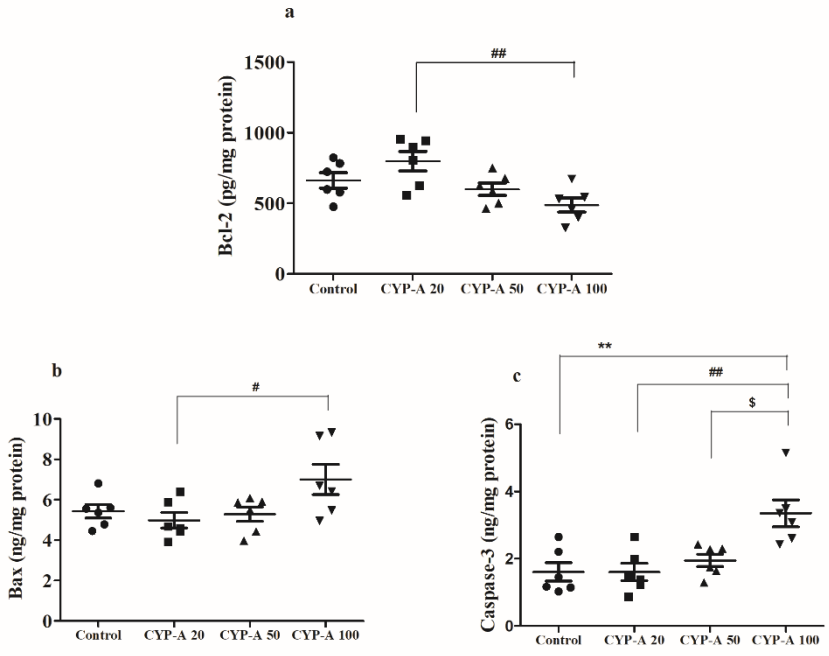

Figure 7. CYP-A impacts apoptotic markers in rats, including (a) Bcl-2, (b) Bax, and (c) Caspase-3. The mean ± SEM (n = 6) is used to express the results. Tukey-Kramer multiple comparisons test was conducted after one-way ANOVA [F(3,20)=5.532, p<0.01 for Bcl-2; F(3,20)=3.423, p<0.05 for Bax; and F(3,20)=8.309, p<0.001 for Caspase-3]. **p<0.01 vs control; \$$p$<0.05 vs CYP-A 50; #$p$<0.05 and ##$p$<0.01 vs CYP-A 20.

3.7. CYP-A Results in Neurotoxicity in Histopathology of Rats' Brain

The effects of CYP-A on brain tissues were investigated at three different dose levels (20, 50, or 100 mg/kg, p.o.). Hematoxylin and eosin (H&E) staining of brain tissue from control group animals revealed normal brain architecture (Figure 8a). In comparison to the control group, CYP-A at all three dose levels (20, 50, or 100 mg/kg, p.o.) caused various changes in brain tissue, including haemorrhages, inflammation, congestion, and necrosis.

A histopathological assessment of rat brain tissue was conducted, categorizing changes based on the severity of inflammation, congestion, and haemorrhages (Scores 0-3). The categorization was as follows: Score 0 indicated no inflammation, congestion, or haemorrhages; Score 1 represented mild inflammation, congestion, and haemorrhages; Score 2 signified moderate inflammation, congestion, and haemorrhages; and Score 3 denoted severe inflammation, congestion, haemorrhages, and necrosis. The severity of alterations was observed to increase with higher doses of CYP-A (Figures 8b-8e). Specifically, the group treated with CYP-A 100 (Figures 8e and 8f; Score 3) exhibited severe haemorrhages, inflammation, necrosis, and congestion compared to the control group (Figure 8a; Score 0). While tissue changes were also observed in the groups treated with CYP-A 20 and CYP-A 50, these changes were less pronounced. The CYP-A 20 group (Figure 8b; Score 1) showed mild inflammation and vascular changes, and the CYP-A 50 group (Figures 8c and 8d; Score 2) showed moderate inflammation and vascular changes compared to those in the CYP-A

100 group (Figures 8e and 8f; Score 3). These findings suggest that CYP-A can cause neurotoxicity and that higher doses of CYP-A result in significant tissue alterations.

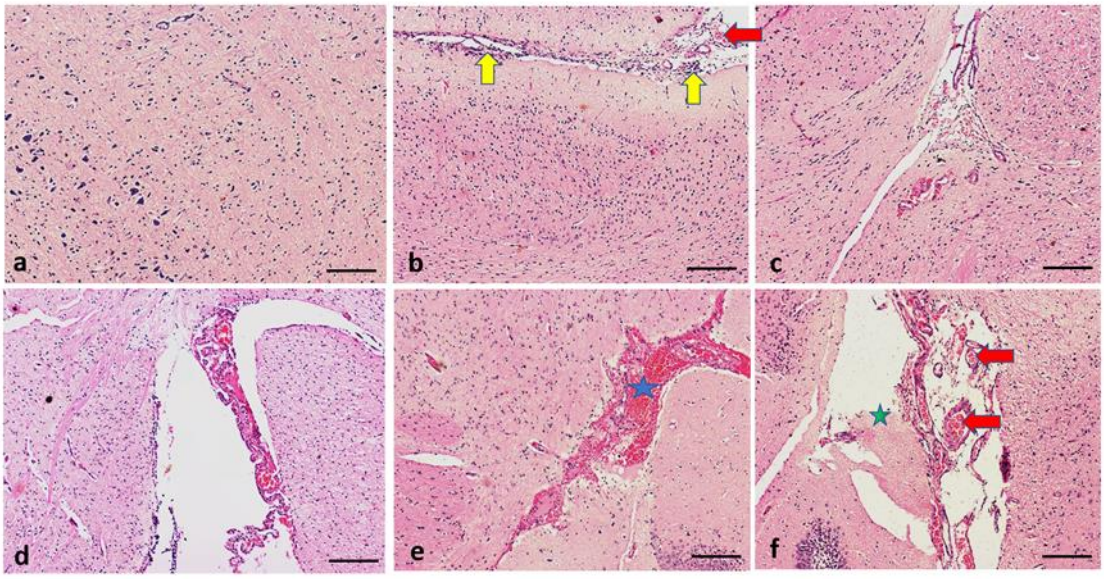

Figure 8. Brain tissue architecture, (a) normal brain tissue architecture as normal neuron and neuroglia cells of control group animals, (b) CYP-A 20 treated rat brain tissue presents different alterations such as mild inflammatory cells (yellow arrow) and congestion (red arrow), (c,d) CYP-A 50 treated group revealed moderate changes in brain tissue architecture as compared to the control group and (e,f) CYP-A 100 treated showed severe changes as congestion (red arrow), inflammation, haemorrhages (blue star) and necrosis (green star) as compared to control and CYP-A treated (20 & 50 mg/kg, p.o) treated group (Original magnification 100×). Scale bar: 50 μm.

4. Discussion

The complexity of CYP-A neurotoxicity is not entirely understood, but it typically manifests in three levels. Grade 1 symptoms include changes in mental state, tremors, or headaches. Grade 2 is characterized by vision problems or cortical blindness, while Grade 3 may involve seizures or comas. The incidence of seizures is estimated to be 1.8%, 5.5%, and 25% among patients who have undergone kidney, bone marrow, and liver transplants, respectively[27]. The mechanism of CYP-A neurotoxicity remains unclear, but it is associated with oral administration and high doses. Additionally, this complication may occur within the therapeutic range of CYP-A[6]. Up to 50% of patients may experience neurotoxicity, which is more common in the early stages of medication usage, especially at high doses[7]. To better understand the neurotoxicity of CYP-A, the present study closely examined the apoptotic process, neuronal inflammation, tolerance to oxidative stress, and cognitive abilities in rats at three dosage levels (20, 50, and 100 mg/kg, p.o). We found that oxidative stress, neuroinflammation, and apoptosis were among the mechanisms underlying neurotoxicity and that these mechanisms were dosage-dependent. Additionally, we observed challenges in cognitive functions particularly prevalent at high doses (50 and 100 mg/kg, p.o) of CYP-A treatment.

Initially, the cognitive performance of the treated rats was assessed using TL time in the EPM as a measure of cognitive ability, with shorter TL values indicating improved memory and learning capacities. The results showed how CYP-A treatments affected TL values over two days. Notably, learning capability was primarily reflected in the TL on the first day, while memory consolidation was evident in the TL on the second day. A closer examination of the data reveals that on both days 1 and 2, rats treated with higher doses of CYP-A (50 and 100) had longer TL times compared to the control group. This suggests that higher doses of CYP-A may negatively impact learning and memory processes, leading to increased TL times.

The present study also examined the effect of CYP-A on cognitive performance using the NOR test, a well-known method for assessing recognition memory in rats. The NOR test leverages rats' natural tendency to explore NO more than familiar ones, indicating their ability to recall previously seen items. Rats are allowed to explore between FO and NO, and their preference for the NO suggests intact recognition memory[28]. This test is effective for evaluating both short- and long-term memory and assessing the impact of various treatments on memory function. Significant variations in FO exploration length were found across the groups. Particularly rats with CYP-A 100 had a considerably shorter exploration period with both FO and NO than the control group. These results imply that CYP-A treatment negatively impacts the recognition memory of rats, Also, the CYP-100 group spent less time exploring the NO, which suggests that they had poorer recognition memory. These findings suggest that CYP-A may have a deleterious effect on cognitive function, particularly in the NOR parameters.

Using the Y-maze test, the results of the study sought to determine how CYP-A treatment affected the behaviours of rats linked to novel arm exploration and environment adaptation. A popular behavioural test for assessing the spatial working memory of rodents, novelty preference, and exploratory behaviour is the Y-maze test[25,26]. Concerning parameters like NEKA and NENA, as well as the TSNA%, variance analysis produced statistically significant results with CYP-A treatments. The Y-maze test findings indicate that CYP-A treatment significantly affects rat behaviours linked to new arm exploration and environment adaptation. The duration of stay in the new arm was shorter, and this difference was statistically significant when compared to the control group. All the doses of CYP-A affected memory impairment compared to the control group. The lower in both NEKA and NENA followed by CYP-A induction explained the weakening of spatial memory in rats. The results suggest that CYP-A may cause cognitive and adaptive deficiencies.

These findings are consistent with previous research on CYP-A's impact on cognitive function. For example, Wang *et al*.[13] found that CYP-A administration was associated with similar deficits in learning and memory in the Morris water maze (MWM) analysis. Additionally, CYP-A treatment reduced the number of BrdU+, BrdU+/DCX+, and BrdU+/NeuN+ cells in the hippocampus and lowered the protein levels of the Shh signaling pathway, including Shh, Smo, and Gli1, all of which are connected to impaired learning and memory functions. In another study, the administration of CYP-A has been demonstrated to

impair working and spatial memory. Furthermore, research involving kidney transplant recipients revealed that CYP-A affected long-term memory in people, which may contribute to the development of Alzheimer's disease (AD). According to this study, those who took CYP-A performed worse on verbal and visual memory tests than people who received other immunosuppressive medications[29]. Moreover, a study on CYP-A exposure revealed that it affected visuospatial learning in the Morris Water Maze (MWM) analysis, both during and after exposure to isoflurane anaesthesia[30]. In conclusion, a recent systematic review of the clinical setup highlighted that continued treatment with calcineurin inhibitors, particularly CYP-A and tacrolimus, is associated with neuronal toxicity. This toxicity is linked to blood-brain barrier impairments, ROS-mediated neuroinflammation, and mitochondrial dysfunctions, all of which contribute to cognitive dysfunctions and AD induction[31].

By examining oxidative and antioxidant indicators such as GSH, CAT, and MDA in brain homogenates, our study also sought to evaluate the effects of CYP-A-induced oxidative damage dose-dependently. Lipids present in the cell membrane are damaged under oxidative stress due to an imbalance between the production of ROS and the body's defence mechanism. In this condition, MDA is liberated as a byproduct of lipid peroxidation, making its level a critical marker of oxidative damage[32]. MDA, a highly reactive three-carbon dialdehyde, can interact with various functional groups on molecules such as RNA, DNA, lipoproteins, and proteins. MDA is a recognized indicator of oxidative stress and has been linked to the aetiology of several human illnesses including neuronal degeneration [33]. Moreover, various factors have been correlated with the neuronal toxic effects of MDA. These include leading to DNA damage through direct adduct formation with cellular nucleic acids, impairing mitochondrial function by disturbing ATP production, and activating various neuronal inflammatory and apoptosis pathways[33,34]. According to our research, CYP-A administration significantly impacted MDA levels in the brain among the treatment groups. Specifically, the administration of 100 mg of CYP-A resulted in a marked increase in MDA levels, indicating extensive oxidative injury to the brain.

Additionally, CYP-A treatments were associated with a substantial change in GSH levels. GSH plays a central role in removing ROS within cells and exists in two forms: endogenous (produced in the body) and exogenous (obtained from the environment). GSH is highly reactive and directly neutralizes reactive species by donating an electron to them, thereby protecting cells from oxidative damage[35]. Furthermore, GSH serves as a substrate for glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide, neutralizing it[36]. In this study, a higher dose of CYP-A 100 led to a significant decrease in brain GSH levels compared to the control group. This reduction in GSH levels is linked to the toxicity of CYP-A and the resulting oxidative stress, both of which can cause various types of cellular damage. Moreover, CYP-A has been shown also inhibit the activity of superoxide dismutase, a critical antioxidant enzyme, further impacting the antioxidant defense system[37,38]. Furthermore, another targeted antioxidant, CAT, also showed reduced levels following treatment with a higher dose of CYP-A 100. This reduction in CAT levels supports the overall impairment of the antioxidant defence system induced by CYP-A. Conversely, CAT

functions as a highly effective antioxidant enzyme, characterized by its hemoprotein structure that incorporates four heme groups, facilitating the conversion of hydrogen peroxide into water and oxygen[39].

From a collective perspective, the alterations in MDA, CAT, and GSH levels in the brain provide clear evidence of CYP-A-induced oxidative damage and oxidative stress. The increased MDA levels, along with reduced CAT and GSH levels, indicate an imbalance between the production of ROS and the brain's antioxidant defence system following CYP-A administration. This oxidative damage results from several processes, including the overproduction of ROS and the compromised antioxidant defence system. Oxidative stress can arise from CYP-A-induced dysregulation of the brain's capacity to manage ROS generation. This oxidative stress can damage lipids, proteins, and DNA, eventually leading to cellular malfunction and death within the brain[40,41].

The purpose of this investigation was to determine whether administering CYP-A would induce inflammation in neurons. To assess this, brain samples were examined for three distinct inflammatory markers: PGE2, NF-κB, and TNF-α. After the administration of CYP-A 100, notable changes were observed in the levels of TNF-α, a prominent pro-inflammatory cytokine. TNF-α is primarily released from macrophages in response to various inflammatory signals. In the CNS, the activations of microglia and astrocytes are closely associated with TNF-α-related neuronal inflammation[42]. The activation of TNF-α also leads to the stimulation of other cytokines, including IL-1β and IL-6, as well as increased glutamate activity. TNF-α also affects BBB permeability, allowing peripheral immune cells to infiltrate the CNS. Additionally, there is evidence that TNF-α can induce neuronal apoptosis, contributing to neurodegeneration[43]. These factors related to TNF-α activation by CYP-A might be associated with the observed neurotoxicity.

Further, NF-κB is an inflammatory mediator from the transcription factor family and is ubiquitously expressed in neurons, constantly its activation triggers neuronal inflammation. It can be able to regulate many inflammatory mediators including cytokines and proinflammatory enzymes, which contribute to neuronal inflammation[44]. The NF-κB signalling pathway is vulnerable to crosstalk and has an impact on several signalling pathways. NF-κB activation triggers inflammation and immunological responses by increasing the expression of genes that encode proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and enzyme cyclooxygenase (COX)-2[45]. Also, it has a regulatory function in different target genes that are involved in cell proliferation and apoptosis[43]. In particular, NF-κB levels in the brain increased significantly after CYP-A 100 therapy in comparison to the control treatment. Additionally, the effect of CYP-A 100 treatments on PGE-2 levels in brain homogenates was evaluated, and significant alterations were recorded. The elevation of PGE-2 levels, an active lipid by-product of the arachidonic acid and COX inflammatory pathway, indicates neuroinflammation and neurotoxicity[46]. Furthermore, the stimulations of cytokines such as TNF-α, IL-1β, and IL-6 are associated with higher levels of PGE-2, which contributes to the inflammatory process in the CNS[47]. These findings suggest that CYP-A

administration induces a significant inflammatory response in the brain, as evidenced by elevated PGE-2 levels and subsequent cytokine activation.

Cumulatively, the current results show that CYP-A therapies significantly affect brain inflammatory markers, as evidenced by changes in TNF-α, NF-κB, and PGE-2 levels at a dosage of 100 mg/kg. The increase in these markers indicates that CYP-A treatment activates inflammatory pathways, potentially leading to neuronal inflammation. According to Wu *et al*.[48] beyond the calcineurin/NF-AT pathway, CYP-A has been shown to impact the activity of AP-1 and NF-κB. Furthermore, Meyer *et al*.[49] found that endothelial progenitor cells (ECFCs) are susceptible to inflammatory responses and functional impairment caused by CYP-A. This is indicated by increased nuclear translocation and phosphorylation of the NF-κB p65 subunit, both of which are associated with inflammation.

The objective of the present study was to elucidate the impact of CYP-A on pivotal apoptosis-associated parameters within rodent brain tissue. Three distinct indicators were assessed: Bcl-2, an anti-apoptotic marker, and the pro-apoptotic markers Bax and caspase-3. A systemic review concluded that it is well established that CYP-A induces apoptosis in various cell types, including renal cells. The review identified five apoptosis-related pathways—Fas/FasL, mitochondrial, endoplasmic reticulum, AngII, and hypertonicity pathways—as being correlated with CYP-A-induced renal cell apoptosis. Furthermore, the activation of caspases is a common intersection for these pathways, forming a central intracellular pathway[50]. In the mitochondrial apoptosis pathway, the pro-apoptotic factor Bax undergoes oligomerization upon exposure to apoptotic triggers. Following conformational changes, Bax inserts into the outer membrane of the mitochondria, resulting in pore formation. This leads to the release of cytochrome C and other pro-apoptotic factors into the cytoplasm. Once in the cytoplasm, cytochrome C activates caspase-9, which in turn processes and activates procaspase-3 into caspase-3. This pathway is a crucial mechanism by which CYP-A promotes apoptosis. The activation of caspase-3, a key stage in the apoptotic process, leads to the cleavage of numerous cellular proteins, ultimately causing cell death[51–54]. In the current study, administration of CYP-A 100 mg/kg significantly increased caspase as well as Bax levels in brain tissues compared to lower doses (20 or 50 mg/kg, p.o.). This suggests that higher doses of CYP-A are more effective at inducing apoptosis in neuronal cells.

On the other hand, to counteract the pro-apoptotic mechanisms discussed above, the anti-apoptotic protein Bcl-2 inhibits the Bax-mediated release of cytochrome c from the mitochondria. Essentially, Bcl-2 maintains the integrity and potential of the mitochondrial outer membrane, preventing the release of pro-apoptotic factors that would lead to cell death[51,52]. In this study, treatment with CYP-A 100 mg/kg showed lower levels of Bcl-2 compared to the lower dose of CYP-A 20 mg/kg. Previous research has shown that Bax and caspase-3 levels rise as a result of CYP-A therapy. Specifically, a study found that after 24 and 48 hours, CYP-A treatment significantly increased caspase-3 activity—more than doubling it—in human prostate cancer cells (PC3 and DU145)[54]. This indicates that CYP-A treatment can induce apoptosis, as evidenced by the activation of caspase-3. In summary,

our research showed that CYP-A treatment in the rat brain results in changes to apoptosis-related markers, such as elevated Bax and caspase-3 levels and decreased Bcl-2 levels. According to these results, CYP-A could potentially trigger apoptotic processes and may play a role in neurodegenerative processes. Further investigation is required to clarify the underlying mechanisms and consider possible treatment approaches to mitigate the apoptotic response induced by CYP-A.

The neurotoxic effects of CYP-A were evidenced by distinct dose-dependent architectural changes in the brain tissues. As the doses increased from 20 to 50 and 100 mg/kg, the severity of brain tissue damage intensified correspondingly. Notably, the highest dose of CYP-A 100 resulted in the most pronounced damage, including severe haemorrhages, extensive inflammation, marked necrosis, and significant congestion. This dose-dependent pattern emphasizes the potential risks associated with CYP-A exposure, especially at higher concentrations. These findings indicate that CYP-A induces neurotoxicity through mechanisms that increasingly damage brain tissue as the dose rises. This is reflected in cognitive deficits observed with the highest dose of CYP-A 100 in various maze experiments focusing on learning abilities and memory types. Furthermore, evidence of elevated oxidative stress, inflammation, and apoptosis markers with higher doses correlated with tissue damage in the histopathological results. This underscores the necessity for cautious dosing and thorough evaluation of CYP-A's potential neurotoxic effects in its therapeutic application.

Although the findings offer interesting insights, it is important to realize some of the limitations. First of all, the duration, although adequate for detecting acute neurotoxic effects, may not encompass the long-term repercussions of being exposed to CYP-A. As well as, the size of our sample, which is determined by practical and ethical factors, may restrict the extent to which our findings may be applied to a larger population. Furthermore, species-specific differences in drug metabolism and neurophysiology naturally limit the portrayal of human neurotoxicity using laboratory rats. Rats are a useful model, but to fairly apply these results to humans, one should take caution and conduct more studies in clinical environments. Previous studies and accepted procedures guided the dosages of CYP-A administered. Still, the exact breadth and intervals may not cover all conceivable dose-response relationships. Examining a larger spectrum of dosages and more exact gradations would help to fully assess the neurotoxic effects of CYP-A in the next study. Moreover, the study largely concentrated on readily apparent neurotoxic consequences and distinct biomarkers. Although comprehensive behavioural assessments and in-depth molecular studies were not within the limits of the study, they are crucial for obtaining a complete knowledge of CYP-induced neurotoxicity. Integrating these elements in future research will yield a more comprehensive depiction.

5. Conclusion

Our research findings indicated that lower doses of CYP-A have a normal impact on behavioural functions in three maze models, whereas higher doses resulted in significant declines in spatial learning and memory ability. This demonstrates a clear relationship

between CYP-A dosage and cognitive impairment. Additionally, the findings indicated that damage to neurons caused by CYP-A might be due to an imbalance in oxidative stress, characterized by an increase in ROS and a decrease in antioxidant defences. This imbalance leads to detrimental effects on neurons. Neuronal death via apoptosis could be directly induced by oxidative stress, as reflected by the increased activity of the caspase-3 protein, the presence of Bax proteins, and the reduction in Bcl-2 levels. Significantly, the higher dose of CYP-A 100 mg/kg exhibits neuroinflammation by elevating inflammatory factors like TNF-α, NF-κB, and PGE2. These factors might contribute to neurotoxic effects, as indicated by increased pro-inflammatory cytokines in the brain. Finally, the histopathological examinations also revealed that higher levels of architectural changes occurred with CYP-A 100. The structural deterioration also exhibited a dose-dependent manner. These observations underscore potential risks associated with CYP-A treatment, notably at elevated dosages, and necessitate vigilant monitoring of cognitive and neurological outcomes in patients. Further investigation is warranted to comprehensively elucidate the underlying mechanisms of CYP-A-induced neurotoxicity and devise strategies to mitigate these detrimental effects.

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