Cytoprotective Effect ofDaidzein, Naringenin, and Icariin against Cisplatin Induced Neurotoxicity in Mice

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ABSTRACT

Introduction: Cisplatin is an effective chemotherapeutic drug with significant dose-limiting neurotoxicity that causes peripheral neuropathy. Although it is hypothesised that DNA platinum adduct production is responsible for both its cytotoxicity in cancer cells and adverseefects in neurons, downstream mechanisms that cause distal axonal degeneration are still unknown.
Objective: The cytoprotective efficacy of Daidzein, Naringenin, and Icariin was investigated against Cisplatin-induced neurotoxicity in female Swiss mice during this work.
Methods: All animals were divided into two sets i.e. pre-cisplatin (BA)and post-cisplatin (AA) administration and treated with Daidzein, Naringenin, and Icariin. Nephrotoxicity was assessed by body weight and biochemical parameters i.e. ALT, lipid peroxidation (MDA level) and Glutathione (GSH).
Results: During this study body weight of the experimental animal changed by 5% maximum observed in both sets. Biochemical brain showed that pre-treated and post-treated with Icariin groups were most effectiveagainst cisplatin induced neurotoxicity as compare to naringenin and daidzein. These findings are also confirmed by histopathology.
Conclusion: The study has proven that pre-treatment with flavonoids was found more effective against cisplatin-induced cytotoxicity as compared to post-treatment.

Keywords: Cytoprotective, Flavonoids, Cisplatin Cytotoxicity, neurotoxicity.
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INTRODUCTION

Cisplatin also known as dichlorodiamino platinum, is an inorganic platinum-based chemotherapeutic drug. it is frequently used in the treatment of a range of solid malignant tumors,including head and neck, lung, testis, ovarian, and bladder cancers.(Dasari and Bernard Tchounwou 2014)Cisplatin has been prescribedfor the treatment of numerous human cancers since its FDA clearance in 1978.(Galluzzi et al. 2012) DNA is the proven primary target of cisplatin and cisplatin adduct formation not only affects several DNA-dependent cellular functions, including inhibition of replication and transcription, cell cycle arrest, and DNA damage resulting in cell death and apoptosis but also may result in mutations.(Basu and Krishnamurthy 2010; Jadon et al. 2019; Yimit et al. 2019; Li et al. 2021)Cisplatin attaches to DNA, leading to the formation of inter-and intrastraland cross-links. Cross-linking causes flawed DNA templates and halts the synthesis and replication of DNA.Cross-linking can further cause DNA damage in cells that divide quickly, such as those found in malignancies. Cross-linking can further cause DNA damage in cells that divide quickly, such as those found in malignancies. Mildly damaged DNA sometimes can be repaired, butsevere DNA damage results in irreparable injury and cell death. Despite being a staple of cancer treatment, cisplatin's use is primarily constrained by two factors: developed resistance to it and severe adverse effects in normal tissues.(Aldossary 2019)

The side effects of cisplatin on healthy tissues, such as neurotoxicity, ototoxicity, nausea and vomiting, and nephrotoxicity, are another important aspect that restricts its use. However, the neurotoxicity caused by cisplatin, which manifests as peripheral neuropathy, diminished recognition, tremor, and ataxia, is a well-defined dose-limiting side effect that restricts its therapeutic utility. Peripheral neurotoxicity develops in approximately 50% of patients receiving cisplatin.(Van Der Hoop et al. 1990)Peripheral neuropathy due to neurotoxicity of cisplatin is characterized by distal axonal degeneration, a conditionsimilar to other axonal peripheral neuropathies. The exact molecular mechanisms that lead to distalaxonal degeneration are not fully understood. However, a number of pathophysiological mechanisms have beenproposed to explain this phenomenon.It is also thought that cisplatin-induced toxicities may be caused by oxidative stress, DNA damage, mitochondrial malfunction, and the production of pro-inflammatory cytokines.(Andres et al. 2014; Wang et al. 2020)Many strategies have been tried over time to reduce these side effectson healthy tissues. One strategy is to synthesize novel cisplatin analogs and screen them for their decreased toxicity in healthy tissues. In this manner, a number of cisplatin analogues with milder side effects have been found, including carboplatin.(Pasetto et al. 2006)During cisplatin treatment, hydrating the patients has also been tried with some degree of benefit.(Cornelison and Reed 1993; Kurihara et al. 1996)Despite these improvements, cisplatin's side effects, particularly neurotoxicity and nephrotoxicity, continue to play a significant role in limiting its use and effectiveness in cancer therapy.

METHODS

Drugs and chemicals: Cisplatin was purchased from the local pharmacy as Cisplatin Injection Celplat-10 (Celon Labs Pvt. Ltd. India). Cisplatin was administered in a dose of 10 mg/kg/body wt, i.pinjected for 14 consecutive days, this dose was selected according to the cisplatin mouse model study of Perse M and Sara J. Holditch**,**et al.(Holditch et al. 2019; Perše 2021)

Daidzein(Purity ≥98%), Naringenin(Purity ≥95%), and Icariin (Purity ≥94%) were purchased from

Sigma Aldrich Chemical Pvt Ltd, Bangalore, India.Amifostinewas purchased from the local market as Amifostine Injection Cytofos 500(Sun Pharmaceuticals Industries Ltd, India)and all other chemicals were of analytical grade. Daidzein, Naringenin, and Icariinwere suspended in the 0.5% CMC suspensionand given orally to the respective groups.

Animals: Adult female Wistar albino mice (20-22g) were used for all experimental procedures.Animals were provided by the Department of Pharmacology, School of Studies in Pharmaceutical Science, Jiwaji University, Gwalior. The animals were housed in standard conditions of temperature (25±20C) and 12:12 h light-dark cycle. The mice were fed a commercial diet and water *ad Libitum*. Mice were left for one week ofacclimatization timebefore the beginning of the experiment. The experiment was approved by the institutional animal ethics committee ofSchool of Studies in Pharmaceutical Science, Jiwaji University, Gwalior, M.P. India (Approval no. IAEC/JU/60 dated 03/06/2019).

Experimental Design: Mice, acclimatized with laboratory conditions were divided into two sets of 36 each. In both sets of the experiment; the animals will divide into six treatment groups with six mice in each group. To the set-I dose of flavonoids and Amifostine were administered before 30 minutes of Cisplatin injection in which BA-I Without treatment (Normal Control), BA-II Cisplatin (Negative Control), BA-III Cisplatin+ Amifostine (Positive Control), BA-IV Cisplatin + Daidzein, BA-V Cisplatin + Naringenin and BA-VI Cisplatin + Icariin. To the set-II dose of Amifostine and flavonoids were administered After 30 minutes of Cisplatin injection in which BA-I Without treatment (Normal Control), BA-II Cisplatin (Negative Control), BA-III Cisplatin+ Amifostine (Positive Control), BA-IV Cisplatin + Daidzein, BA-V Cisplatin + Naringenin and BA-VI Cisplatin + Icariin.

Cisplatin(CP)**(**10mg/kg)(Perše 2021) and Amifostine(200mg/kg)(Wills et al. 2007)were injected intraperitoneally (i.p.) for 14 consecutive days.Daidzein (40mg/kg)(Eun Jeong Choi 2009), Naringenin (50mg/kg)(Hermenean et al. 2013)and Icariin (30mg/kg)(Li et al. 2015)administrated orally by gavage every day, 30 minutes before and after cisplatin injection to respective set. These selected doses of flavonoids were previously reported and not to produce any significant toxicity.The animals were immediately kept in groups of three in metabolic cages after the final cisplatin dose in order to collect urine continuously throughout the day.Neurotoxicitywas assessed by determining various histopathology and biochemical parameters i.e. body weight,Lipid peroxidation and Glutathione (GSH) in mice before and after cisplatin administration.

Collection and storage of blood and urine samples:

Urine samples were takenwhile theanimals wer ehousedin metabolic cages. During the time of urine collection, animals had free access to drinking water. A drop of concentrated hydrochloric acid was mixedwith the urine and thenkeep stored at 4˚C. Blood was drawn from each animal at the end of the experimentby puncturing the retro-orbital plexus. For the serum analysis, blood samples were allowed to coagulate for 45 minutes at room temperature. Serum was separated by centrifuge the sample at 3000 rpm at 4˚C for 15 minutes and utilized for the evaluation of several biochemical parameters.

Biochemical analysis inblood and urine sample: Various biochemical parameters were estimated for neuroprotective activity e.g., Lipid peroxidation and Glutathione (GSH). All parameters were estimated usingcommercially available kits and following the manufacturer's instructions.

Histological examination: After the collection of urine and blood samples physical methods of euthanasia were used for animal scarification. Animal packed in a chamber having chloroform dipped cotton when animal inhaled an excess

number of vapors and it got anesthetized, followed by cervical dislocation. After sacrifice, the whole brain tissue was collected carefully anddipped in 10%formalin solution and embedded in paraffin. The embedded tissues were then divided into sections that were 3 µm thick, placed onglass slides, and incubatedfor 30 minutes at 75 °C.To rehydrate the materials, a graded ethanol series (95%, 85%, and 70% ethanol) was used, after being deparaffinized using xylene for 10 min. Following washing, the specimens were incubated with Haematoxylin for 2 minutes, rinsed in running tap water for 1 minute, and then incubated with acid alcohol for 1 second. Afterward, the specimen samples were incubated with ammonia water solution for 1 second and then rinsed inrunning tap water for 10 min. The specimens were dehydrated using 70%, 80%, 90%, and 100% ethanol after counterstaining with Eosin solution for 90 seconds. Finally, the specimens were mounted with a mountingmedium and examined under a microscopetodetermine the extent ofthe tissue damage.

Statistical analysis: All data are expressed as means ± SEM (standard error of the mean). A statistical package for social sciences (SPSS) computer programme (version 22) was used to conduct the statistical study. To clarify the significance between group means, a one-way analysis of variance (ANOVA) test was employed, followed by a Tukey-Kramer post hoc test for multiple comparisons.At $p < 0.05$, differences were considered significant.

RESULTS

Effect of Daidzein, Naringenin, Icariin and cisplatin on body weight

During this study body weight of the experimental animal changedby 5% maximum observed in both sets (table no 1, 2 and figure no 1).

Table 1: change in body weight(SET-I)

Group	Treatment	Body weight (gm)		% Change in body weight
		Initial	Day 14	
BA - 1	Vehicle	20.6 ± 0.78	21.9 ± 0.44	6.13
BA - 2	Cisplatin	21.1 ± 0.41	20.7 ± 0.36	-1.90
BA - 3	Cisplatin+ Amifostine	20.5 ± 0.88	21.2 ± 0.23	3.41
BA - 4	Cisplatin + Daidzein	20.8 ± 0.55	21.2 ± 2.52	1.92
BA - 5	Cisplatin + Naringenin	21.5 ± 0.34	21.9 ± 0.36	1.83
BA - 6	Cisplatin + Icariin	22.2 ± 0.15	22.6 ± 0.25	1.80

Table 2: change in body weight(SET-II)

Group	Treatment	Body weight (gm)		% Change in body weight
		Initial	Day 14	
AA - 1	Vehicle	21.4 ± 0.78	22.5 ± 1.14	5.14
AA - 2	Cisplatin	20.2 ± 0.41	19.9 ± 1.06	-1.49
AA - 3	Cisplatin+ Amifostine	21.5 ± 1.88	22.1 ± 1.23	2.79
AA - 4	Cisplatin + Daidzein	19.9 ± 1.00	20.2 ± 1.12	1.12
AA - 5	Cisplatin + Naringenin	19.8 ± 0.45	20.1 ± 1.27	1.52
AA - 6	Cisplatin + Icariin	20.6 ± 1.26	20.9 ± 1.15	1.46

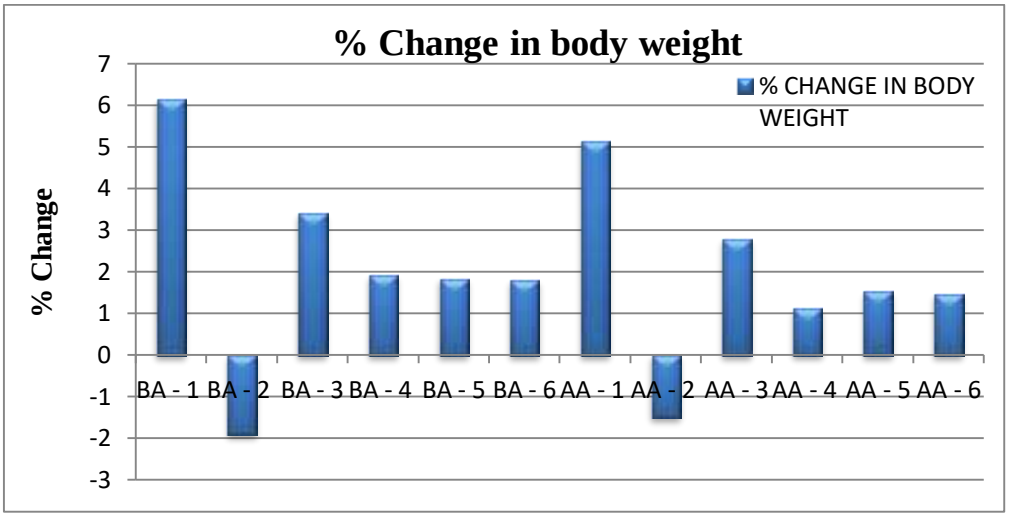

Figure1: Percentage change in body weight

Collected urine volume ranges from 2.0 to 2.82 ml and % decrease 12 to 24.82 %, physically clear to light yellow color observed (table no 3, 4 and figure no 2).

Table 3: Urine collection and physical observation (SET-I)

Group	Treatment	Volume of urine (ml)	% Decrease	Physical observation
BA - 1	Vehicle	2.82 ± 0.21	-	Clear
BA - 2	Cisplatin	2.12 ± 0.24	24.82	Pale yellow
BA - 3	Cisplatin+ Amifostine	2.48 ± 0.36	12.06	Light yellow
BA - 4	Cisplatin + Daidzein	2.46 ± 0.33	12.77	Light yellow
BA - 5	Cisplatin + Naringenin	2.43 ± 0.25	13.83	Light yellow
BA - 6	Cisplatin + Icariin	2.12 ± 0.44	24.82	Light yellow

Table 4: Urine collection and physical observation (SET- II)

Group	Treatment	Volume of urine (ml)	% Decrease	Physical observation
AA - 1	Vehicle	2.52 ± 0.21	-	Clear
AA - 2	Cisplatin	2.16 ± 0.24	14.29	Pale yellow
AA - 3	Cisplatin+ Amifostine	2.04 ± 0.36	19.05	Light yellow
AA - 4	Cisplatin + Daidzein	2.16 ± 0.33	14.29	Light yellow
AA - 5	Cisplatin + Naringenin	2.21 ± 0.45	12.30	Light yellow
AA - 6	Cisplatin + Icariin	2.13 ± 0.34	15.47	Light yellow

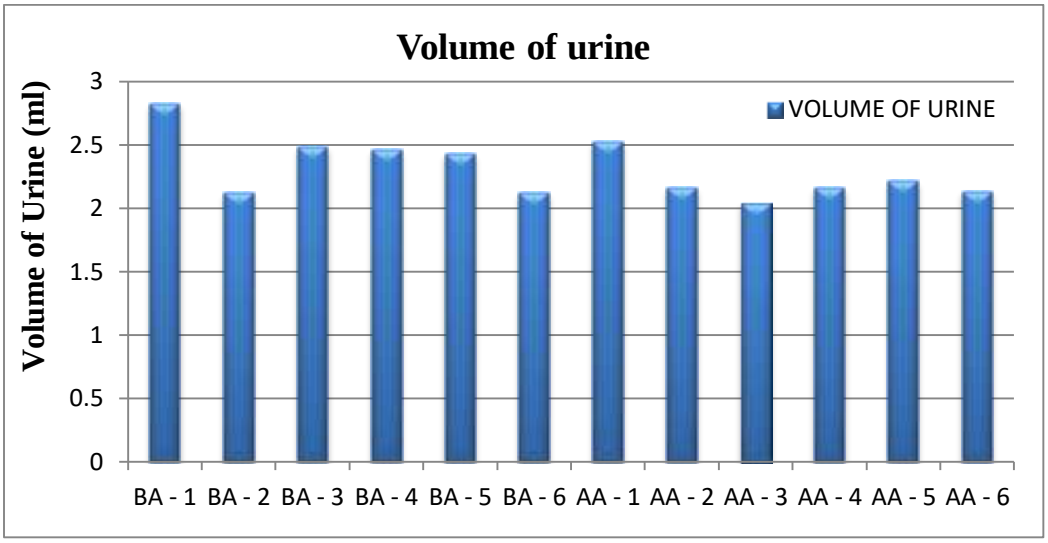

Figure 2: Volume of urine collected from both sets

a. Significance difference as compared to BA-1 (Vehicle)

b. Significance difference as compared to BA-2 (Cisplatin)

Effect of Daidzein, Naringenin, Icariin on Cisplatin-induced Neurotoxicity

Effect of Daidzein, Naringenin, and Icariin on Cisplatin-induced Neurotoxicity was detected by estimation of Lipid Peroxidation (LPO) by measuring MDA Level and reduced glutathione (GSH) in mice brain tissues by biochemical tests and histopathology study. A significant effect was shown in table no. 5,6 and figure 3.

Table5: Brain tissue analysis for lipid peroxidation (MDA) and reduced glutathione (GSH) tests (SET- I)

Group	Treatment	MDA (nmol/g tissue)	GSH (nmol/100mg tissue)
BA - 1	Vehicle	22.3 ± 1.1	158.6 ± 17.4
BA - 2	Cisplatin	56.6 ± 2.6a	85.7 ± 18.6a
BA - 3	Cisplatin + Amifostine	26.8 ± 1.8b*	130.4 ± 21.8b**
BA - 4	Cisplatin + Daidzein	31.3 ± 1.4b**	112.5 ± 14.5 b**
BA - 5	Cisplatin + Naringenin	37.6 ± 3.2a**	97.7 ± 13.7a**
BA - 6	Cisplatin + Icariin	29.2 ± 1.6b*	123.2 ± 12.9b*

All values are mean ± SEM, n = 6. *p<0.05, **p<0.01,

a. Significance difference as compared to BA-1 (Vehicle)

b. Significance difference as compared to BA-2 (Cisplatin)

Table6: Brain tissue analysis for lipid peroxidation (MDA) and reduced glutathione (GSH) tests (SET- II)

Group	Treatment	MDA (nmol/g tissue)	GSH (nmol/100mg tissue)
AA - 1	Vehicle	20.4 ± 1.3	173.2 ± 20.2
AA - 2	Cisplatin	47.4 ± 2.1a	92.23 ± 19.3a
AA - 3	Cisplatin + Amifostine	21.8 ± 1.5b*	162.3 ± 13.5b**

AA - 4	Cisplatin + Daidzein	25.3 ± 1.4b**	123.2 ± 22.2b**
AA - 5	Cisplatin + Naringenin	34.6 ± 0.3a**	102.8 ± 11.2 a**
AA - 6	Cisplatin + Icariin	23.4 ± 0.5b*	147.23 ± 18.3b**

All values are mean ± SEM, n = 6. *p<0.05, **p<0.01,
a. Significance difference as compared to AA-1 (Vehicle)
b. Significance difference as compared to AA-2 (Cisplatin)

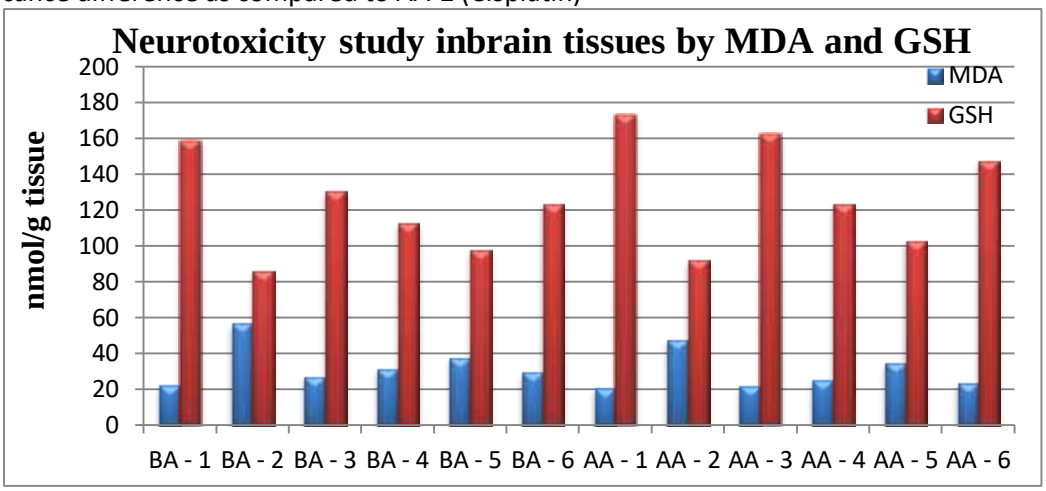

Figure 3: Brain tissue analysis for MDA and reduced GSH tests

Brain Histopathology

Tissues obtained from the brain of control group mice displayed regular morphology of cell (BA-1& AA-1), Histological analysis of the brain from cisplatin-treated mice showed severe and widespread necrosis which leads to loss of architecture. (BA-2 & AA-2), Histological analysis of the brain from mice group cisplatin-treated mice pretreated and post-treated with Amifostine (positive control) displayed minimal changes in regular morphology (BA-3 & AA-3), Histological analysis of brain from cisplatin-treated mice pretreated and post-treated with Daidzein, Naringenin, Icariin (BA-4, BA-5 BA-6 & AA-4, AA-5, AA-6) showed less histopathological changes. Greater improvements were seen in BA-4 & AA-4 and BA-6 & AA-6. All observations are similar for both sets (I and II) as sown in figures 4&5.

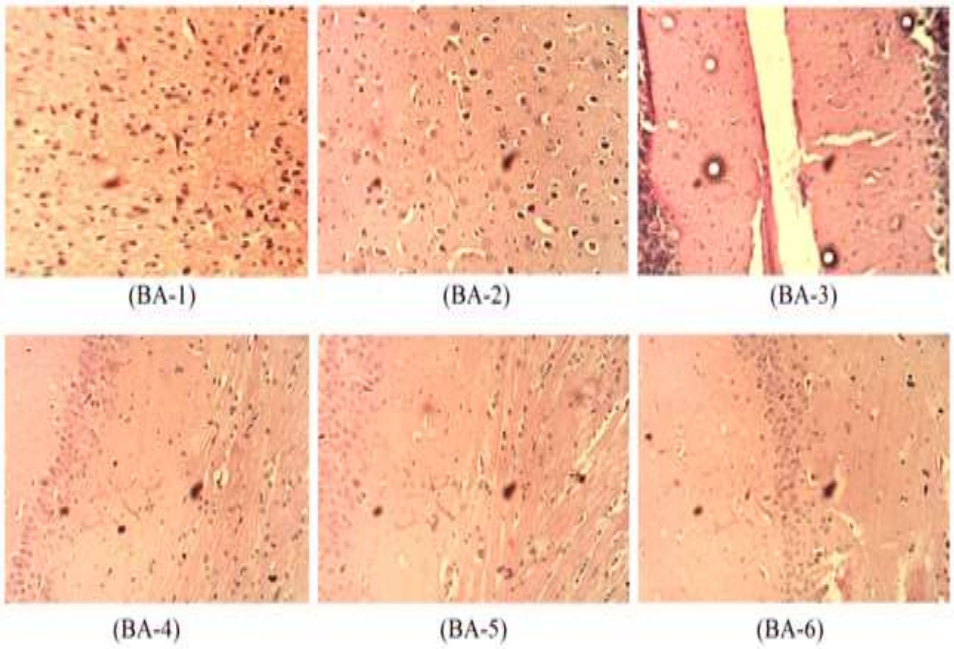

Figure 4: Histopathology of the brain obtained from set-I animals

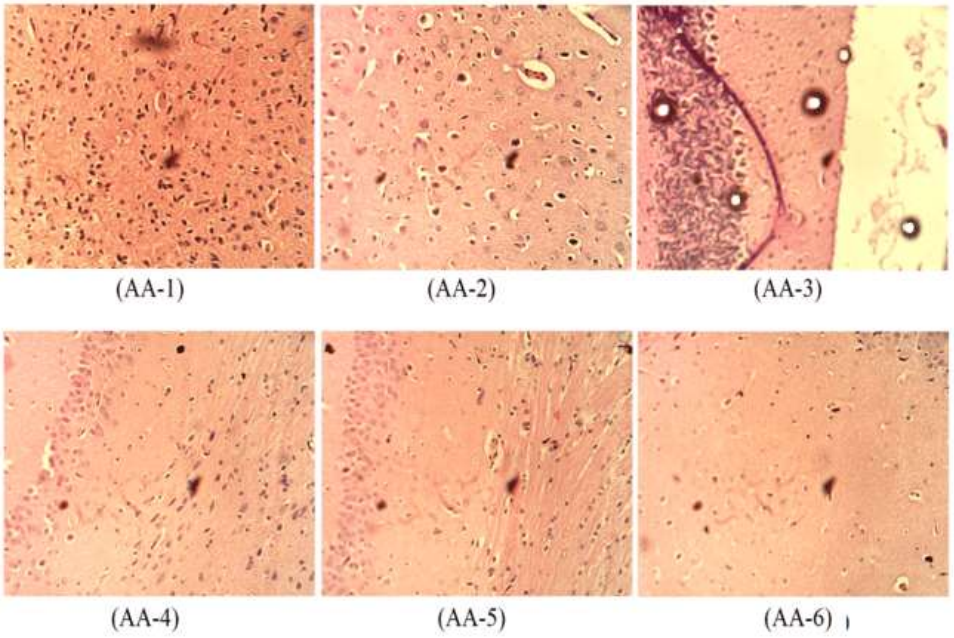

Figure 5: Histopathology of the brain obtained from set-II animals

DISCUSSION

Evaluation of Cytoprotective Potential of Daidzein, Naringenin, Icariin Flavonoids Against Cisplatin Induced Neurotoxicity in Mice. A total of 36 Wistar albino mice divided into two sets were taken and acclimatized to laboratory conditions through standard procedure. The Neurotoxicity of cisplatin are the major dose limiting side effects of this drug. Neurotoxicity was assessed by determining various

biochemical parameters i.e. body weight, Lipid peroxidation (MDA) and Glutathione (GSH) in mice before and after cisplatin administration. Histopathology findings in different tissues of the mice revealed cell degeneration and necrosis on the 14th day after the execution of a single dose of cisplatin per day.

Effect of Daidzein, Naringenin, and Icariin on Cisplatin-induced Neurotoxicity was detected by estimation of Lipid Peroxidation (LPO) by measuring MDA Level and reduced glutathione (GSH) in mice brain tissues by biochemical tests and histopathology study.(Andres et al. 2014)

Histological studieswerecarried out in the brain tissues to examine the impact of cisplatin on these tissues. The previous reports have also demonstrated the toxic effect of cisplatin in oxidative stress and injury to brain tissue.(Yimit et al. 2019)In the current investigation, it was found that cisplatin's cytotoxic properties caused the destruction of brain neurons. The Daidzein, Naringen, and Icariinontreatment also greatly lessens the brain tissue damagecaused by the administration of cisplatin.

CONCLUSION

Finally, based on our research, we can conclude that pretreatment with flavonoids was found more effective against cisplatin-induced cytotoxicity as compared to posttreatment. Furthermore,Icariin groups were most effective against neurotoxicitylikely due to their antioxidant andanti-inflammatory actions. However, the results of our study are restricted to relationships in female mice only, necessitating molecular research to expand on the mechanisms and validate our findings.

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CONFLICTS OF INTERESTS

There are no conflicts of interest.

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ABBREVIATIONS

FDA: Food and Drug Administration;**ANOVA:** One-way analysis of variance; **BA:** Before Administration; **AA:** After Administration; **SEM:**Scanning Electron Microscope; **MDA:** Malondialdehyde; **GSH:**Glutathione.

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