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Ginkgo biloba Alleviates Cisplatin-Mediated Neurotoxicity in Rats via Modulating APP/Aβ/P2X7R/P2Y12R and XIAP/BDNF-Dependent Caspase-3 Apoptotic Pathway

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Abstract: Neurotoxicity is an obvious adverse effect in Patients encountering a complete course of chemotherapy. The present work is conducted to evaluate the neuroprotective effect of *Ginkgo biloba* (*Ginkgo*) against the neurotoxicity induced by Cisplatin (Cis) in rats. Forty male Wistar albino rats were arranged into four groups: (1) Control group, rats were given saline; (2) Cis group, rats were injected by Cis 2 mg/kg body weight i.p., twice a week starting on the fifth day for thirty days; (3) Ginkgo group, rats were administered *Ginkgo* (50 mg/kg orally) daily for thirty days; and (4) Ginkgo+Cis group, rats received *Ginkgo* (50 mg/kg orally) daily and on the fifth day, rats were injected with Cis (2 mg/Kg body weight i.p.) twice a week for thirty days. Cis significantly increased Gamma glutamyltransferase (GGT) and Acetyl Cholinesterase (CHE) as compared to the control group and also disturbed cerebral oxidative/antioxidant redox. Co-administration of *Ginkgo* and Cis reversed the adverse effect of Cis on the brain tissue. Moreover, co-administration of *Ginkgo* and Cis ameliorated Cis induced brain damage by reducing Amyloid precursor protein (APP), amyloid β (Aβ), P2Y12R and P2X7R mRNA expressions and proteins. Furthermore, *Ginkgo* regulated XIAP/BDNF expressions with a consequent decrease of caspase-3 and DNA fragmentation%. The current results concluded that concurrent treatment with *Ginkgo* can mitigate neurotoxicity mediated by Cis in experimental animals through exhibiting antioxidant effect by restoring cerebral oxidative/antioxidant redox and anti-apoptotic effect via regulating cerebral APP/Aβ/P2Y12R/P2X7R and XIAP/BDNF signaling pathways.

Keywords: cisplatin; neuro toxicity; *Ginkgo biloba*; XIAP; APP; BDNF; purinergic receptors; caspase-3

1. Introduction

Cisplatin (cis-diamminedichloroplatinum II) (Cis), is a heavy metal compound that comprises a central atom of platinum surrounded by two chloride molecules and two ammonia molecules. Its molecular formula is CL2-H6-N2-Pt [1]. Plasma proteins such as albumin, transferrin and gamma globulin, irreversibly bound to the platinum component of Cis [2].

Cis is considered one of the most essential anti-cancer chemotherapeutic agents which can be concerned with the management of numerous human malignancies in different organs [3]. One of the

most important mechanisms of Cis is its ability to bind with purine bases of deoxyribonucleic acid (DNA), hinder the repair mechanisms of DNA and boost DNA damage, in addition to prohibition of cell proliferation, and death of tumor cell [4]. Cis mainly targets DNA and many cytoplasmic components, such as proteins, thiol peptides and RNA [5].

One of the major apparent complications of Cis chemotherapy is the brain toxicity [6]. The cerebral toxicity of the Cis is considered dose-dependent [7]. Cis has a powerful penetrating power into the blood–brain barrier (BBB), and so it can debilitate mature neurons in the brain [8]. Hydrogen peroxide and hydroxyl radicals are examples of reactive oxygen species (ROS) which are formed by Cis. The formed free radicals interact with DNA, proteins and fats, resulting in lipid peroxidation and destruction of DNA [9].

One of greatest key mechanisms of Cis toxicity is the induction of oxidative stress. Under normal conditions, ROS are controlled by cells through harmonizing the production of ROS with their removal by scavenging system. Oxidative stress results in extreme damage of cellular proteins, lipids and DNA, leading to critical cellular injuries. Cis can induce mitochondrial oxidative damage with a disruption of sulfhydryl group of mitochondrial protein, hindering calcium uptake and weakening the mitochondrial membrane potential [10], decreasing the antioxidant defense system and reducing glutathione (GSH) [11].

Indeed, too much ROS production, DNA injury, inflammation, mitochondrial dysfunction, and also programmed cell death in the nervous system are considered the chief mechanisms in which neurotoxicity is provoked by Cis [12]. Many previous studies recorded the toxic mechanism of Cis on the nervous system but there is no satisfactory information to explain the mechanistic pathway of Cis induced-brain apoptosis.

Nowadays, a great attention is given to the amyloid precursor protein (APP) and β amyloid (Aβ) as they have a pivotal role to play in the development of Alzheimer's disease (AD). Free-radical oxidative stress, neuronal lipids peroxidation, proteins denaturation and DNA fragmentation were detected in AD brain areas in which Aβ is abundant [13]. APP and Aβ are considered sensitive biomarkers in neuronal damage and neuronal cell death [14]. It is reported that accumulation of Aβ directly activates caspase-3 concerned with the death of neuronal cells [15]. Also, it increases the expression of Purinergic receptors especially P2Y12R and P2X7R [16]. These receptors became a target for examination of the sites of chronic inflammation, neuro-degeneration, and neuropsychiatric troubles [17] as they enhance the formation of neuro-immune cells [18] and the release of IL-1β; one of the neuro-degenerative and neuro-inflammatory mediators [19].

On the contrary, there are many protective mechanisms and mediators which can protect against cerebral injuries and apoptosis. One of the most important inhibitor apoptosis proteins (IPAs) is XIAP. It directly binds and inhibits the caspases which carry out the cell-death program [20]. Another important neuro-protective protein is brain-derived neurotrophic factor (BDNF). BDNF belongs to neurotrophin family proteins which can be detected in both the peripheral and central nervous systems [21]. It has an essential function in survival, development, differentiation and plasticity of neurons. It could protect neurons from induced neuronal cell oxidative stress and apoptosis [22]. Many studies have revealed that cleaved caspases and caspase-3 mediate the release of the cytokines interleukin-1β (IL-1β) and IL-18. In addition, inflammatory caspases modulate distinct forms of programmed cell death [23]. Liu et al. stated that both cleaved caspase-3 and caspase-3 are sensitive biomarkers for apoptosis and different tumor stages [24].

The previously mentioned research has provoked the need to find novel, effective and safe neuroprotective agents to protect against the cis-induced cerebral apoptosis. So we aimed to study the anti-apoptotic effect of a well-known antioxidant herbal plant; *Ginkgo biloba* (*Ginkgo)* on Cis-induced cerebral apoptosis. *Ginkgo* is used as a conventional herbal medicine to cure anxiety, headaches, depression, and poor memory in Asia and Europe [25]. *Ginkgo* is composed chemically of flavonoid glycosides (22−27%), terpenetrilactones (5.4−6.6%), ginkgolides (2.8−3.4%), bilobalide (2.6−3.2%) and trace ginkgolic acid [26,27].

Noticeable cerebral antioxidant, anti-ischemic, anti-apoptotic effects of *Ginkgo* have been previously reported [28,29]. The antioxidant effect of *Ginkgo* could be attributed to its ability to reduce cerebral H_2O_2 and ROS production especially superoxide anion in the brain cell [30–32]. These influential properties of *Ginkgo* could be attributed to the flavonoid contents of *Ginkgo* [33]. A previous report recorded that *Ginkgo* has a power anti-apoptotic effect in rat brains with acute cerebral infarction [34]. Added to that, *Ginkgo* has potent antioxidant and anti-inflammatory effects in the cerebral infarction [35]. No available studies concern the effect of *Ginkgo* on inhibitor apoptosis protein (IAPs) or BDNF in the brain toxicity induced by Cis.

We aimed to verify whether *Ginkgo* therapy may eliminate the toxic effect of Cis on the brain tissue. Thus, this study was designed to assess the effect of *Ginkgo* as a neuroprotective agent on neurotoxicity induced by Cis in the rat model by assessing the antioxidant condition, certain gene expressions and anti-apoptotic specific protein markers in the brain tissue of experimental animals.

2. Materials and Methods

2.1. Drugs and Chemicals

Ginkgo was obtained as a commercial preparation Ginkgo Biloba® (Future Pharmaceuticals for industries for EMA Pharma, Cairo, Egypt). *Ginkgo* contains 24% Ginkgoflavones glycosides and 6% terpenoids. *Ginkgo* was given orally using stomach gavage. Cis was received from Sigma Chemicals (Sigma-Aldrich Louis, MO, USA).

2.2. Animals

Forty male Wistar Albino rats weighing (180–250 g) were received from the animal facility at El Nahda University (Beni-Suef, Egypt). Rats were adapted to laboratory conditions for two weeks before the start of the experimental work, then, rats were put in metal cages (each one contained 3 rats), kept under standard laboratory conditions with an optimum temperature (25 ± 2 °C), humidity (70%), 12 h of dark/light cycle and free access to rat chow and drinking water. The present work was approved by the Experimental Animal Ethics Committee at the faculty of Veterinary Medicine, Beni-Suef University, Egypt and all procedures used during dealing with animals were in harmony with the National Institutes of Health (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and the body weights were recorded weekly.

2.3. Experimental Design

As shown in Figure 1 and after the accommodation period, rats were randomly divided into four groups (n = 10) as follows:

Control group: Rats were injected with 0.9% saline 0.5 mL i.p., twice a week on the 5th day, for 30 days.

Cis group: Rats were given Cis 2 mg/kg body weight i.p., twice a week starting on the 5th day for 30 days [36].

Ginkgo group: Rats received *Ginkgo* (50 mg/kg body weight PO) daily for 30 consecutive days [37].

Ginkgo+Cis group: Rats received *Ginkgo* (50 mg/kg body weight PO) daily 30 days [38]. On the 5th day, rats were injected with Cis 2 mg/Kg body weight IP twice a week for 30 days [36].

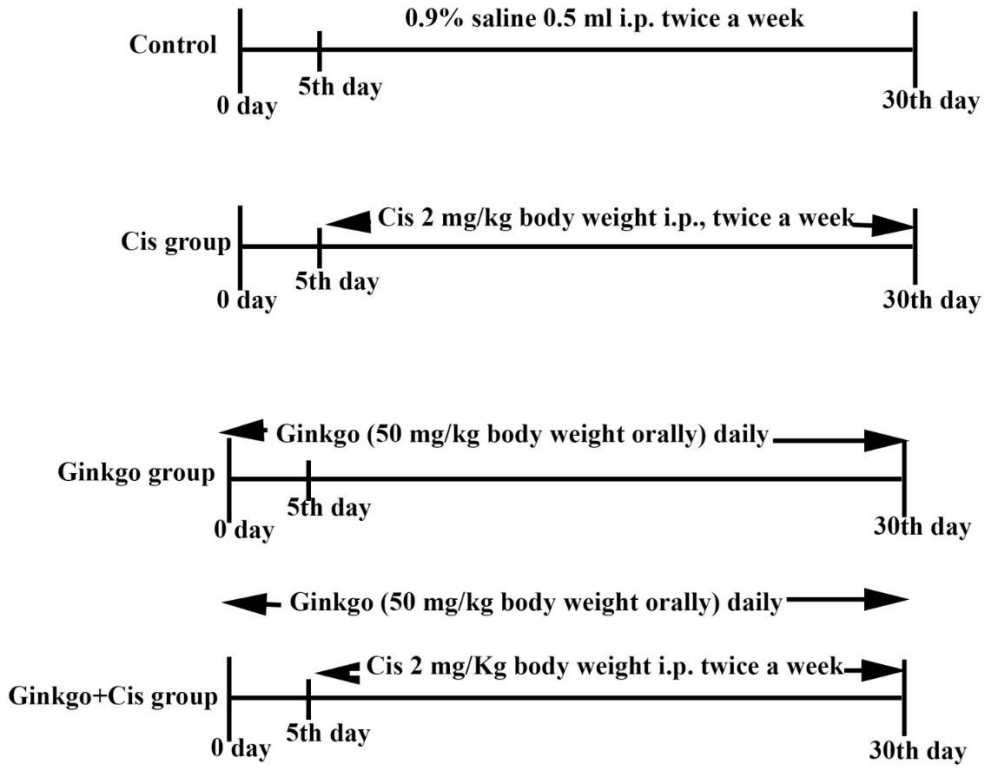

Figure 1. Experimental design including all treated groups. Experimental design outlining the cisplatin (Cis) induced cerebral damage model and *Ginkgo* treatment protocol where $n = 10$ rats for each group. Control group: Rats were injected with 0.9% saline 0.5 mL i.p. twice a week on the 5th day, for 30 days. Cis group: Rats were given Cis 2 mg/kg body weight i.p. twice a week starting on the 5th day for 30 days. Ginkgo group: Rats received *Ginkgo* (50 mg/kg body weight orally) daily for 30 consecutive days. Ginkgo+Cis group: Rats received *Ginkgo* (50 mg/kg body weight orally) daily for 30 days, and on the 5th day, rats were injected with Cis 2 mg/Kg body weight i.p. twice a week for 30 days. All measured parameters were blind examined to confirm the accuracy of the results.

Twenty-four hours after the last treatment, the rats were fasted overnight and then sacrificed by decapitation. The brain tissues were collected, weighed then washed with phosphate-buffered saline (PBS) and divided into two parts. The first part was used to obtain a uniform suspension, 0.5 g of brain tissue was suspended in 5 mL PBS (pH: 7) and homogenized by using tissue homogenizer (Ortoalresa, Spain). The supernatant was kept at −80 °C for further biochemical evaluation. The second part was kept for molecular investigations (Western blot analysis and real-time polymerase chain reaction (RT-PCR) analyses) according to the instruction kits.

2.4. Methods

2.4.1. Biochemical Investigations

All used kits were purchased from Sigma-Aldrich Chemicals, St Louis, MO, USA. Acetyl Cholinesterase activity (CHE) was measured by using CHE assay kit (Cat. No. 119BJ11A25) according to [39]. Gamma glutamyl transferase activity (GGT) was measured by using GGT assay kit (Cat. No. 6A03K07840) according to [40]. Reduced glutathione concentration (GSH) was measured by using GSH assay kit (Cat. No. 099M4064V) according to [41]. Super oxide dismutase activity (SOD) was measured by using SOD assay kit (Cat. No. BCCC1068) according to the method of [42]. Total antioxidant capacity (TAC) was measured by using TAC assay kit (Cat. No. 059M4154V) according to the method of [43]. Malondialdehyde content (MDA) was measured by using MDA assay kit (Cat. No. 6A20K07390) according to the method of [44].

2.4.2. Western Blot Technique for Measurement of (APP, Aβ, X1AP and Caspase-3)

The concentration of cerebral APP, Aβ, X1AP and caspase-3 were quantified through immune blotting with the corresponding antibody and then the proteins were separated according to their molecular weight by gel electrophoresis. We used TGX Stain-Free™ Fast-Cast™ Acrylamide Kit (sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)) which was provided by Bio-Rad Laboratories, TNC, USA, Catalog No. 161-0181. The SDS-PAGE TGX Stain-Free Fast Cast was prepared according to the manufacturer's instructions. Bands were visualized using Clarity™ Western ECL substrate (Bio-Rad, USA cat#170-5060), and the intensity of the bands was assessed against that of β-actin with the image analysis software Chemi Doc MP imager (Markham Ontario L3R 8T4 Canada) [45,46].

2.4.3. Determination of BDNF, P2Y12R and P2X7R Gene Expression by Real-Time-Polymerase Chain Reaction (RT-PCR)

By using RNeasy Purification Reagent (Qiagen, Valencia, CA, USA), RNA of the brain tissue was isolated. Primers specific for BDNF, P2Y12R and P2X7R were used and are listed in Table 1. Real time quantitative PCR was used to determine gene expression according to the instructions for Applied Biosystems version 3.1 software and SYBR Green I (Step One™, USA). All data are expressed relative to the β-actin gene [47].

Table 1. Primers used for real-time quantitative PCR.

	Primer Sequence	
BDNF	Forward: 5′-AGTGATGACCATCCTTTTCCTTAC3′ Reverse: 5′-CCTCAAATGTGTCATCCAAGGA-3′	Gene ID: 24225 Accession number of chromosome: NC_005102.4
P2Y12R	Forward primer: 5-CCAGTGCATCCCTAAATATTCC-3 Reverse primer: 5-CCAGCGTTCCCATATACCAG-3	Gene ID: 6803 Accession number on chromosome: NC_005101.4
P2X7R	Forward primer: GAACCTCGAGTGAGCCACAACCAGAACACT Reverse primer: GACAAGATCTATGGCCCAAGGAGCTCGGT-3	Gene ID: 29665 Accession number of chromosome: NC_005111.4
Beta actin	Forward: 5′-GCACCCAGCACAATGAAGATCAAG-3′ Reverse: 5′-TCATACTCCTGCTTGCTGATCCAC-3	Gene ID: 11461 Accession number of chromosome: NC_000071.6

2.4.4. DNA fragmentation%

The percentage of DNA fragmentation in the brain tissue was measured according to the method described by [48], and the percentage was calculated by using the following equation:

$$\text{DNA fragmentation\%} = \frac{\textit{Optical Denisty of Supernatant}}{\textit{Optical Denisty of Sediment} + \textit{Optical denisty of supernatant}} \times 100$$

2.5. Statistical Analysis

All obtained data are described as arithmetic means ± standard error (SE). The results were assessed by SPSS 20 (SPSS, Chicago, IL, USA). One-way ANOVA followed by Tukey's post hoc test were done to evaluate and compare the significance between testing groups. Values of $p < 0.05$ were referred to as significant.

3. Results

Administration of *Ginkgo* alone in the Ginkgo group caused no significance at ($p < 0.05$) as compared to control group in all measured parameters.

3.1. Effect of Ginkgo on CHE and GGT Activities in Brain Toxicity

The activities of cholinesterase (Figure 2A) and GGT (Figure 2B) were significantly ($p < 0.05$) increased in the Cis group in comparison with the control group. Administration of Ginkgo with Cis in Ginkgo+Cis group decreased the activities of CHE and GGT compared to Cis only in Cis group at ($p < 0.05$).

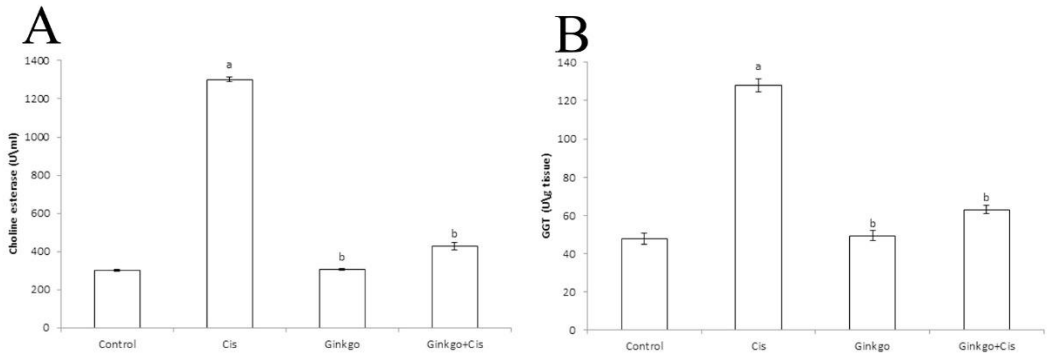

Figure 2. Changes of Cholinestrase (CHE) and Gamma glutamyl transferase (GGT) activities of different groups. The effect of Cis, *Ginkgo*, and their combination on choline esterase (**A**) and GGT (**B**). Data are presented as mean ± SE ($n = 10$). a indicates a significant difference compared to control group, b indicates a significant change compared to Cis group. a and b indicate statistical significance at $p < 0.05$ using ANOVA followed by Tukey–Kramer as a post ANOVA test.

3.2. Effect of Ginkgo on Oxidant/Antioxidant Parameters in Brain Toxicity

Cis administration caused a significant decrease of GSH concentration (Figure 3A), SOD activity (Figure 3B), TAC concentration (Figure 3C) and a significant increase of MDA concentration (Figure 3D) in comparison with the control group ($p < 0.05$). Administration of *Ginkgo* with Cis reversed the adverse effect of Cis as Ginkgo+Cis group indicated a significant boost of GSH concentration, SOD activity, TAC concentration and a significant reduction of MDA in comparison with the Cis group ($p < 0.05$).

3.3. Effect of Ginkgo on APP, Aβ, XIAP and Caspase-3

Cis caused a significant increase of APP (Figure 4A) and Aβ (Figure 4B), decrease of XIAP (Figure 4C) and increase of caspase-3 (Figure 4D) in comparison with the control group ($p < 0.05$). *Ginkgo* reversed the effect of Cis as *Ginkgo* administration in the Ginkgo+Cis group decreased APP, Aβ and caspase-3 and increased XIAP in comparison with the Cis group ($p < 0.05$).

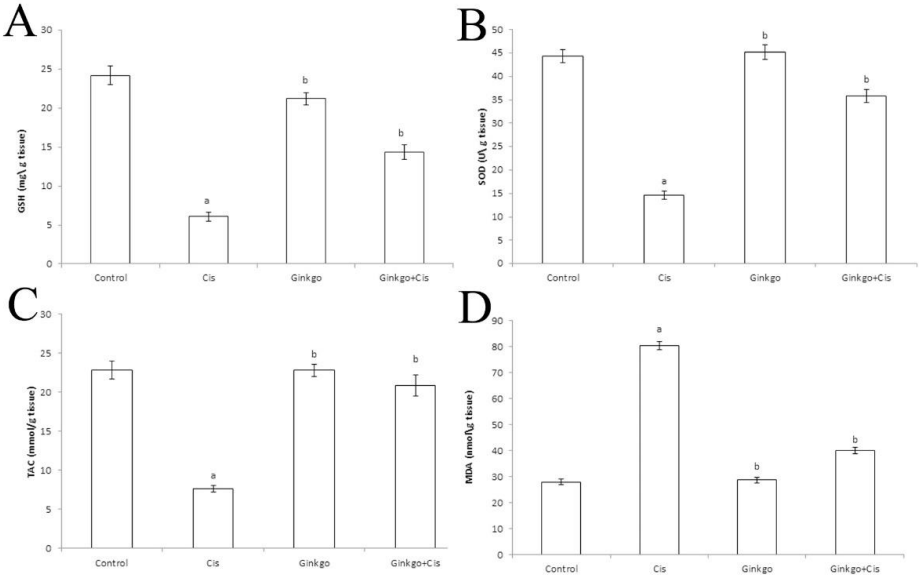

Figure 3. The changes of oxidant/antioxidant parameters of different groups. The Effect of Cis, Ginkgo, and their combination on reduced glutathione (GSH) (**A**), super oxide dismutase (SOD) (**B**), Total antioxidant capacity (TAC) (**C**) and Malondialdehyde (MDA) (**D**). Data were presented as mean ± SE (n = 10). a indicates a significant difference compared to control group, b indicates a significant change compared to Cis group. a and b indicate statistical significance at $p < 0.05$ using ANOVA followed by Tukey–Kramer as a post ANOVA test.

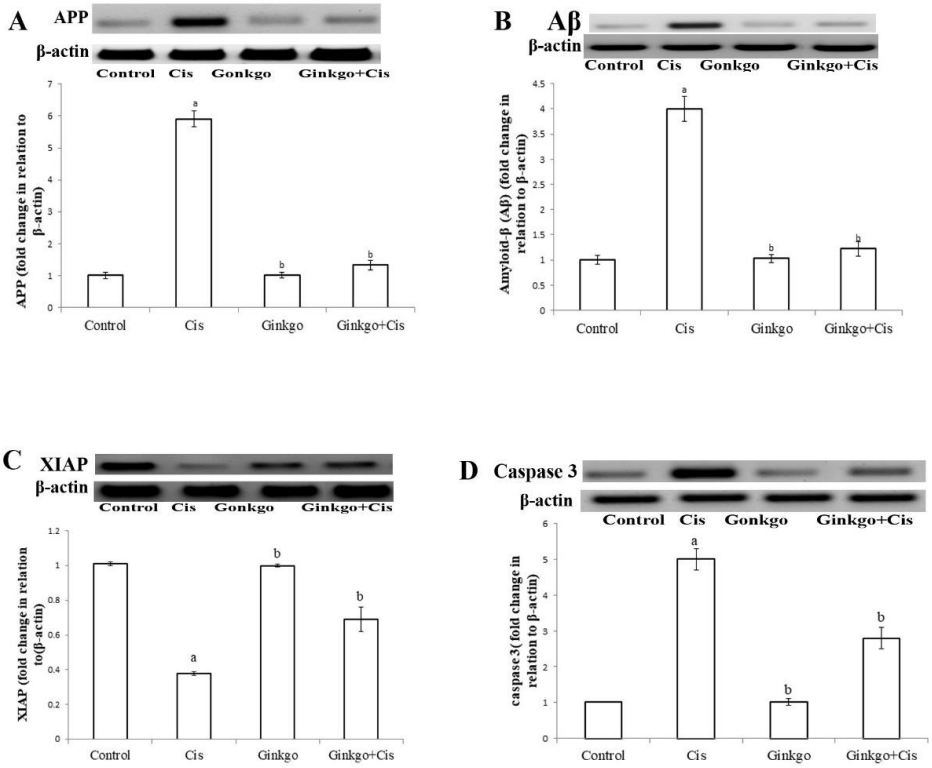

Figure 4. The changes of amyloid precursor protein (APP), Aβ, XIAP and caspase-3 in different groups. The effect of Cis, *Ginkgo*, and their combination on the expression levels of APP (**A**), Amyloid β (Aβ) (**B**), XIAP (**C**), caspase-3 (**D**) in rat brain tissues. Data present as mean ± SE (n = 10). a indicates a significant difference compared to control group, b indicates a significant change compared to Cis group. a and b indicate statistical significance at $p < 0.05$ using ANOVA followed by Tukey–Kramer as a post ANOVA test.

3.4. Effect of Ginkgo on BDNF, P2Y12R and P2X7R mRNA Expressions in Brain Toxicity

Administration of Cis caused a significant reduction of BDNF mRNA expression ($p < 0.05$) in comparison with the control group (Figure 5A). Moreover, Cis caused a significant increase of P2Y12R (Figure 5B) and P2X7R (Figure 5C) mRNA expressions ($p < 0.05$) in comparison with the control group. *Ginkgo* caused a significant augmentation of BDNF mRNA expression and decreased P2Y12R and P2X7R mRNA expressions in the Ginkgo+Cis group in comparison with the Cis group ($p < 0.05$).

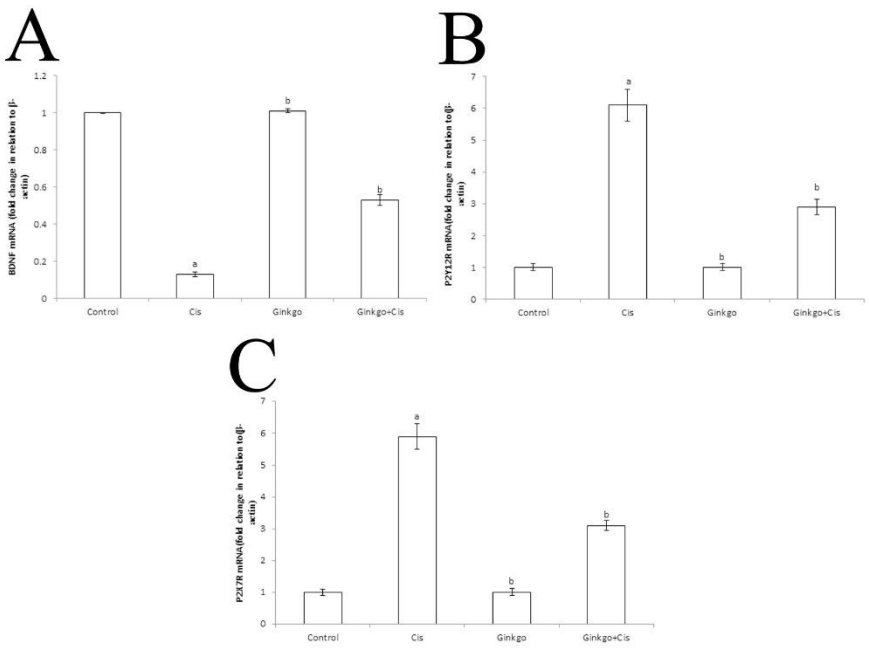

Figure 5. The changes of BDNF, P2Y12R and P2X7R mRNA expressions in different group. The Effect of Cis, *Ginkgo*, and their combination on the expression levels of BDNF (**A**), PY12R (**B**) and P2X7R (**C**) in rat brain tissues. Data were presented as mean ± SE ($n = 10$). a indicates a significant difference compared to control group, b indicates a significant change compared to Cis group. a and b indicate statistical significance at $p < 0.05$ using ANOVA followed by Tukey–Kramer as a post ANOVA test.

3.5. DNA Fragmentation%

Cis caused a significant increase of DNA fragmentation% (Figure 6) in comparison with the control group ($p < 0.05$). *Ginkgo* ameliorates the effect of Cis as *Ginkgo* decreased DNA fragmentation% in comparison with Cis group ($p < 0.05$).

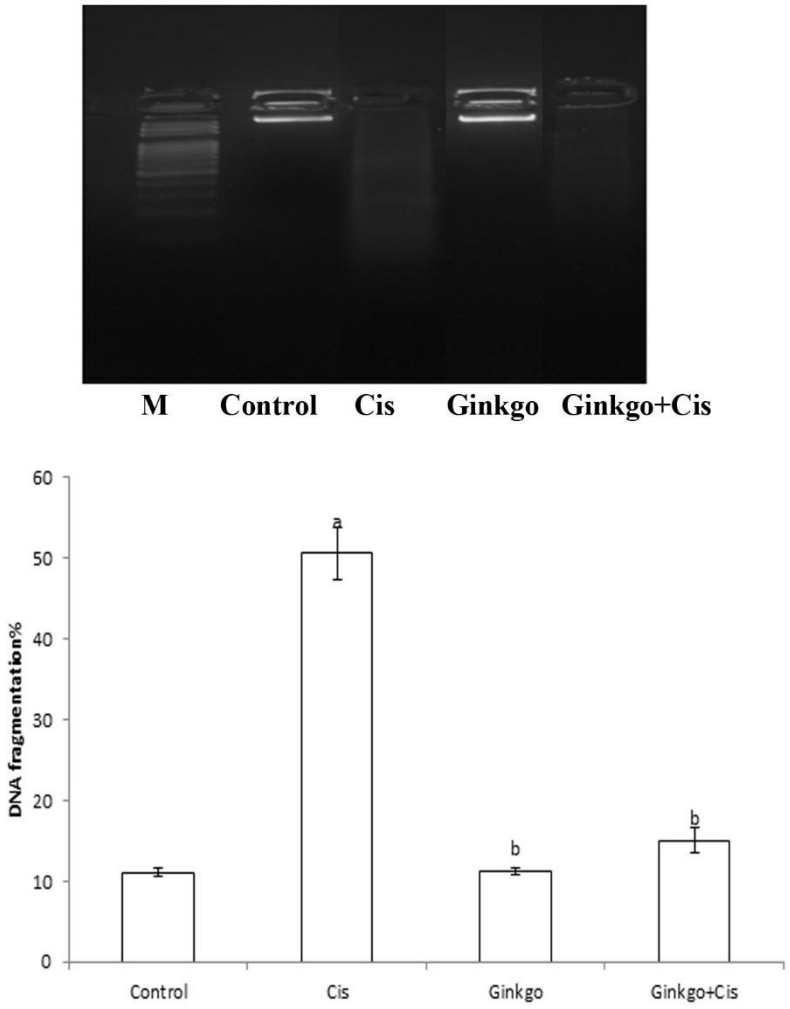

Figure 6. The changes of DNA fragmentation% in different groups. The effect of Cis, *Ginkgo*, and their combination on the expression level of DNA fragmentation% in rat brain tissues. Data present as mean ± SE (n = 10). a indicates a significant difference compared to control group, b indicates a significant change compared to Cis group. a and b indicate statistical significance at $p < 0.05$ using ANOVA followed by Tukey–Kramer as a post ANOVA test.

4. Discussion

Although Cis is a potent chemotherapeutic drug, it has many harmful complications. In the existing study we focused on the toxic effect of Cis on the brain; the main target of Cis. It crosses the BBB and inhibits the proliferation of neuronal stem cells [3]. During Cis therapy, a marked decrease in the hippocampus neurons number has been identified [49].

Our results showed that Cis causes severe cerebral damage which is manifested by a significant increase of CHE and GGT activities in the Cis group in comparison with the control group. These findings are in agreement with [50–52]. References [53,54] reported that GGT is posited as an early and a delicate marker for brain damage. CHE is an important enzyme involved in acetyl choline catabolism at cholinergic synapses into acetic acid and choline [55]. Also, CHE is considered a target enzyme for Cis induced cerebral damage as the inhibition of CHE activity leads to the disturbance of the neurotransmitter acetylcholine [56] and an obvious disturbance in the nerve impulse transmission will take place [57].

Treatment with *Ginkgo* significantly reduced CHE and GGT activities compared to the Cis group. This effect might be explained by the efficacy of *Ginkgo* to scavenge the free radicals and suppress the leakage of enzymes through plasma membranes [58].

Furthermore, our results showed that Cis causes severe cerebral oxidative stress which is revealed by the severe depletion of GSH concentration [59], SOD activity, TAC with a concurrent augmentation of MDA. These findings are in accordance with [60]. Stimulation of lipid membrane peroxidation via augmentation of free oxygen radicals generation together with down regulation of antioxidant defense could explain the oxidative harmful effects of Cis [61]. Based on the existing work, the potent antioxidant outcome of *Ginkgo* on Cis-stimulated cerebral oxidative stress was so clear and could be inferred by the significant reduction of MDA [62] and increase in cerebral antioxidant defense GSH and SOD [63]. The antioxidant properties of *Ginkgo* are related to its efficacy to work as a free radical scavenger by preventing the lipid peroxidation [58]. The bio-flavonoid contents of *Ginkgo* have the upper hand in its antioxidant properties [64] through the direct scavenging effect on ROS, removing pro-oxidant transitional metal ions and boosting antioxidant proteins [65].

Apoptosis, which is clarified as a programmed cell death, controls cell repair and the removal of injured cells [66]. Cis induces neuronal loss by promoting neuronal apoptosis and inhibiting neurogenesis [67].

A novel finding is the ability of Cis to increase the production of APP and accumulation of Aβ, which is generated by proteolytic processing of the APP via β-secretase (β-amyloid cleavage enzyme) and γ-secretase complex [68]. Over accumulation of Aβ is one of the most acceptable hypotheses explaining the induction of neuronal oxidative damage and cell expiration in Alzheimer's disease (AD) [15,69,70]. The neuronal apoptosis of AD is distinguished by the presence of many apoptotic markers which are found in the brains of AD sufferers after death, such as inducing caspase activities and DNA fragmentations [71,72].

Based on our findings, Cis increases the expression of some purinergic receptors such as P2Y12R and P2X7R. It is recorded that purinergic receptors significantly increased the neurotoxicity [73]. They are implicated in several CNS disorders, such as neuronal damage [74] and Alzheimer's disease [75]. These receptors are activated by binding certain nucleotides that are released from injured neurons [76], and then activated in microglia [77]. Various pro-inflammatory mediators are generated by the activated microglia such as cyclooxygenase-2 (COX-2), tumor necrosis factor (TNF-a), and interleukin 6 (IL-6) [78], leading to neuronal damage, disability of tissue renovate and neuronal lesions [79]. A previous study reported that over concentration of Aβ in the brain tissue leads to the over expression of P2Y12R and P2X7R in the brain tissue [16].

Another apoptotic pathway of Cis is the inhibition of XIAP. XIAP belongs to the IAP family [80]. Reference [81] stated that XIAP has a powerful neuroprotective effect by reducing cerebral apoptosis. Our results showed that Cis administration diminished cerebral XIAP concentration with a concurrent up-regulation of caspase-3 which activates apoptotic cell death through a pathway of signal transduction Figure 7. The down-regulation of XIAP inhibits the phosphorylation of the tropomyosin linked kinase-B (TrkB) receptor; one of the most important membrane receptors of BDNF [82] and this could explain the significant reduction of BDNF expression in Cis treated rats. Added to that, Peng stated that too much accumulation of Aβ reduces the BDNF expression [83]. The reduction of BDNF inhibits Bcl-2 which is an important factor inhibiting apoptosis. Thus, caspase-3 is augmented and the intrinsic pathway of apoptosis is initiated in the brain tissue [84].

From what has been previously mentioned, using anti-apoptotic agent such as *Ginkgo* is a logic approach to protect against cis induced cerebral apoptosis. *Ginkgo* has a powerful protective effect against the induced neuronal apoptosis which may be developed due to many sets of conditions, such as oxidative stress [85], and aggregation of Aβ [86]. Our data reported that co-administration of *Ginkgo* and Cis reduced APP causes a consequent reduction of Aβ accumulation in the brain tissue [87,88].

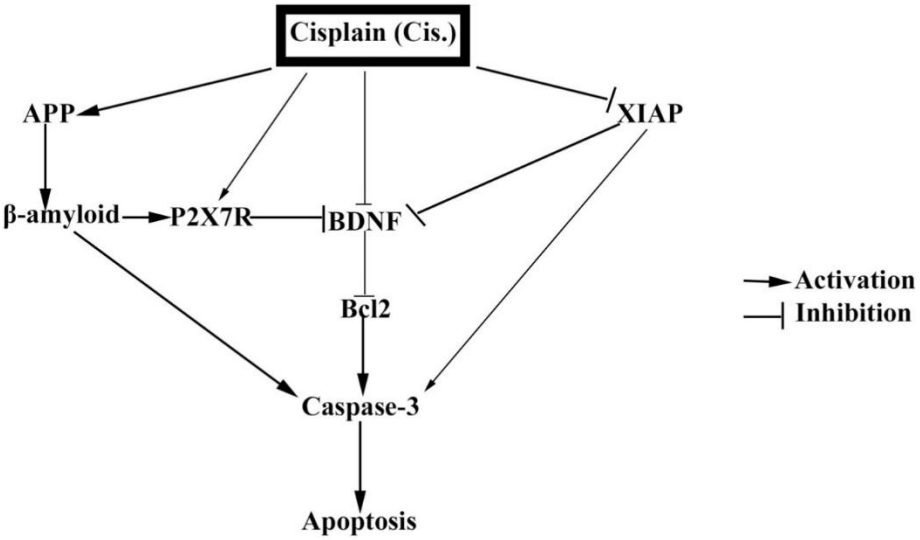

Figure 7. The mechanistic cascades of Cis induced brain apoptosis. The integration of possible mechanistic cascades of Cis induced brain apoptosis. Cis directly activates APP, β-amyloid and P2X7R. On the contrary, Cis can inhibit BDNF directly and indirectly via inhibiting XIAP. The decrease of DDNF is subsequently associated with the decrease of Bcl2, and so caspase-3 is activated and finally cerebral apoptosis is induced.

Our obtained results showed a significant reduction of P2Y12R, P2X7R and up-regulation of XIAP in the Ginkgo+Cis group. These changes were followed by a significant increase of BDNF. The overexpression of BDNF is in accordance with [89] who reported that *Ginkgo* protects against acrylamide induced brain toxicity.

The increased BDNF expression promotes the neuronal regeneration [89], inhibits many inflammatory mediators, up-regulates anti-apoptotic proteins such as Bcl-2 [90], thus blocking apoptotic markers such as caspase-3 [91].

5. Conclusions

The results of the existing study verify that concurrent treatment with *Ginkgo* attenuated Cis-induced cerebral apoptosis in rats. Thus, *Ginkgo* is considered an effective adjuvant for Cis as it has the ability to inhibit cerebral oxidative stress and apoptosis via regulating XIAP and BDNF levels and inhibiting APP, P2Y12R, P2X7R and caspase-3 in the brain tissue.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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