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Protective Effect of Daidzein on Ifosfamide-Induced Neurotoxicity *Via* Improving Some Selected Oxidative Stress Parameters in Male Rats [#]

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Abstract

Ifosfamide is an alkylating agent and an analog of cyclophosphamide, used as a single agent or in combination with other agents to treat a wide variety of malignancies. Central nervous system (CNS) toxicity has been reported in approximately 10%–30% of patients receiving intravenous infusions of ifosfamide. Daidzein, a natural isoflavone with estrogen-like activity, has various properties, such as anticancer, and antiinflammation activities. In this study, the possible protective effects of daidzein on ifosfamide-induced neurotoxicity in male rats were examined by the determination of changes in selected oxidant–antioxidant markers of male rats' brain tissue.

Twenty-eight (28) apparently-healthy Wistar male rats weighing (120-150gm) allocated into 4 groups (n=7) were used in this study. Rats orally-administered 1% tween 20 dissolved in distilled water/Control (**Group I**); rats were orally-administered daidzein suspension (100mg/kg) for 7 days (Group II); rats intraperitoneally-injected with a single dose of ifosfamide (500 mg/kg) (Group III); rats orally-administered for 7 days with the daidzein (100mg/kg) before a single intraperitoneal dose of ifosfamide (500 mg/kg) at day 7 (Group IV). Twenty-four (24) hours after the end of treatment, determination of the malondialdehyde, reduced glutathione, and superoxide dismutase enzyme activity levels in the rats' brain tissue homogenate were performed; in addition to the histopathological examination of the brain tissues sections. Results showed that the levels of malondialdehyde in brain tissue were significantly-increased (P<0.05) in (Group III/ifosfamide-only) rats compared to such level in the rats' brain tissue of controls (Group I). Furthermore, the brain tissue level of the malondialdehyde was significantly-decreased (P<0.05) in rats of Group IV (orally-administered DZN prior to IFO) compared to such tissue level in rats of Group III. Moreover, the brain levels of each of the reduced glutathione and the superoxide dismutase enzyme activity were significantly-decreased (P<0.05) in (Group III) compared to each level in those of Group I. Additionally, the brain levels of each of the antioxidant parameters was significantly-increase (P<0.05) in Group IV rats compared to each of these tissue levels in rats of Group III.

As a results, daidzein could have a protective effect against ifosfamide-induced neurotoxicity in rats *via* improving some selected oxidative stress parameters in male rats.

Keywords: Daidzein, Ifosfamide, Neurotoxicity, Oxidative Stress

التاثير الوقائي للدايدزين على السمية العصبية المستحثة بواسطة الايفوسفاميد عن طريق تحسين بعض محددات الاجهاد التاكسدي المختارة في ذكور الجرذان[#]

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الخلاصة

الايفوسفاميد هو عامل مؤلكل ، يستخدم كعامل منفرد أو بالاشتراك مع عوامل أخرى لعلاج مجموعة متنوعة من الأورام الخبيثة. تم الإبلاغ عن سمية الجهاز العصبي المركزي في حوالي(١٠-٣٠٪) من المرضى الذين يتلقون الحقن في الوريد من الايفوسفاميد.الدايدزين وهو إيسوفلافون طبيعي له نشاط شبيه بالإستروجين ، وله خصائص مختلفة ، مثل الأنشطة المضادة للسرطان والالتهابات. ان التاثيرات الوقائية المحتملة للدايدزين على السمية العصبية المستحثة بواسطة الايفوسفاميد في ذكور الجرذان تم دراستها عن طريق تحديد التغيرات في بعض المحددات المؤكسدة ومضادات التأكسد المختارة في نسيج دماغ الجرذان الذكور. تم اجراء الدراسة باستخدام ثمان وعشرين (٢٨) جرذا من ذكور الويستار تزن ١٢٠-١٥٠غم وتم تقسيمها عشوائيا الى أربع مجموعات (كل مجموعة مؤلفة من ٧ جرذان) على النحو التالي: **المجموعة الاولى** تم اعطاؤها فمويا (١٪ توين ٢٠ مذاب في الماء المقطر/اليوم) عن طريق انبوب التجريع لمدة ٧ أيام متوالية؛ **المجموعة الثانية**: تم اعطاؤها الدايدزين فمويا عن طريق انبوب التجريع بجرعة مقدارها (١٠٠ملغ لكل كغم من وزن الجسم/اليوم) لمدة ٧ أيام متوالية؛ **المجموعة الثالثة**: حقنت داخل الصفاق بجرعة واحدة من الايفوسفاميد (٥٠٠ ملغ/ كغم من وزن الجسم)؛ **المجموعة الرابعة**: تم اعطاؤها الدايدزين فمويا (١٠٠ملغ لكل كغم من وزن الجسم/اليوم لمدة ٧ أيام والايفوسفاميد الذي حقن داخل الصفاق في اليوم السابع.

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بعد ٢٤ ساعة من اخرانتهاء المعالجة، تم تحديد مستويات المالوندايالديهايد، مختزل الكلوتثيون وأنزيم السوبرأوكسيد ديسميوتايز في متجانس نسيج دماغ الجرذان بالاضافة الى دراسة مقاطع نسيجية للنسيج الدماغي .
اظهرت النتائج ان مستوى المالوندايالديهايد في نسيج الدماغ كان مرتفعا بشكل معنوي في المجموعة الثالثة مقارنة مع المجموعة الاولى، بالاضافة الى ان مستواه قد انخفض بشكل معنوي في نسيج دماغ جرذان المجموعة الرابعة مقارنة بمستواه في النسيج الدماغي لجرذان المجموعة الثالثة ، كما ان المستويات لكل من مختزل الكلوتاثيون وانزيم سوبراوكسيد دسميوتايز قد انحفض بشكل معنوي في النسيج الدماغي لذكور جرذان المجموعة الثالثة مقارنة بالمجموعة الأولى، بالاضافة الى ذلك فان المستويات لكل مضاد أكسدة قد زادت بشكل معنوي في الانسجة الدماغية لذكورجرذان المجموعة الرابعة مقارنة بتلك المستويات في نسيج دماغ جرذان المجموعة الثالثة.
بينت هذه الدراسة الى ان الدايدزين لديه تأثير وقائي ضد السمية العصبية المستحثة بواسطة الايفوسفاميد في الجرذان عن طريق تحسين بعض محددات الاجهاد التاكسدي المختارة في ذكور الجرذان.
الكلمات المفتاحية: الدايدزين، الايفوسفاميد ،السمية عصبية،الاجهاد التأكسدي

Introduction

"Neurotoxicity" term indicates any harmful act on the chemistry, structures, and functions of "the central nervous system (CNS) (brain) or peripheral nervous system" (PNS) brought by exposure to toxic materials, whether they are naturally or artificially formed. These materials can disrupt or kill the nerves necessary for processing and transmitting information in "the brain and other parts of the nervous system" [(1)].

The ability of a particular chemical to enter a specific area of the nervous system is determined by the characteristics of both the tissue and the chemical. Moreover, the blood-brain barrier serves as a protective mechanism that restricts the entry of many chemicals into the brain. [(2)].

Ifosfamide (IFO) is an oxazaphosphorine alkylating and immunosuppressive agent and it is an analog of cyclophosphamide (CP); is used to treat a variety of neoplasia, including sarcoma, lymphoma, and germinal tumors [(3,4,5)].

Mechanism of action

Ifosfamide (IFO) is a prodrug that requires activation by microsomal liver enzymes to produce active metabolites, namely IFO mustard and 4-hydroxy-IFO, which exert their cytotoxic effects. According to researchers, these active metabolites operate through two cytotoxic mechanisms. Firstly, they can induce cellular damage by creating inter-strand or intra-strand crosslinks, leading to apoptosis of damaged cells. Secondly, they can enhance the synthesis of reactive oxygen species (ROSs), which cause irreversible DNA damage and cessation of protein synthesis [(6)].

Side Effect

Its major adverse effects include nausea, vomiting, myelosuppression, interstitial pneumonitis, hemorrhagic cystitis, alopecia, and arrhythmias [(7). IFO] was also recognized to have the potential to produce neurotoxicity and encephalopathy [(8)], which can manifest as tiredness, disorientation, blurred vision,
seizures, and auditory or visual paranoid hallucinations among other symptoms. [(7,9)]. Besides; that and according to studies the incidence of IFO-associated neurotoxicity has been expected to occur in 10–30% of adults and 3–19% of children [(10, 12)] and typically, the signs of neurotoxicity were discovered within the hours of 12 and 146 following the injection of IFO [(11)].

The phenomenon of oxidative stress (OS) is brought on by an imbalance between the biological system's capacity to detoxify these reactive products and the formation and buildup of reactive oxygen species (ROSs) in cells and tissues [(13).]

According to the researcher, neurotoxicity may be linked to the pathogenesis of a metabolite of IFO, known as chloroacetaldehyde (CAA) [(14)]; The neurotoxic acts formed by such metabolites are via - reduction in the CNS' level of reduced glutathione (GSH) and the cessation of oxidative phosphorylation in the mitochondria, which formed a disturbance in the fatty acid metabolism Additionally, during regimen with the chemotherapy, an important issue is to minimize the toxic effects of such therapy in healthy cells [(15)]; thus, in this context, there is a need for natural compounds that can effectively-reduce the neurotoxicity- induced by IFO.

Daidzein (DZN) is a polyphenolic compound of the isoflavone family that is present in red clover (Trifolium pratense), alfalfa (Medicago sativa), soy, and some legumes (Leguminosae). In addition, DZN has been shown to have antioxidant, anti-inflammatory, and cardio protective properties [(16)], as well as to protect against cancers of the large intestine, chest, and prostate.

Pharmacological uses

the pharmacological uses and the effects of DZN on human health are as follows:

1.Antidiabetic Decreased type 2 diabetes risk and may possibly improve glycemia control in overweight patients [(17)].

2.Menopausal symptoms: Hot flash frequency lowering, intensity, and fatigue, and reduce of anxiety scores [(18)].

3.Anti-osteoporotic: rise in lumbar spine bone mineral density, a decline in bone resorption indicators, an uptick in markers of bone formation, and an increase in calcium retention [(19)].

Side Effect

Generally, isoflavones are well-tolerated; Their gastrointestinal (GI) side effects, which primarily include moderate nausea, bloating, diarrhea, and constipation [(20)]

By assessing a few chosen OS parameters in male Wistar rats, this study aimed to determine the

potential protective impact of DZN orally given on IFO-induced neurotoxicity.

Materials and Methods

Drugs, chemicals and kits with their Suppliers

Ifosfamide powder was obtained from Picasso/China. Daidzein (DZN) pure powder was purchased from Macklin/China. Diethyl Ether (Alpha Chemika, India), and Phosphate buffered saline (PBS) (Santa Cruz biotechnology /USA) were also used in the study.

Moreover, all enzyme-linked immunosorbent assay (ELISA) kits used in the current study, were obtained from Nanjing Pars Biochem CO., Ltd /China and include:

Rat reduced glutathione (GSH) Elisa kit, rat Malondialdehyde (MDA) Elisa kit,

rat Super oxidase dismutase (SOD) enzyme Elisa kit.

Preparation of Daidzein Suspension

Daidzein suspension was prepared according to the method of Vinarov *et al* (2018) [21]; where

1.One tenth of ml (0.1 ml) of Tween 20 was added to 9.9ml of distilled water to prepare surfactant solution.

2- Two hundred milligrams (200mg) of DZN powder were weighed and added to the surfactant solution. then,

3.Mixed well for 5 minutes by Vortex

Animals and Experimental Design

The scientific and ethical committees of the University of Baghdad's College of Pharmacy gave their approval to this work. Twenty-eight (28) mature Wistar male rats weighing 120–150g that appeared healthy were procured from the animal house of the College of Pharmacy at the University of Baghdad and kept there under regular temperature, humidity, and light/dark cycle conditions. Rats were housed for a week before the experiment and fed commercial pellets and tap water as needed during the trial. The experimental male rats were allocated into 4 groups of 7 rats each, according to a random choice process.

Group I. Male rats were orally-administered 1% tween 20 dissolved in distilled water (DW) *via* rats' oral gavage for 7 days. This group represents the control group.

Group II- Male rats were orally-administered DZN suspension at a dose (100mg/kg/day) [22] *via* rats' oral gavage for 7 consecutive days.

Group III- Male rats were orally-administered 1% tween 20 dissolved in D.W *via* rats' oral gavage for 7 days; and a single IP dose of IFO 500mg/kg [11], was injected on day 7.

Group IV-Male Rats were orally-administered a DZN suspension daily for 7 consecutive days by oral gavage at a dose (100mg/kg/day) and a single I.P dose of (500mg/kg) IFO, which was injected on day 7.

Preparation of Brain Tissue Homogenate

All rat groups were sacrificed by cervical dislocation under diethyl ether anesthesia 24 hours after the last dose of the drug; the brain was swiftly removed, cut in half vertically for biochemical and microscopic analysis; the excess blood was then removed by rinsing the brain with PBS/(pH=7.4), followed by drying with filter paper, and it was divided into two parts:

Part One: since the weight of the brain tissue was determined before homogenization, the first part was employed to prepare the homogenate of brain tissue. Each rat's brain tissue was placed in a 15 ml plastic test tube containing cold PBS solution (pH=7.4); the tissue weight in grams to the PBS volume in milliliters was then converted to a homogenate using an electrical homogenizer; the brain homogenate was then centrifuged using a cold centrifuge for 20 minutes at 10,000 rpm at +4 C°, and the supernatant fluid was collected, kept frozen for later use for the quantitative measurement of MDA, GSH, and SOD levels [23,24].

Part Two: was used for histological examination; where, each male rat's brain was removed after necropsy and used for histopathological analysis using the technique described by Çelik et al (2020) [24] by using paraffin sections. The brain fragments were fixed in a10% formaldehyde solution, embedded in paraffin, segmented, and then stained with hematoxylin and eosin. Moreover, morphological analysis of samples of brain tissue was performed using a light microscope [25].

Statistical Analysis

Data were expressed as Mean±SD. The statistical significance of the differences among various groups was explored by one-way analysis of variance (ANOVA) test by SPSS statistics version 25. The differences were statistically regarded significant when *P*-value was less than (0.05).

Results

Effects of Daidzein (DZN) on the Malondialdehyde (MDA) Level in Rats' Brain Tissue Homogenate

Table 1 and figure 1 showed that, there was a non-significant difference ($P>0.05$) in the level of MDA in brain tissue homogenate in male rats (Group II) compared to the corresponding tissue level in control (Group I) rats. The mean ±SD of MDA were (1.59±0.4, 2.21±0.36) mmol/L levels, respectively. Furthermore, a single I.P dose of IFO (500mg/kg) injected into male rats (Group III) produced a significant ($P<0.05$) increase in the MDA level in brain tissue homogenate compared to the corresponding tissue level in control (Group I)

rats. The Mean±SD of MDA levels were respectively, (3.31±1.04, 2.21±0.36) mmol/L.

Additionally, there was a significant decrease (P<0.05) in the MDA level in the brain tissue of male rats (Group IV compared to such level in the brain tissue of (Group III). The mean± SD of MDA in brain tissue were respectively, (1.87 ±0.33, 3.31±1.04) mmol/L levels.

Table 1. Levels of selected oxidative stress (os) parameters in each brain tissue rats' groups. malondialdehyde (mda); reduced glutathione (gsh); superoxide dismutase (sod) enzyme.

Group	No.	MDA (mmol/L)	GSH (ng/L)	SOD (ng/L)
I (Control)	7	2.21 ± 0.36 [a]	55.6 ± 7.4 [a]	61.3 ± 4.03[a]
II (Daidzein)	7	1.59 ± 0.4 [a]	48.7 ± 5.23 [a]	50.03 ± 10.1[a]
III Ifosfamide (IFO) (500mg/kg)	7	3.31 ± 1.04 [*b]	25.3 ± 9.1 [* b]	35.1 ± 5.78[*b]
IV (IFO and DZN)	7	1.87 ± 0.33 [a]	45.1 ± 6.4 [a]	48.9 ± 12.37[a]

Data were expressed as Mean±SD by one-way ANOVA tests. (*) represent as significant difference in comparison with the control group. Non-identical superscripts (a, and b) within the columns are significantly different (P<0.05).

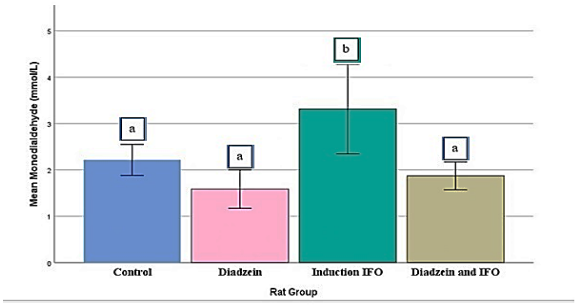

Figure 1. The Malondialdehyde (MDA) Level in Brain Tissue of each Rats' Group. Values with non-identical small letters (a, and b) are significantly different (P<0.05). Values with an identical small letter (a) are non-significantly different (P>0.05).

Effects of Daidzein (DZN) on the Reduced Glutathione (GSH) Level in Rats' Brain Tissue Homogenate

Table 1 and figure 2 showed that, there was a non-significant difference (P>0.05) in the brain tissue level of GSH in (Group II) rats compared to such tissue level in control male (Group I) rats. The Mean±SD of GSH levels were (48.7±5.23, 55.6± 7.4) ng/L, respectively.

Moreover, table 1 and figure 2 showed that a single I.P dose of IFO (500mg/kg) injected to rats (Group III), produced significant decrease (P<0.05) in the GSH level in brain tissue of male rats compared to the corresponding tissue level in control (Group I) rats. Mean±SD of GSH levels were respectively, (25.3±9.1, 55.6±7.4) ng/L.

Additionally, there were significant increase (P<0.05) in the GSH level in brain tissue of male rats (Group IV compared to that level in brain tissue of rats (Group III); where, Mean±SD of GSH brain levels were (45.1±6.4, 25.3±9.1) ng/L, respectively.

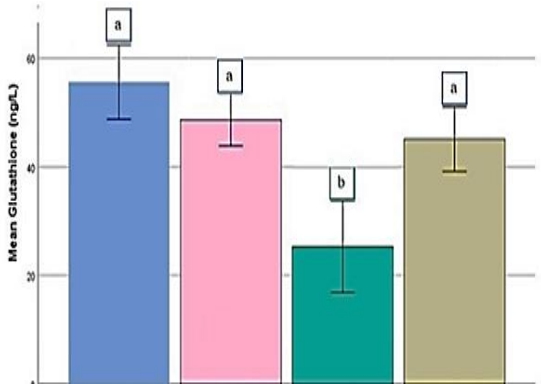

Figure 2. The Reduced Glutathione (GSH) Level in Brain Tissue of each Rats' Group. Values with non-identical small letters (a, and b) are significantly different (P<0.05). Values with an identical small letter (a) are non-significantly different (P>0.05).

Effects of Daidzein (DZN) on the Superoxide Dismutase (SOD) Enzyme levels in Rats' Brain Tissue Homogenate

Table 1 and figure 3 showed that, there were non-significant difference (P>0.05) in the brain tissue SOD level in rats (Group II) compared to such enzyme level in brain tissue of control male (Group I) rats. Mean±SD of MDA levels were (50.03 ±10.1, 61.3±4.03) ng/L, respectively.

Furthermore, table 1 and figure 3 also showed that in rats injected with a single dose of (500mg/kg) IFO (Group III), there were significant reductions (P<0.05) in the activity levels of SOD in brain tissue homogenate of rats compared to the corresponding activity level in control (Group I) male rats. Mean±SD of SOD brain tissue levels was (35.1±5.78, 61.3±4.03) ng/L, respectively.

Additionally, there was a significant increase (P<0.05) in SOD levels in the brain tissue of Group IV rats compared to the corresponding level in brain tissue homogenate of rats in (Group III); since, the levels were respectively, (35.1±5.78, 48.9±12.37) ng/L.

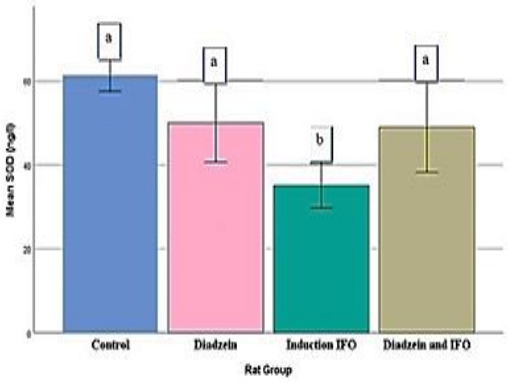

Figure 3. The Superoxide Dismutase (SOD) enzyme activity levels in Brain Tissue of each Rats' Group. Values with non-identical small letters (a, and b) are significantly different (*P*<0.05). Values with an identical small letter. (a) are non-significantly different (*P*>0.05).

Histopathological examination of rats' brain tissue

The brain section of male rats that were orally administered 100mg/kg/day DZN by oral gavage for 7 consecutive days (Group II), as shown in Figure 4B, exhibited normal characteristics. This included normal neuronal pyramidal cells with open-face nuclei and basophilic cytoplasm, with blood capillaries scattered between neurons. These features were similar to those observed in the brain section of control rats (Group I) as depicted in Figure 4A. "Besides, in section of male rats' brain IP injected with a single dose (500mg/kg) of IFO, there were neuronal degeneration, and irregular darky-stained cells, with frequent pyknotic nuclei that were surrounded with prominent halos arrows (Figure 4C).

Additionally, in the section of brain tissue of rats orally-administered DZN suspension daily by oral gavage at a dose (100mg/kg/day) for 7 consecutive days and a single I.P dose of (500mg/kg) IFO, which was injected on day 7 (Group IV); although there was a congestion of blood vessels and darky-stain nucleus surrounded by the halo, but there was no neuronal degeneration, and hyper-cellularity were not observed (in other word, the brain of normal architecture). (Figure 4D).

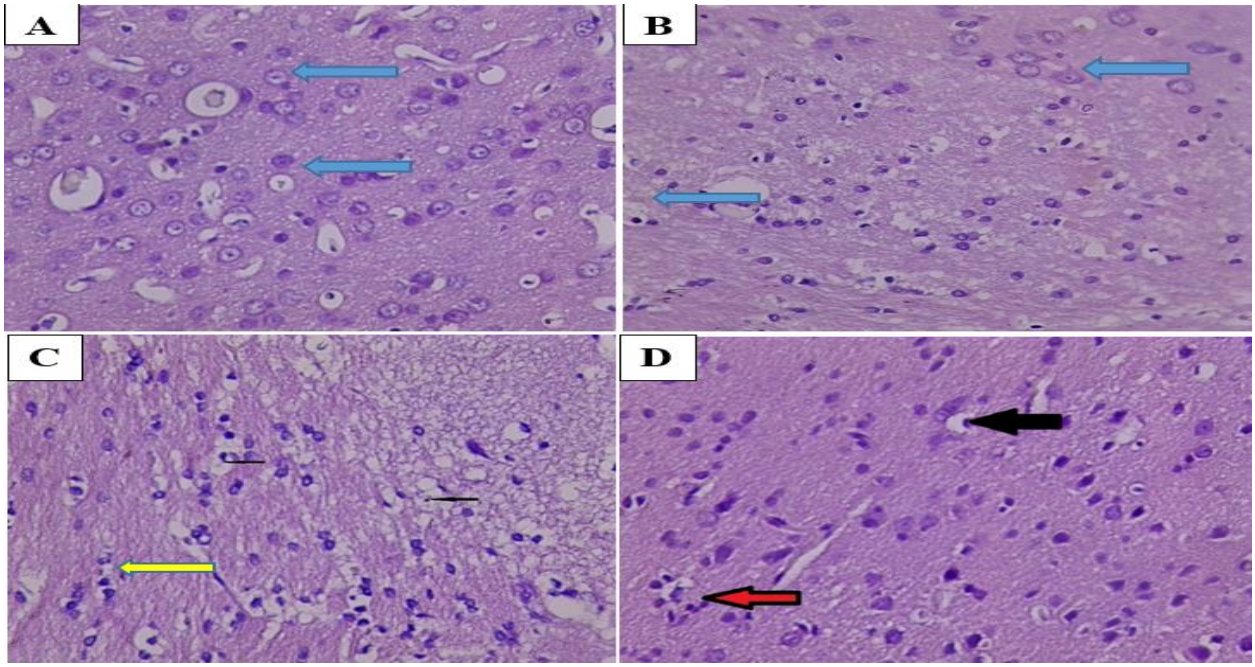

Figure 4. Histopathological section of brain tissue in various expCerimental rats' groups; (Hematoxylin and eosin; X40). (A) The control/Group I; (B) (DZN alone/Group II; (C) IFO/Group III; (D) The DZN and IFO/Group IV. Blue arrow refers to normal neuronal cell. Yellow arrow refers to inflammatory cells (Lymphocyte). Black arrow refers to darkly stained nucleus with surrounding Halo. Red arrow refers to mild congestion of blood vessels.

Discussion

Ifosfamide (IFO) is an alkylating chemotherapy medication that is used to treat a variety of neoplasia, including sarcoma, lymphoma, and germinal tumors [(3)]. Its major adverse effects include nausea, vomiting, myelo-suppression, interstitial pneumonitis, hemorrhagic cystitis, alopecia, and arrhythmias [(7)]. The equivalent chemotherapy medication was also recognized to have a risk of neurotoxicity and encephalopathy [(8)].

The formation of hazardous levels of Chloroacetaldehyde, an IFA metabolite that may pass the blood-brain barrier and have inhibitory effects on the CNS, was assumed to be linked to the neurotoxicity caused by IFO [3,10, 27]. Furthermore, it was hypothesized that Chloroacetaldehyde could inhibit mitochondrial oxidative phosphorylation, which could lead to impaired fatty acid metabolism in the inner mitochondrial membrane's electron transport chain [28, 29]. Since the neurons are more vulnerable to oxidative damage because the brain is rich in polyunsaturated fatty acids [12].

The results of the current study (table 1) showed that IP injection of IFO (Group III rats) caused significant -increase (P<0.05) in brain tissue level of MDA (figure 1), and also -decrease in brain tissue level of the GSH and SOD levels (figures 2 and 3, respectively) in comparison with such levels in control Group I rats.these result in line with other result obtained by Ilyas *et al*(2021)[24] .

Moreover, the histological results of the present study demonstrated that there were degenerative changes (neuronal degeneration) in the brain tissue cells in male rats IP injected with a single dose of IFO (500mg/kg), and irregular darky-stained cells, with frequent pyknotic nuclei that were surrounded with prominent halos arrows;these change showed withthin 24 hours; and these results are similar to those obtained by other researcher Çelik *et al* (2020) [25].

The current study showed that in Group II rats orally-administered DZN (100mg/kg) suspension for 7 consecutive days, there were non-significant differences (P>0.05) in the levels of MDA, GSH, SOD compared to each of such levels in brain tissue of Group I/Control rats; these results are consistent with those obtained by Tomar *et al* (2020) study [26].

The current study also showed that in Group IV rats/ orally-administered DZN for 7 days prior to IFO, there was a significantly-decrease (P<0.05) the level of MDA (table 1and figure 1), -increase. the levels of GSH, and SOD levels in brain tissue homogenate of male rats (table 1 and figures 2, 3. respectively). Researchers reported that DZN has antioxidant effects; since, it can -lower the ROSs and -decrease the lipid peroxidation process and thus a decrease in the levels of MDA in different rats' organs (kidneys, and brain), respectively, can be promoted [26, 30].

Moreover, the results of the current study concerning Group IV are in accordance with another study; where, the levels of GSH in the brain tissue increased by DZN administration despite of using a different animal model to induce epilepsy [31], rats' model of AD induced by Streptozotocin [32], and on reserpine-induced PD in rats [33].

Antioxidant enzymes like SOD have been documented to reduce the potential harm that oxidants may bring to cells, and it has been shown that a decrease in this enzyme's activity is linked to an increase in free radical formation during the metabolism of IFOs [34]. Moreover, GSH depletion with an increase in lipid peroxidation has been well-documented critical component in the pathophysiology of IFO-induced neurotoxicity, and Chloroacetaldehyde can also consume GSH in the brain [11, 12,35].

Moreover, the results of table 1 and figure 3 showed that in Group IV rats, there was an increment in the SOD level in rats' brain tissue homogenate compared with the corresponding levels in IFO injected rats (Group III). Results of this study resembled those of others but with the utilization of different rats' models (Alzheimer induced by Streptozotocin) [32], and (creation of focal cerebral ischemia in rats) [30]. The proposed mechanism by which DZN act as an antioxidant might be due to the activation of the Nrf2(nuclear factor erythroid 2–related factor 2) pathway that was responsible for the production of antioxidant element like SOD enzyme [36].

Additionally, in the present study, the section of the brain tissue histology of Group IV rats (Figure 4D), showed normal brain architecture with no observed hyper-cellularity. The suggestion could be related to the anti-oxidant effect of DZN [37]; DZN increase the level of GSH which function as natural anti-oxidant in the brain [33] and these results are confirmed by those of Zheng *et al* (2022) study by the utilization of other models of neuronal injury; where researchers suggested that, DZN caused neuronal regeneration after traumatic nerve injury [38].

Conclusion

According to the results obtained from this study, IFO produced considerable adverse effects on rat brains; and the orally-administered DZN (100 mg/kg/day) for 7 days has a protective effect against IFO-induced neurotoxicity *via* the improvement of -OS mechanism (reduction of MDA/and elevation of both GSH and SOD) and -in the histological analysis of brain tissue of rats.

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Conflicts of Interest

The authors have no conflict of interests.

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Ethics Statements

It was approved by the Ethical Committee of the College of Pharmacy/University of Baghdad before the start of the study

Author Contribution

The first author did the practical work and result analysis.The second author supervised the whole work.

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