NEUROTOXIC EFFECT OF CISPLATIN ON HIPPOCAMPAL POSTNATAL DEVELOPMENT AND THE POTENTIAL PROTECTIVE EFFECT OF PANAX GINSENG EXTRACT

BY

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ABSTRACT

Background: Platinum compounds have been considered the 1st line drugs in the treatment of pediatric cancers despite their high organ toxicity. Conversely, Panax ginseng is known for its antioxidant, anti-inflammatory, and anti-stress properties. **This study aimed** to examine the histopathological and biochemical effects of cisplatin ± ginseng whole extract on hippocampal postnatal development, through investigation of oxidative stress, inflammatory, apoptotic pathways, effects on intracellular calcium homeostasis, and the subsequent effects on neurotransmitter levels. **Methods:** Rats' offspring were allocated into four groups (Control group, Gin group (200 mg/kg orally from postnatal day (PD) 7, Cis group (single dose of 5 mg/kg, S.C at PD 10), and Gin/Cis group). At PD11 and PD17, male pups were weighed, tested for open field test then decapitated and hippocampal samples were prepared for both histological examination and tissue homogenate analysis **Results**: cisplatin significantly decreased BW and activity, histopathological changes (pyramidal and granular cells were fewer, shrunken, and deeply stained, with pyknotic nuclei and pericellular vacuolations), and a decrease in calbindin protein expression with immunohistochemistry. Additionally, Cis significantly increased oxidative stress (elevated MDA and decrease SOD levels), inflammatory, and apoptotic markers (TNF-α, IL-1β, and P53), but significantly decreased acetylcholine and GABA activity. All these alterations were more prominent on PD17 while prior/co-administration of ginseng provided considerable protection against such neurotoxic effects. **Conclusion**: wherefore there is no choice in using cisplatin as a chemotherapeutic agent, prior/co-administration of ginseng is recommended to alleviate the expected neurotoxic effect.

Keywords: *Calbindin, Cisplatin, CNS Development, Ginseng, Neurotoxicity.*

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INTRODUCTION

Platinum compounds, particularly cisplatin, have been the considered 1st line drugs in the treatment of many pediatric malignancies, despite their grave side effects such as neurotoxicity (especially hippocampal neuronal damage and cognitive impairment), hepatotoxicity, ototoxicity, and nephrotoxicity ***(Choi et al., 2013; Hassan et al., 2016; Liu et al., 2021).*** Although a new generation of less toxic cisplatin analogs such as carboplatin has been introduced ***(Stathopoulos, 2010),*** cisplatin is still particularly effective against solid CNS tumors (e.g., neuroblastoma, osteosarcoma, and some brain tumors) and non-CNS tumors (e.g. leukemia) ***(Ghosh, 2019).***

Platinum-based therapy exerts its principle cytotoxicity by covalently binding DNA purine bases to generate intra-and interstrand crosslinks with subsequent activation of DNA damage that's aggravated by the activation of the tumor suppressor p53, raising of intracellular reactive oxygen species with the subsequent inflammatory response which finally trigger an apoptotic cascade ***(Gorgun et al., 2017; Han et al., 1999).***

Calcium ion signaling is important in neurogenic processes like cell growth, apoptosis, migration, specialization, and synaptogenesis (Lohmann, 2009). However, raised levels of [Ca2+] are participating in neurodegenerative processes and neuropathies ***(Mattson, 2007).*** Cis is binding to sulfhydryl groups of proteins as enzymes, pumps, and channels, altering calcium homeostasis ***(Florea and Büsselberg, 2009)***, it also shows different effects on calbindin (CB) (a calcium buffer

protein) which is essential to handle the unwanted raised intracellular calcium concentration, downregulation of calbindin leads to restricting in activated calcium pump which could damage neuron function and morphology ***(Piccolini et al., 2013).***

Panax ginseng has several valuable properties as immune-modulation, anti-inflammation, lipid-depression, anti-oxidation, anti-diabetes, and anti-tumor ***(Florea and Büsselberg, 2009; He et al., 2018).*** Ginsenosides, are the main derivatives of ginseng ***(Reay et al., 2010)***. In vitro and in vivo, Ginseng and ginsenosides have been utilized to prevent cancer in a variety of experimental settings ***(Ahuja et al., 2018; He et al., 2018).*** The robust anti-inflammatory effect of ginseng is principally mediated by the suppression of IL1β, IL2, IL4, IL6, Cox2, and TNFα cytokines ***(Jung et al., 2013; Yang et al., 2014)***.

Panax ginseng Meyer extract has been long prescribed in ayurvedic Chinese medicine for ameliorating several neurological disorders like amnesia, aging, depression ***(Chen et al., 2014),*** cognitive disorders ***(Chu et al., 2014)***, and degenerative disorders ***(Iqbal et al., 2020)***. The whole ginseng extract comprises additional active constituents including polysaccharides, peptides, polyacetylenic alcohols, and fatty acids ***(Shahrajabian et al., 2019).***

Most of the research about the protective value of ginseng against cisplatin toxic effects tested individual ginsenosides especially Rb1, Rb3, and Rg1 ***(Ong et al., 2015; Iqbal et al., 2020).*** However, the accessibility of the standardized ginseng extract G115 has enabled animal experiments and human medical trials to produce dependable and reproducible results ***(Chen et al., 2020).*** Its safety on neonatal experimental animals with expected safety in children was tested as well ***(Gonzales et al., 2016).***

Aim of the work: This study not only evaluated the structural and functional toxic effects of cisplatin on the early postnatal development of the hippocampus in male albino rats' pups, but also studied the proposed underlying mechanisms implicated in these toxic changes including oxidative stress, inflammatory cytokines, cell apoptosis regulatory proteins, and Ca2+ homeostasis. As well, we investigated the probable neuroprotective role of prior and concomitant administration of concentrated, standardized Ginseng whole extract (G115 100 mg), derived from roots of the highest quality of genuine Panax Ginseng C.A. Meyer, rather than the usual testing of individual ginsenosides against the hippocampal neurotoxicity caused by cisplatin exposure during early neonatal life.

MATERIAL AND METHODS

- **Material:**

I- Chemicals:

Cisplatin (Cis): (1 mg/ml sterile concentrate) was purchased from Sigma-Aldrich Co, Germany, rat were received as a single subcutaneous injection at a dose of 5 mg/kg body weight, dosing according to previous studies (Almutairi et al., 2017, Rezvanfar et al., 2013). **Ginseng** (Gin): **Panax ginseng** (G115) was purchased from EIPICO Pharmaceutical Company, Egypt. Each capsule contains 100 mg of ginseng extract. It was suspended in 1 % carboxy-methylcellulose (CMC) in water and administrated orally by gavage at a dose of 200 mg/kg daily for 10 consecutive days- a safe dosing regimen according to previous studies ***(Kim et al., 1999; Park et al., 2013).***

II- Animals:

They were attained from the Faculty of Medicine's Breeding Animal House and were kept under standard environmental and laboratory conditions with standard rat food. Rats were acclimatized in separate cages (two females and one male in each cage), and matting between them was allowed. 18 females became pregnant and continued pregnancy with a gestational length of about 22 days. Experiments were conducted out in accordance with appropriate guidelines and regulations of the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC committee), with approval number **ZU-IACUC/3/F/148/2021.**

- **Experimental protocol:**

Eighty Offspring (male pups) were allocated randomly into 4 equal experimental groups (20 pups each). (Control group): pups were kept without any treatment; (Gin group): pups were received ginseng orally at a dose of 200 mg/kg from day 7 postnatal till the last day of the experiment; (Cis group): pups received a single subcutaneous injection of Cis at a dose of 5 mg/kg ***(Almutairi et al., 2017; Rezvanfar et al., 2013)*** in the nape at postnatal day (PD) 10; and (Gin/Cis group): pups were received a single subcutaneous injection of Cis at a dose of 5 mg/kg in the nape on the 10^{th} PD plus ginseng at a dose of 200 mg/kg daily for ten consecutive days by gavage from 7^{th} PD. It was intended to start ginseng administration before cisplatin injection and then continued after. Ginseng administration is not recommended before neonatal day 7 ***(Okamura et al., 1994).***

- In all groups, 10 pups from each group at postnatal day PD11 and 10 pups at PD17 (i.e: one day and one week after cisplatin injection) were weighed, deeply anesthetized by intraperitoneal injection of thiopental 50 mg/kg ***(Altun et al., 2019)*** and sacrificed. In each pup, the vault of the skull was rapidly removed; the cerebrum with covering meninges was carefully dissected and divided into two cerebral hemispheres. Half of the samples were immediately immersed and fixed in buffered formalin 10% for 24 h then prepared for histological examination, and the other half were immediately frozen on dry ice and kept at -80°C for tissue homogenate analysis.

- **Ethical approval:** All animal experiments were carried out in accordance with relevant guidelines and regulations of the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC committee), approval number **ZU-IACUC/3/F/148/2021.**

- **Methodology:**

I. Anthropometric measures: Body weight measurement: each animal was put in a closed plastic container and was weighed the day before the experiment (PD 6) and on the last day before sacrifice (PD11 or PD17). For each labeled rat, the results were recorded. After sacrifice, the whole brains were harvested and weighted before dissection (relative brain weight was calculated as "brain weight/body weight %" (b./B. wt.%).

II. Behavioral and general activity test: Open field test: The apparatus composed of a white box (60 x 60 x 40 cm) in which animals were placed singly in the center and given 5 minutes to explore. The amount of time spent in the inner zone and the number of entries were employed as indicators of anxiety-like behavior, after which, rats were returned to their cages and the floor of the box was cleaned with 70% ethyl alcohol and allowed to dry between tests to eliminate olfactory bias. This test assessed horizontal locomotion (number of squares crossed), vertical locomotion (number of rearings), and self-grooming behavior (number of times the rat cleaned its body). Each animal was given two trials with a 30-minute interval and the average time was taken ***(Pan et al., 2006; Stevens et al., 2016).***

II. Homogenate tissue analysis

All tissues were kept at +4 ºC throughout the preparation, the cerebral cortex was removed, and hippocampal tissues were homogenized as the previously described technique by ***Owoeye et al. (2014).***

The preserved homogenate was used for colorimetric assessment of oxidative stress: malondialdehyde (MDA) and superoxide dismutase (SOD) (BioDiagnostic, Giza, Egypt). The apoptotic protein: Rat Tumor Protein p53 (P53). The inflammatory markers: Tumor Necrosis Factor Alpha (TNF-α), Interleukin-1β (IL-1β), and the neurotransmitters acetylcholine (Ach) and gamma-aminobutyric acid (GABA), were assessed by ELISA specific experimental kits (MyBioSource, ELISA Test Kits).

III.Histological and morphometrical analysis (Hematoxylin & Eosin Stain):

For the histological study, hippocampal tissues were preserved in 10% neutral buffered formalin for 24 hours. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene, and embedded in molten paraffin. Parasagittal sections of 5 µm thicknesses were cut by a

rotary microtome and placed on clean slides. For histological examination, sections were stained with hematoxylin and eosin (H&E). For each sample, eight slides were prepared and all the slides were given a similar code or number allowing for blind examination. Slides were examined by the light electric microscope (LEICA ICC50 W), Anatomy Department, Faculty of Medicine, Zagazig University. Assessment Images were analyzed using ImageJ software using 5 different non-overlapping fields from each slide at 400× magnification, and the following parameters were measured: 1) The thickness of the pyramidal cell layer (PCL) in CA1 and CA3 regions and the granular cell layer (GCL) of the dentate gyrus (DG). 2) The total number of vesicular neurons (pyramidal or granular) per field in each region ***(Alsemeh et al., 2020; Barkur and Bairy, 2016).***

The degree of lesions was graded blindly by pathologist according to extension of pathological changes from one to five as follows: 1 = minimal (<1%); 2: slight (1–25%); 3 = moderate (26–50%); 4 = moderate/severe (51–75%); 5 = severe/high (76–100%) ***(Wu et al., 2019).***

III. Immunohistochemical Analysis:

For the Immunohistochemistry study, hippocampal samples were prepared according to ***Piccolini et al., (2013),*** and immunocytochemical labeling was executed by monoclonal polyclonal anti- calbindin-28kD (1:2,000; Sigma) on 5µm thick sections. Eight slides were prepared for each sample and all the slides were given a similar code or number allowing for blind examination. Consequently, the slides were counterstained with Mayer's hematoxylin and equipped with a coverslip. Photomicrographs were taken with a light microscope (at ×400 magnification). ImageJ software was used for the calculation of the area % CB immunoreactivity per field. In addition, the optical density (OD) of CB immunoreactivity was measured in the molecular layer (ML), granular cell layer (GCL), and Polymorphic cell layer (POL) of the DG region, the values correspond to the intensity of the transmitted light and were measured on a scale going from 0 (100% transmitted light) to 3.5 (0% transmitted light) for each pixel ***(Piccolini et al., 2013).*** For all parameters, 5 different non-overlapped fields from each section were examined.

STATISTICAL ANALYSIS

SPSS program (Statistical Package for Social Science) version 18.0 was used for statistical analysis of the biochemical and morphometric data collected. Data were checked for normally distributed and represented in mean ± standard deviation (SD). One-way ANOVA was used to compare the mean values of different groups. Multiple comparisons were estimated by post-hoc test. Furthermore, the t-test was utilised to demonstrate significance between the two age control groups. With 95 percent degrees of freedom, a value of $p<0.05$ was statistically significant, a value of $p<0.001$ was extremely statistically significant, and a value of $p>0.05$ was non-statistically significant ***(Khan and Goel 2021).***

RESULTS

Effect of Cis ± Gin on body and brain weight

At PD11, neonatal rats treated with cisplatin (Cis group) showed a significant decrease in body weight (-12.4%) compared with control and Gin groups, while brain weight and Relative brain weight (b./B. wt. %), the difference remained negligible. Gin consumption before and during cisplatin treatment protected the Gin/Cis group against weight loss **(Table 1).**

While, at PD17, Cis group showed a highly significant decrease in body weight (-22.8%) with a significant decrease in brain weight (-16.3%) and b./B. wt. % (-12.5%) compared with control groups. When comparing the percentage of difference (% of dif.) between PD11 and PD17, the PD17 rats exhibited more bodyweight loss and significant brain weight loss. Consumption of Gin before and parallel with cisplatin in Gin/Cis group resulted in partial bodyweight loss protection and full protection against brain weight loss **(Table 1).**

Table (1): Effect of Cis ± Gin on body and brain weight in different study groups.

Parameter	at postnatal day 11					at postnatal day 17				
	Con. group (n10)	Gin group (n10)	Cis group (n10)	Gin/Cis group (n10)	P value	Con. group (n10)	Gin group (n10)	Cis group (n10)	Gin/Cis group (n10)	P value
Body wt. day before (gm)	14.4±3.1	14.5±2.9	13.5±2.6	12.6±2.8	0.4181	15.1±2.8 [e]	13.7±2.3	14.4±2.7	12.9±2.5	0.2787
Body wt. last day (gm)	19.3±1.7	20.5±4.1	16.9±3.2 [a]	17.2±2.1	0.0265 *	27.1±1.9 [g]	29.4±1.6	20.9±2.7 [b]	24.5±2.1 [ad]	0.0000 **
% Of dif. [#]			-12.4%			40.4%		-22.8%	-9.6%	
Brain weight (gm)	0.69±0.1	0.71±0.05	0.69±0.07	0.69±0.15	0.9593	0.86±0.04 [g]	0.89±0.07	0.72±0.11 [a]	0.78±0.18	0.0074 *
% Of dif. [#]						24.6%		-16.3%		
b./B. wt.%	3.35±0.7	3.57±0.74	4.3±0.8	3.72±0.91	0.0647	3.2±0.29	3.4±0.32	2.8±0.4 [a]	3.1±0.3	0.0026 *
% Of dif. [#]								-12.5%		

[e] non-significant vs PD11, [f] significant vs PD11, [g] highly significant vs PD11.
[#] When significant difference: mean of (Cis or Gin/Cis) group - mean of control)/ mean of control %, mean of control group PD17 - mean of control PD11/mean of control PD11%.Data are expressed as mean±SEM, n = 10.
*One-way ANOVA, P > 0.05: no significant differences, *P < 0.05: significant differences,*
***P < 0.001: highly significant differences. Post-hoc test (significance between different groups):*
[a] significant vs control group, [b] highly significant vs control group.
[c] significant vs Cis group, [d] highly significant vs Cis group t-test: PD11 vs PD17.

- **Effect of Cis ± Gin on Behavioral and general activity test: Open field test:**

Cisplatin significantly reduced locomotor activity (the transitions, rearings, and self-grooming) when compared with the control group. As rats crossed lower squares and had fewer frequencies of rearing and self-grooming. All of the animals' locomotor activity reduced with time. In all groups, it was much greater in the first minute of observation than in later minutes.

When comparing the percentage of the difference between PD11 and PD17, the PD17 rats exhibited more decrease in all locomotor parameters (-52.8% vs -34.2% at PD11 regarding transitions, -59.6% vs -50% at PD11 regarding rearings, and -57.8% vs -48% at PD11 regarding self-grooming). Ginseng supplementation in Gin/Cis group showed partial improvement in locomotor parameters in both PD11 and PD17 groups **(Table 2).**

Table (2): Effect of Cis ± Gin on Behavioral and general activity test: Open field test in different study groups.

Parameter	at postnatal day 11					at postnatal day 17				
	Con. group (n10)	Gin group (n10)	Cis group (n10)	Gin/Cis group (n10)	P value	Con. group (n10)	Gin group (n10)	Cis group (n10)	Gin/Cis group (n10)	P value
No. of transitions [@]	11.4±2.8	12.5±3.2	7.5±1.6 [a]	8.6±2.5 [a]	0.0003 **	17.8±3.4 [g]	20.5±2.8	8.4±1.7 [b]	14.6±4.6 [c]	0.0000 **
% Of dif. [#]			-34.2%	-24.6%				-52.8%	-18%	
No. of rearings [@]	3.2±0.4	2.9±0.8	1.6±0.5 [b]	2.2±0.3 [bc]	0.0000 **	5.2±0.5 [g]	5.9±0.7	2.1±0.8 [b]	4.2±0.7 [ad]	0.0000 **
% Of dif. [#]			-50%	-31.3%				-59.6%	-19.2%	
No. of self-grooming [@]	2.5±0.4	3.1 ±0.7	1.3±0.4 [b]	1.9±0.2 [ac]	0.0000 **	4.5±0.4 [g]	5.2 ±0.8	1.9±0.7 [b]	3.9±0.6 [d]	0.0000 **
% Of dif. [#]			-48%	-24%				-57.8%	-13.3%	

*[@] Per 5 minutes, [#] When significant difference: mean of (Cis or Gin/Cis) group - mean of control)/ mean of control %, mean of control group PD17 - mean of control PD11/ mean of control PD11 %. Data are expressed as mean ± SEM, n = 10. One-way ANOVA, P>0.05: no significant differences, *P<0.05: significant differences, **P<0.001: highly significant differences. Post-hoc test (significance between different groups): [a] significant vs control group, [b] highly significant vs control group. [c] significant vs Cis group [d] highly significant vs Cis group t-test: PD11 vs PD17. [e] non-significant vs PD11, [f] significant vs PD11, [g] highly significant vs PD11*

- **Effect of Cis ± Gin on Oxidative Stress Markers:**
  Evaluation of cisplatin effects on Malondialdehyde (MDA) as a lipid peroxidation marker in hippocampal tissue revealed a significant increase in MDA up to 110% in the PD17 pups vs 58.5% in those of PD11. Moreover, cisplatin's effects on superoxide dismutase (SOD), as an antioxidant marker in hippocampal tissue, revealed a significant decrease in SOD activity up to -42.9% at PD17 vs -15% at PD11. Nevertheless, ginseng supplementation in (Gin group) revealed an increase in SOD activity at PD11 (33.1%). Ginseng supplementation in Gin/Cis group showed improvement in SOD activity and MDA levels in both PD11 and PD17 groups **(Table 3).**
- **Effect of Cis ± Gin on inflammatory and apoptotic Markers:**
  Evaluation of cisplatin effects on TNF-α, IL-1β, and P53 markers in hippocampal tissue revealed that cisplatin treatment significantly increased TNF-α up to 36.8% at PD17 vs 19% at PD11, IL-1β activity up to 70% at PD17 vs 67.5% at PD11, and significantly increased P53 activity up to 96.5% at PD17 vs 98.7% at PD11. Nevertheless, after ginseng supplementation in Gin/Cis group, there were significant differences against the cisplatin group in TNF-α, IL-1β, and P53 levels at PD17, while at PD11, Gin/Cis group showed significant differences against the cisplatin group in IL-1β and P53 levels with an insignificant difference in TNF-α level **(Table3).**
- **Effect of Cis ± Gin on neurotransmitter:**
  Evaluation of cisplatin's effects on the neurotransmitters gamma-aminobutyric acid (GABA) and acetylcholine (ACh) in hippocampal tissue revealed that cisplatin treatment significantly decreased GABA activity up to -39.3% at PD17 vs -19.2% at PD11. Likewise, cisplatin decreased ACh level up to -43.7% in the PD17 group vs -37.8% in the PD11. Nevertheless, GABA and ACh levels in both PD11 and PD17 groups after ginseng supplementation in Gin/Cis group showed significant differences against both control and cisplatin groups **(Table 3).**

Table (3): Effect of Cis ± Gin on oxidative, inflammatory, apoptitic markers, and neurotransmitters in different study groups.

Parameter	at postnatal day 11					at postnatal day 17				
	Con. group (n10)	Gin group (n10)	Cis group (n10)	Gin/Cis group (n10)	P value	Con. group (n10)	Gin group (n10)	Cis group (n10)	Gin/Cis group (n10)	P value
Oxidative markers										
MDA (nmol/g tissue)	11.86±0.86	11.01±0.63	18.8±1.94 b	14.85±1.1 bd	0.0000 **	11.53±0.56	12.09±0.6	24.3±1.3 b	17.78±1.9 bd	0.0000 **
% Of dif. #			58.5%	25.2%				110%	54.2%	
SOD (U/g tissue)	3.41±0.44	4.54±0.42 b	2.9±0.21 a	3.1±0.23	0.0000 **	3.71±0.4	4.23±0.3	2.12±0.44 b	2.98±0.33	0.0000 **
% Of dif. #		33.1%	-15%					-42.9%		
Inflammatory markers										
TNF-α (pg/g tissue)	206.3±19.3	199.2±17.5	245.6±28.4 a	223±20.5	0.0001	241.2±18.6	213.5±15.9	329.9±32 b	281±32.4 ad	0.0000 **
% Of dif. #			19%					36.8%	16.5%	
IL-1B (pg/g tissue)	49.8±6.4	49.6±6.6	82.4±7.1 b	61.8±8.8 a d	0.0000 **	55.2±8.5	57.5±7.1	93.8±9.7 b	61.4±9.6 d	0.0000 **
% Of dif. #			67.5%	24.1%				70%	11.2%	
Apoptotic marker										
P53 (pg/g tissue)	23.2±4.4	21.2±3.7	46.1±6.3 b	31.9±4.9 a d	0.0000 **	28.2±3.6	27.5±4.4	55.4±7.4 b	38.7±3.9 ad	0.0000 **
% Of dif. #			98.7%	37.5%				96.5%	37.2%	
Neurotransmitters										
GABA (pg/g tissue)	109.3±9.7	104.7±11.6	88.3±8 b	94.3±11.3 bd	0.0002 **	123.2±12.6	122.5±14.1	74.8±8.3 b	105.7±15.6 ad	0.0000 **
% Of dif. #			-19.2%	-13.7%				-39.3%	-14.2%	
ACH (nmol/g tissue)	109±13.4	96.3±10.8	67.7±9.07 b	88.2±11.3 ad	0.0000 **	144.3±8.9	127.1±9.5	81.2±9.8 b	102.8±13.1 bd	0.0000 **
% Of dif. #			-37.8%	-19.1%				-43.7%	-28.8%	

 # When significant difference: mean of (Cis or Gin/Cis) group - mean of control)/ mean of control %, mean of control group PD17 - mean of control PD11/ mean of control PD11 %. Data are expressed as mean ± SEM, n = 10.
*One-way ANOVA, P > 0.05: no significant differences, *P < 0.05: significant differences, **P < 0.001: highly significant differences. Post-hoc test was used to show significance between different groups: [a] significant vs control group, [b] highly significant vs control group. [c] significant vs Cis group, [d] highly significant vs Cis group.*

- **<u>Effect of Cis ± Gin on histological examination of hippocampi:</u>**
  Histological examination of hippocampi of the control and Gin groups revealed that the hippocampal formation was formed of the Cornu Ammonis (CA) and dentate gyrus (DG). The CA was further subdivided into four regions (CA1, CA2, CA3, and CA4) (**Figs.1: a1, a2),** CA1 and CA3 regions formed of polymorphic cell layer (POL), pyramidal cell layer (PCL) "the main cell layer contained pyramidal cells with a large rounded vesicular nucleus with prominent nucleoli, tightly packed in CA1 (4-6 layers) and loosely packed in CA3 (2-5 layers)", and Molecular layer (ML) which contained branched apical dendrites of PCL **(Fig. 1: b1, b2 & fig. 2: a1, a2).** Additionally, the dentate gyrus (DG) was observed as a triangular structure with opened concave part directed to Cornu Ammonis. DG consisted of three layers, molecular cell layer (ML), granular cell layer (GCL) contained many densely arranged granule cells with round pale vesicular nuclei, and polymorphic cell layer (POL) **(Figs.3: a1, a2).** In all regions, glial cells were present in different layers either deeply or lightly stained. Comparing the histological structure of hippocampi at PD11 and PD17, the cornu ammonis and dentate gyrus made more external bulging at PD17. Moreover, the thickness of GCL showed a significant increase at PD17, while PCL showed no significant difference between PD11 and PD17 but, no. of vesicular neurons/ field was significantly decreased at PD17 in all regions as granular and pyramidal cells increase in size at PD17 with more prominent apical dendrites **(Figs.1, 2, 3 & Table 4).**
- In comparison with the age-matched controls, histological examination of hippocampi

revealed pathological changes in Cis group at PD11 and PD17, which are more obvious at PD17. Different regions showed that pyramidal and granular cells were fewer, shrunken deeply stained, or swollen; moreover, some cells had pyknotic nuclei, pericellular vacuolations, and empty areas. Additionally, neuropil lost its normal homogeneity with the appearance of vacuolations and dilated blood vessels **(Fig. 1: d1, d2, fig. 2: c1, c2 & fig. 3: c1, c2).**

- The histopathological alterations were confirmed morphometrically, comparing the thickness of PCL/GCL, at PD17, there were a significant decrease (-22.5%) in the thickness of PCL in CA1 and a significant decrease (-13.9%) in the thickness of GCL in DG. Other regions at PD11 or PD17 showed no significant difference in thickness of PCL/GCL. In contrast, there were a significant decrease in total number of vesicular neurons/fields in all regions; CA1 (-51.2% at PD17 vs -15.9% at PD11), CA3 (-39.3% at PD17 vs -34.4% at PD11), and DG (-55.9% at PD17 vs -53.5% at PD11) **(Table 4).**

These histological findings were improved with concomitant usage of Gin in Gin/Cis group, as at PD11 and PD17, most pyramidal and granular cells gained their normal structure having large rounded vesicular nuclei with prominent nucleoli, but few shrunken or deeply stained cells and dilated congested blood vessels were still present **(Fig. 1: e1, e2, fig. 2: d1, d2 & fig. 3: d1, d2).** This histological improvement was confirmed morphometrically in different regions **(Table 4).**

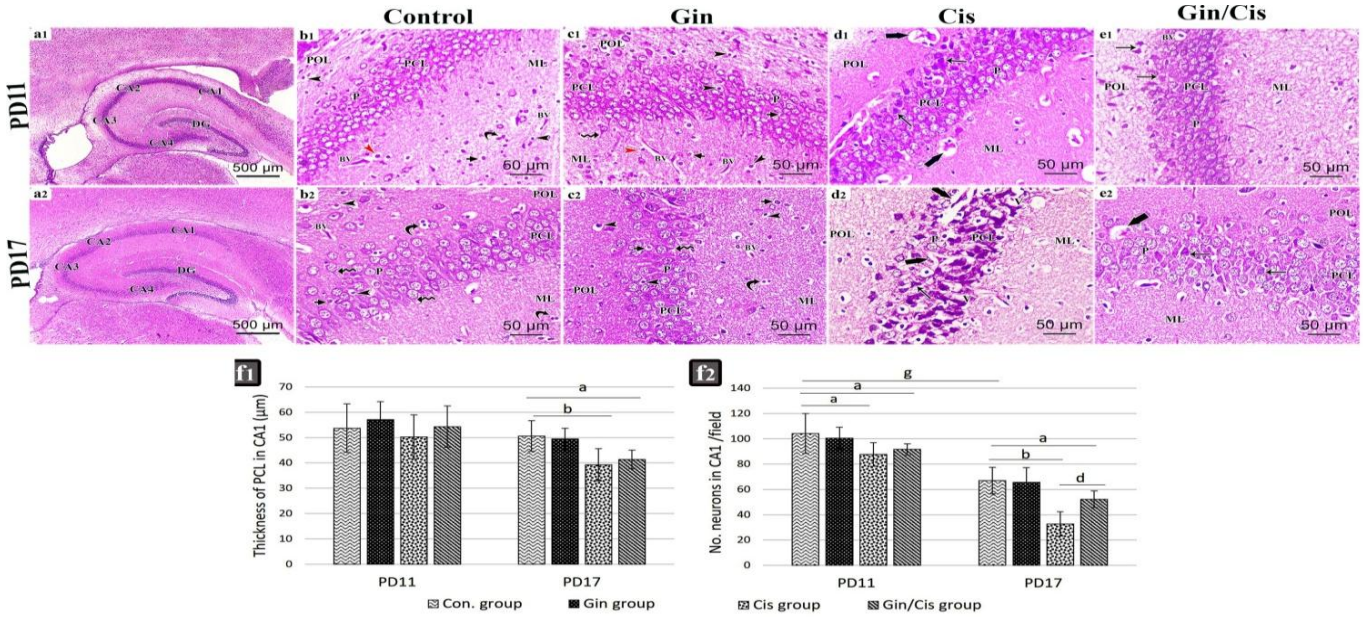

Fig. (1): *H & E-stained sections of hippocampal formation at PD11 (**a1**) and PD17 (**a2**) are showing that the hippocampus is formed of the Cornu Ammonis (CA) and dentate gyrus (DG). The CA is further subdivided into four regions (CA1, CA2, CA3 and CA4). CA1 of **control** and **Gin** groups at PD11 (**b1, c1**) and PD17 (**b2, c2**) are showing; polymorphic layer (POL), pyramidal cell layer (PCL) containing tightly packed pyramidal cells (P) with large rounded vesicular nuclei and prominent nucleoli and Molecular layer (ML) containing branched apical dendrites of PCL (zigzag arrow). Dark (arrow heads) or lightly (short arrows) stained glial cells with characteristic perinuclear halos are present in different layers and perivascular (red arrowhead). Glial mitotic cell divisions (curved arrows) and blood vessels with narrow perivascular halos (BV) are also seen. CA1 of **Cis** group at PD11 (**d1**) and PD17 (**d2**) are showing many shrunken pyramidal cells with darkly stained pyknotic or elongated nuclei (thin arrows) which are more obvious at PD17. Also, there are normal pyramidal cells (P) with lightly stained nuclei which are fewer at PD17 and dilated congested blood vessels (thick arrows). At PD17, pericellular vacuolations and unoccupied areas (V) are evident at PCL. CA1 of **Gin/Cis** group at PD11 (**e1**) and PD17 (**e2**) are showing many normal pyramidal cells (P) with large rounded vesicular nuclei and prominent nucleoli but few shrunken and deeply stained pyramidal cells (thin arrows) are also present. At PD17, dilated congested blood vessels (thick arrow) are still present (H&E, Bar= 50 µm &500 µm).* ***f1&f2:*** *Morphometrical comparing between thickness of PCL and total number of vesicular neurons/fields of CA1 at PD11 and PD17. Data are expressed as mean ± SEM, n = 10. [a] significant vs control group, [b] highly significant vs control group, [c] significant vs Cis group, [d] highly significant vs Cis group, [f] significant vs PD11 and [g] highly significant vs PD11.*

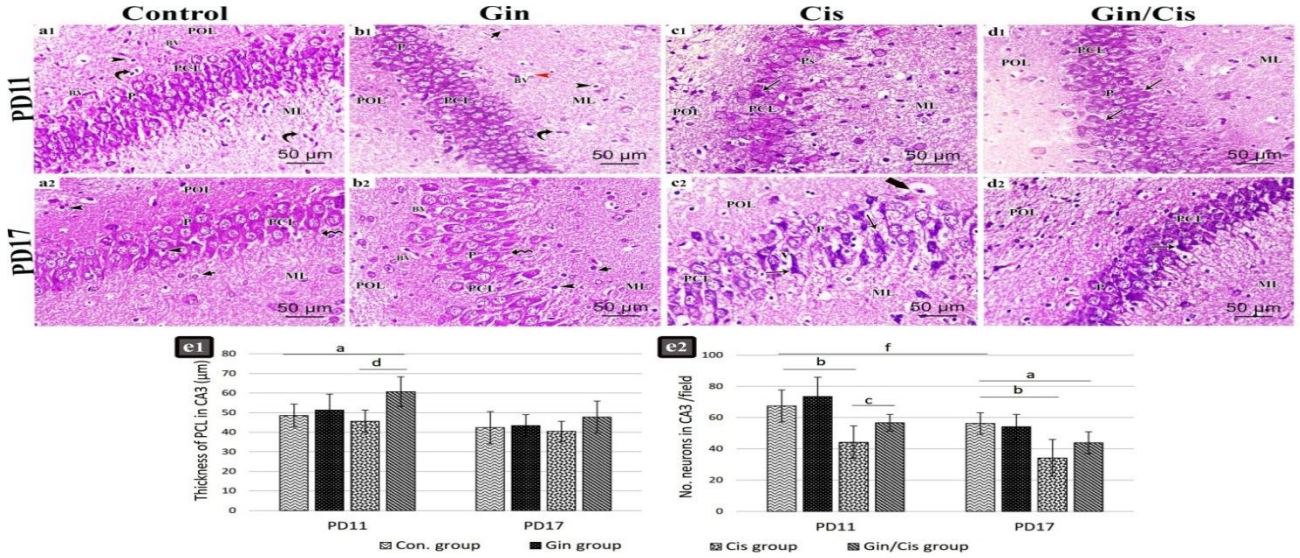

Fig. (2): *H&E-stained sections of CA3 of hippocampal formation at PD11 and PD17. CA3 of C**ontrol** and **Gin** groups at of PD11 (**a1, b1**) and PD17 (**a2, b2**) are showing; polymorphic layer (POL), pyramidal cell layer (PCL) containing tightly packed pyramidal cells (P) with large rounded vesicular nuclei and prominent nucleoli and Molecular layer (ML) containing branched apical dendrites of PCL (zigzag arrow). Dark (arrow heads) or lightly (short arrow) stained glial cells with characteristic perinuclear halos are present in different layers and perivascular (red arrowhead). Glial mitotic cell divisions (curved arrows) and blood vessels with narrow perivascular halos (BV) are also seen. CA3 of **Cis** group at PD11 (**c1**) and PD17 (**c2**) are showing neuropil of POL and ML layers losing its normal homogeneity with appearance of vacuolations and dilated congested blood vessels (thick arrow). At PD11, most pyramidal cells (Ps) are swollen and other is pyknotic (thin arrow). At PD17, there are many shrunken distorted pyramidal cells with darkly stained pyknotic or elongated nuclei (thin arrows), few normal pyramidal cells (P) pericellular vacuolations and unoccupied areas (V). CA3 of **Gin/Cis** group at PD11 (**d1**) and PD17 (**d2**) are showing many normal pyramidal cells (P) but few shrunken and deeply stained pyramidal cells (thin arrows) are also present (H&E, Bar= 50µm). **e1&e2:** Morphometrical comparing between thickness of PCL and total number of vesicular neurons/fields of CA3 at PD11 and PD17. Data are expressed as mean ± SEM, n = 10. [a] significant vs control group, [b] highly significant vs control group, [c] significant vs Cis group, [d] highly significant vs Cis group, [f] significant vs PD11 and [g] highly significant vs PD11.*

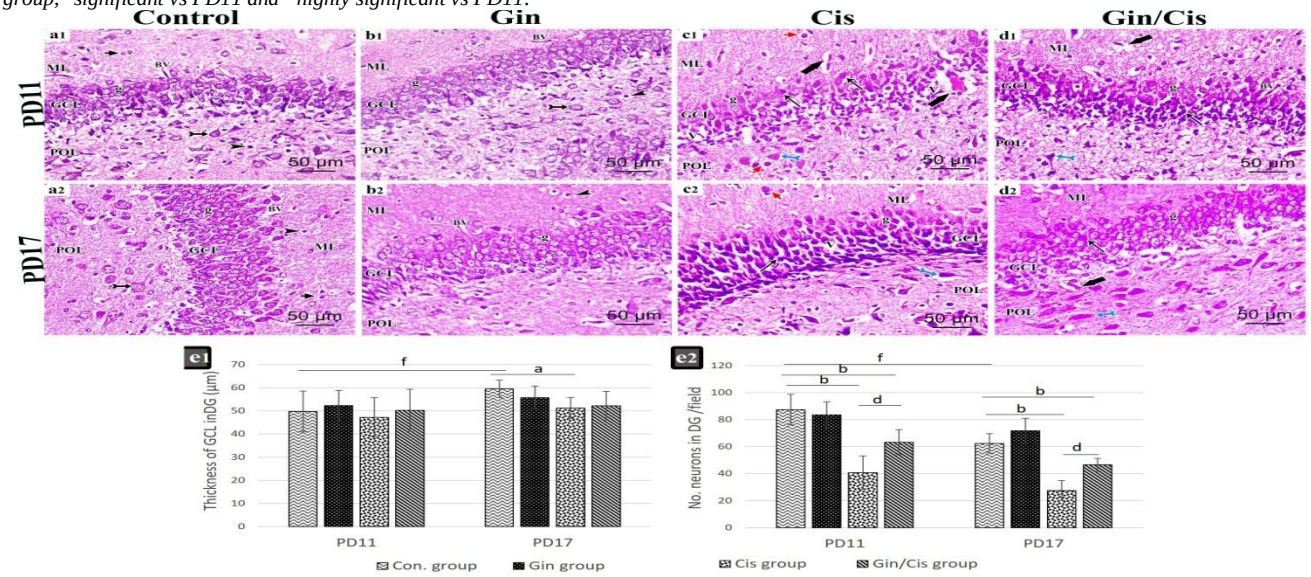

Fig. (3): *H&E-stained sections of DG of hippocampal formation at PD11 and PD17. DG of C**ontrol** and **Gin** groups at of PD11 (**a1, b1**) and PD17 (**a2, b2**) are showing; polymorphic layer (POL) containing mossy neurons with lightly stained nuclei (bifid arrow), granular cell layer (GCL) containing compactly arranged granule cells (g) with rounded pale vesicular nuclei and Molecular layer (ML). Glial cells with dark (arrow head) or lightly (short arrow) stained nuclei with characteristic perinuclear halo are present in different layers. Blood vessels (BV) with narrow perivascular halos are also seen. DG of **Cis** group at PD11 (**c1**) and PD17 (**c2**) are showing many shrunken granule cells with darkly stained pyknotic nuclei (thin arrows) especially in deep layers and more obvious at PD17. Also, there are normal granule cells (g) with lightly stained nuclei which are fewer at PD17. Pericellular vacuolations and unoccupied areas (V) are evident at GCL. Neuropil of ML and POL is losing its normal homogeneity and having dilated congested blood vessels (thick arrows) and giant cells (red short arrow). Some mossy neurons in POL are distorted having dark stained nuclei (blue bifid arrow). DG of **Gin/Cis** group at PD11 (**d1**) and PD17 (**d2**) are showing many normal granule cells (g) with lightly stained nuclei but some shrunken and deeply stained granule cells (thin arrow) are also present. Blood vessels are either normal with narrow perivascular hallo (BV) or dilated congested (thick arrow). Dark stained mossy neurons are still present in POL (blue bifid arrow) (H&E, Bar= 50 µm). **e1&e2:** Morphometrical comparing between thickness of GCL and total number of vesicular neurons/fields of DG at PD11 and PD17. Data are expressed as mean ± SEM, n = 10. [a] significant vs control group, [b] highly significant vs control group, [c] significant vs Cis group, [d] highly significant vs Cis group, [f] significant vs PD11 and [g] highly significant vs PD11.*

Table (4): Morphometric analysis for H&E and immunohistological examination in different study groups.

	at postnatal day 11					at postnatal day 17				
Parameter	**Con. Group (n10)**	**Gin group (n10)**	**Cis group (n10)**	**Gin/Cis group (n10)**	**P value**	**Con. group (n10)**	**Gin group (n10)**	**Cis group (n10)**	**Gin/Cis group (n10)**	**P value**
Thickness of PCL in CA1 [μ]	53.7±9.6	57.1±7.1	50.3±8.7	54.3±8.2	0.3648	50.6±6.1 e	49.5±4.2	39.2±6.4 b	41.3±3.7 a	0.0000 **
% Of dif. [#]								-22.5%	-16.6%	
Thickness of PCL in CA3 [μ]	48.5±5.8	51.3±8.2	45.6±5.7	60.7±7.6 ad	0.0001 **	42.3±8.2 e	43.3±5.6	40.4±5.2	47.7±8.2	0.1337
% Of dif. [#]				25.2%						
Thickness of GCL in DG [μ]	49.7±8.8	52.3±6.5	47.2±8.5	50.2±9.1	0.5948	59.5±3.8 f	55.7±5	51.2±4.5 a	52.2±6.2	0.0025 *
% Of dif. [#]						4.5%		-13.9%		
No. vesicular neurons in CA1 [&]	104.2±15.7	100.5±8.6	87.6±9.3 a	91.6±4.5 a	0.0031 *	67±10.4 g	65.6±11.6	32.7±9.6 b	52.2±6.5 ad	0.0000 **
% Of dif. [#]			-15.9%	-12.1%		-35.7%		-51.2%	-22.1%	
No. vesicular neurons in CA3 [&]	67.5±10.2	73.5±12.3	44.3±10.4 b	56.7±5.3 c	0.0000 **	56.3±6.9 f	54.2±7.8	34.2±11.8 b	43.9±7.1 a	0.0000 **
% Of dif. [#]			-34.4%	-16%		-16.6%		-39.3%	-22%	
No. vesicular neurons in DG [&]	87.5±11.4	83.5±9.8	40.7±12.4 b	63.3±9.3 bd	0.0000 **	62.4±7.3 f	71.8±9.2	27.5±7.4 b	46.6±4.8 bd	0.0000 **
% Of dif. [#]			-53.5%	-27.7%		-28.7		-55.9%	-25.3%	
Pathological score	1±0	1±0	2.7±1.3 b	1.9±1.2	0.0002 **	1±0	1±0	3.2±1.2 b	1.7±0.8 d	0.0000 **
Are % Calbindin	39.7±11.8	42.5±9.6	10.7±3.9 b	27.4±15.4 c	0.0000 **	60.3±9.5 g	62.8±10.7	37.8±6.8 b	41.4±8.5 b	0.0000 **
% Of dif. [#]			-73%	-31%		51.9%		-37.3	-34.1%	
Calbindin OD: ML/DG	0.82±0.13	0.71±0.09	0.52±0.13 b	0.68±0.24	0.0017 *	1.47±0.12 g	1.36±0.34	0.84±0.16 b	1.15±0.21 ac	0.0000 **
% Of dif. [#]			-36.6%			79.3%		-42.9%	-21.8%	
Calbindin OD: GCL/DG	2.15±0.56	2.07±0.2	0.54±0.13 b	1.43±0.9 ac	0.0000 **	3.06±0.75 f	3.1±0.69	2.65±0.8	2.46±0.43	0.1165
% Of dif. [#]			-74.9%	-33.5%		42.3%				
Calbindin OD: POL/DG	0.43±0.14	0.37±0.05	0.2±0.07 b	0.31±0.1 ac	0.0000 **	1.24±0.27 g	1.27±0.19	0.92±0.28 a	1.03±0.3	0.0136 *
% Of dif. [#]			-53.5%	-27.9%		188.4%		-25.8%		

*[μ] Length in μ. [#] When significant difference: mean of (Cis or Gin/Cis) group - mean of control)/ mean of control % and mean of control group PD17 - mean of control PD11/ mean of control PD11 %. [&]Vesicular cells Per 400 field, PCL: pyramidal cell layer, GCL: granular cell layer, CA: Cornu Ammonis, DG: dentate gyrus, ML: molecular layer, Pol: Polymorphic cell layer. Data are expressed as mean ± SEM, n = 10. [1]One-way ANOVA, P > 0.05: no significant differences, *P < 0.05: significant differences, **P<0.001:highly significant differences. [2]Post-hoc test was used to show significance between different groups: [a] significant vs control group [b] highly significant vs control group. [c] significant vs Cis group, [d] highly significant vs Cis group. [3] t-test was used to show significance between control group: PD11vs PD17. [e] non-significant vs PD11, [f] significant vs PD11, [g] highly significant vs PD11.*

- **Effect of Cis ± Gin on Calbindin immunoreactivity and morphometrical analysis**:

At PD11 and PD17, DG of control and Gin groups showed progressive expression of CB in granule cells of GCL (especially in external layers) and their dendrites in ML. Additionally, at PD17, CB was expressed at POL. DG of Cis groups showed poor expression of CB, while Cis/Gin groups showed moderate CB expression. Moreover, CB labeling intensity was increased at PD17 versus PD11 **(Fig. 4).** Morphometrical analysis area % CB immunoreactivity showed a significant increase at PD17 (51.9%) Vs PD11 and control groups, in addition to a significant decrease in the Cis

group (-73% at PD11 vs -37.3% at PD17). Calbindin optical density (OD) evaluation showed the distribution of the OD values in the DG of PD11 and PD17. The intensity of CB labeling decreased after Cis treatment, especially in the granule cells (-74.9%, -42.9% at PD11 and PD17 respectively). These morphometrical findings were improved with concomitant usage of Gin in Gin/Cis group **(Fig. 4 and Table 4).**

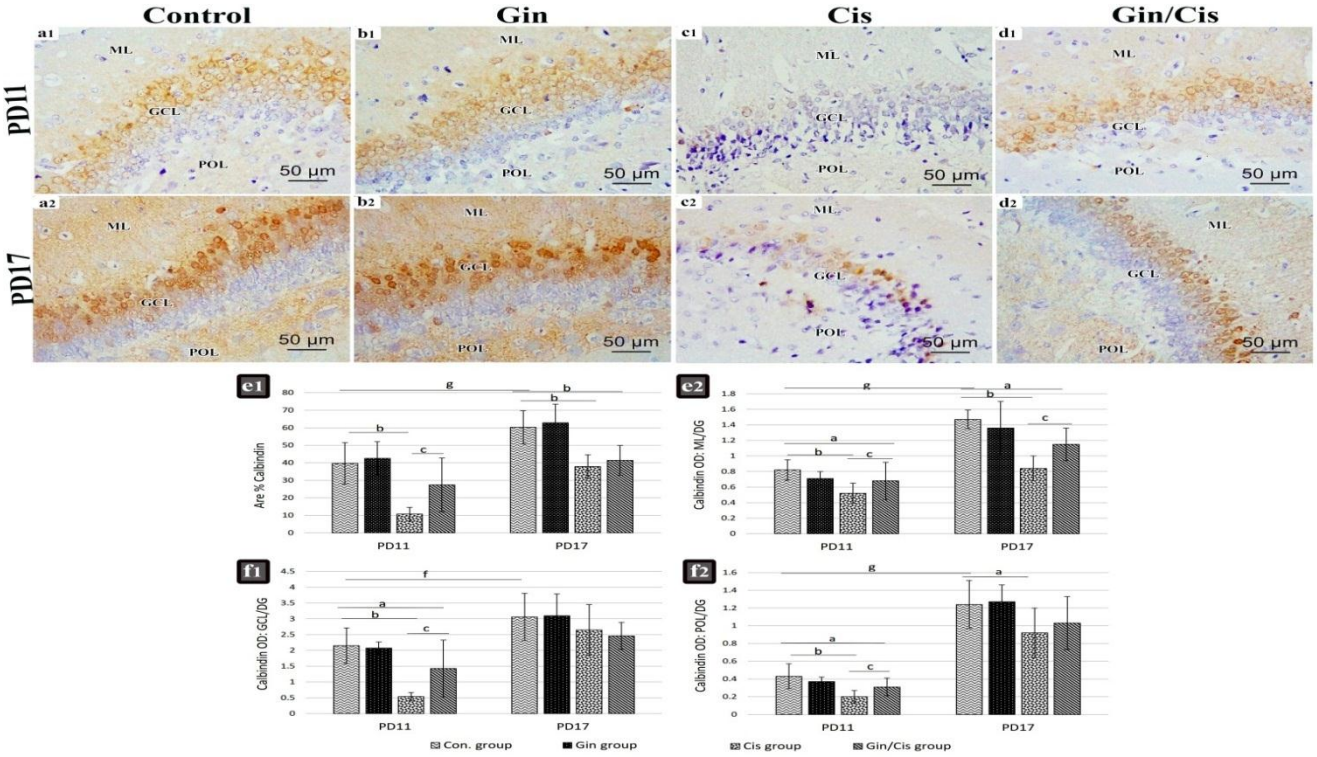

Fig. (4): *Calbindin (CB) immuno reactivity in hippocampal DG at PD11 and PD17 at different groups: Positive stained granule cells are taking brown color while negative stained granule cells are taking blue color. (Scale bar =50 µm).* ***e1&e2:*** *Morphometrical comparing between area% of calbindin immunoreactivity in hippoampal DG at PD11 and PD17.* ***f1&f2****: calbindin optical density in GCL of hippoampal DG at PD11 and PD17. Data are expressed as mean ± SEM, n = 10.* [a] *significant vs control group,* [b] *highly significant vs control group,* [c] *significant vs Cis group,* [d] *highly significant vs Cis group,* [f] *significant vs PD11 and* [g] *highly significant vs PD11.*

DISCUSSION

Cisplatin is an effective antitumor chemotherapeutic drug that was shown to cross the blood-brain barrier, suppressing neurogenesis and causing neuronal damage, a toxic effect that is more evident in immature CNS than in adult one ***(Andres et al., 2014, Son et al., 2015).*** Increased oxidative stress, proinflammatory cytokines, mitochondrial malfunction, DNA damage, and apoptotic cell death are all involved in cisplatin toxicity in the hippocampal and cerebellar areas with resultant disrupted neurotransmitter activity plus axonal shrinkage and demyelination as examples of morphological alterations in neurons***. (Saral et al., 2021).*** On the other hand, Korean Red Ginseng (Panax ginseng C.A. Meyer) has been found as a particular neuroprotective supplement by being an anti-inflammatory agent, significantly decreasing the mRNA expression of TNF-α, IFN-γ, interleukin (IL)-1β, and IL-6, acting as an antioxidant, modulating neurotransmitter activity, and suppressing the apoptotic pathways ***(Iqbal et al., 2020)*** with the extract no observed adverse effect level (NOAEL) of 2,000 mg/kg/day for even a 4-week duration exposure ***(Park et al., 2013).***

While several ginseng extract derivatives, mainly the ginsenosides, have proven their protective action against different neurological disorders ***(Chen et al., 2019b; Ong et al., 2015)*** including cisplatin-induced neurotoxicity ***(Chen et al., 2019a)***; and the whole Korean

ginseng extract has shown effective amelioration of several neurological injuries ***(Iqbal et al., 2020)*** and disorders ***(Gonzales et al., 2016)***, the effect of the whole extract of Korean ginseng (Panax ginseng C.A. Meyer) on cisplatin-induced neurotoxicity, particularly in pediatric developing hippocampus, hasn't yet been examined. Therefore, we developed an animal model of pediatric cisplatin-induced hippocampal injury to evaluate its functional and structural deleterious effects revealing the possible underlying mechanisms, and used the whole extract of Panax ginseng C.A. Meyer to control this cisplatin neurodevelopmental toxicity in early childhood.

Exposing 10-day old male pups to a single dose of Cis injection was done and then evaluated its toxic actions on the rats' general condition and development of hippocampi after one day (PD11) and seven days (PD17) of injection, we also investigated the probable protection of the pre and concomitant treatment with Panax ginseng C.A. Meyer extract. Because rats at postnatal days 7–10 are almost equal to a term human child in developmental brain damage models, the best time to administer the harmful insult to babies is on PD10 and later. Rats at PD17 are commonly used to imitate toddler disease since this is the age when the brain has the most synapses and cerebral blood flow ***(Semple et al., 2013).***

The most sensitive predictor of toxicant adverse effects is frequently body weight ***(Salih and Al-Baggou, 2020)***. In this study, At PD11 and PD17, there was a significant decrease in body weight of Cis groups compared with the control and Gin groups. These results were in concordance ***with Mokhtar et al. (2022)*** and also studies done on adult rats ***(Ko et al., 2019).*** The weight loss induced by Cis may be caused by decreased appetite ***(Hesketh et al., 2003),*** diarrhea and toxicity of the gastrointestinal tract ***(Owoeye et al., 2018),*** alteration of lipid metabolism in the liver ***(Garcia et al., 2013),*** or excessive degradation of tissue proteins ***(Salih and Al-Baggou, 2020).*** While consumption of Gin before and parallel with cisplatin in Gin/Cis group led to partial protection from this weight loss. These results match those of a study done by ***Lobina et al. (2014)*** where Gin protected rats against cisplatin-induced cachexia.

In general, a change in organ weight after exposure to a chemical indicates that this chemical is hazardous ***(Owoeye et al., 2018).*** In the current study at PD17, Cis group showed a significant decrease in brain weight and brain weight/body weight % compared with control groups. While at PD11, brain weight and brain weight/body weight % remained insignificantly different which may be due to the short duration between Cis injection and sacrifice.

Open-field tests at PD11 and PD17 in Cis groups showed a decrease in all locomotor parameters regarding transitions, rearing, and self-grooming. This is due to reduced nervous and muscular activities as reported by ***Akman et al. (2015); Ali et al. (2014) and Pan et al. (2006).*** Ginseng supplementation in Gin/Cis group showed partial improvement in locomotor parameters in both PD11 and PD17 groups. This is parallel to results reported by ***Lee et al. (2002)*** as Gin prevents neuronal cell death.

Because the brain has a significantly larger quantity of the iron deposit and lipid content of myelin sheaths, a high rate of oxidative metabolism, and a limited antioxidant capacity, it is especially prone to oxidative damage caused by many medications and poisons, including cisplatin ***(Uttara et al., 2009).*** Our results showed that cisplatin significantly increased the oxidative stress markers in the form of elevated lipid peroxidation product MDA levels and reduced the SOD antioxidant enzyme activity in hippocampal tissue homogenate; this was parallel with the results reported by ***Oz et al. (2015).*** These findings in the Cis group are a direct result of Cis-induced oxidative stress with subsequent exhaustion of the cellular enzymatic antioxidant system. Oxidative stress plays a pivotal role in the Cis-related anticancer effect and, as well, its induced neurotoxicity ***(Abdel-Wahab and Moussa, 2019; Arafa and Atteia, 2020).***

Besides oxidative stress, there was a significant increase in the inflammatory mediators as tumor necrosis factor-α (TNF-α) which was found before by ***Almutairi et al. (2017) and Zaki et al. (2018)*** in neural tissues, and in other tissues like kidneys ***(Alhoshani et al., 2017).*** Another inflammatory cytokine, interleukin-1β (IL-1β), was found significantly high after Cis treatment parallel to previous studies ***(Arafa and Atteia, 2020).*** Cisplatin was reported to induce oxidative stress together with elevated both TNF-α and IL-1β in neural tissues by several studies ***(Chen et al., 2019a; Saral et al., 2021),*** and this is, indeed, the main mechanism of its both therapeutic and toxic actions. Oxidative stress strongly stimulates inflammatory-related pathways implicated in Cis damage with the release of vital proinflammatory cytokines including TNF-α, and IL-1β that are involved in the initiation of the apoptosis cascade ***(Abdel-Wahab and Moussa, 2019; Liu et al., 2022).*** Cisplatin caused an increase in inflammatory cytokines especially TNF-α and IL-1β, with a decrease in antioxidant enzyme activities (SOD) in other tissues rather than the brain like kidneys ***(Wei et al., 2021),*** testes ***(di Pietro et al., 2012***), and intestines ***(Zhang et al., 2020).***

The apoptotic marker, tumor suppressor protein p53 was also found to be significantly elevated in the Cis group of rats and this Cis-related effect was reported before in the brain tissues ***(Tsuchida et al., 2020)*** in addition to other organs such as kidneys ***(El-Kashef and Sharawy 2018; Hasan et al., 2021)*** and intestines ***(Zhang et al., 2020).*** It has been proved that the cisplatin cytotoxic effect is mediated mainly by activating the p53 apoptotic pathway through induced oxidative stress ***(Jiang et al., 2007)*** and increased inflammatory mediators, like IL-1β and TNF-α ***(Hen et al., 1999).***

As a result of the previous oxidative, inflammatory and apoptotic events, Cis induces disrupted hippocampal neurotransmission. Our study revealed decreasing both ACh and GABA levels in hippocampal tissue homogenate of Cis-treated rats. Several other authors in the literature showed similar Cis-induced elevated oxidative stress markers, elevated inflammatory mediators TNF-α and IL-1β, and the decreased acetylcholine level due to elevated acetylcholine esterase enzyme activity and triggered peroxidation ***(Chen et al., 2019a; Saral et al., 2021)*** with resultant impaired cognitive and behavioral activities. However, ACh was reported to be paradoxically elevated due to decreased acetylcholine esterase enzyme activity by ***Arafa and Atteia (2020); Oz et al. (2015).*** Cis group showed also a decreased GABA level which is parallel with the findings of previous studies ***(Hasan et al., 2021).***

Both Ach and GABA are of special importance in studying the neurophysiological functions and neuropathological changes of the hippocampus, that's why evaluation of their level changes is essential for detecting Cis neurodevelopmental toxicity. It's well established that ACh is the main excitatory transmitter in the brain and GABA is the main inhibitory transmitter and both affect the main hippocampal function of memory and learning ***(Ghafari et al., 2017).*** Both ACh and GABA interfere with the action of each other and it's recommended to be evaluated together; GABA causes tonic inhibition of ACh release controlling network excitability responsible for the oscillatory behavior. In turn, GABAergic interneurons get cholinergic innervation from the basal forebrain's medial septum-diagonal band complex ***(Pepeu and Blandina, 1998).***

Cholinergic system deficiencies resulted in memory and cognitive function impairment ***(Acquas et al., 1996)*** which are particularly relevant in defining cisplatin neurodevelopmental toxicity. In addition, ACh acts as a neurodevelopmental growth factor and plays calcium-mediated actions such as neurotransmitter release, signal transduction cascade modulation, cell survival, and apoptosis. Arousal of cortical and hippocampal ACh is connected with stress, fear, novelty, and learning processes, irrespective of changes in motor activity ***(Dashniani et al., 2020).*** Cisplatin, through its effects on

neurotransmitters and cognitive function of the developing brain, was found to be a useful tool to study CNS development and understand how hippocampal neuronal networks react to an injury, which gives particular importance to our study on developing male pups during their early postnatal life.

Our results showed that prior and coadministration of oral whole Panax ginseng extract, at a dose of 200 mg/kg ameliorated Cis-induced oxidative stress, inflammatory cytokine rise, and apoptotic pathway activation in hippocampal tissue homogenate.

The antioxidant effect of ginseng was reported before against cisplatin neurotoxicity ***(Chen et al., 2019a)*** and other neurotoxic insults ***(Chen et al., 2014)***. Panax ginseng was intentionally administered before the cisplatin to increase the bioavailability of the antioxidants augmenting the anti-oxidative capacity of the animals at the time of cisplatin exposure. This antioxidant effect of ginseng is closely related to its ginsenoside content. Ginsenosides can protect the outer membrane of mammalian cells by intercalating into the plasma membrane, changing its fluidity, affecting its function, and inhibiting lipid peroxidation by chelating transition metals, scavenging free radicals, and restoring the GSH ***(Kang et al., 2007).***

Both cisplatin's anticancer effect and related organ toxicity are mainly ROS-dependent. However, the use of natural antioxidants, like Panax ginseng C.A. Meyer extract, is believed not to decrease the antitumor efficacy of Cis. ROS levels are generally higher in cancer cell lines than in noncancer cell lines because of a higher metabolic release of ROS or a damaged ROS control system which makes them more susceptible to oxidative stress effects reducing their proliferation rate ***(Mailloux et al., 2013).*** Moreover, mitochondrially-targeted antioxidants, including natural ginseng extract, act as a novel therapeutic approach against cisplatin cytotoxicity without affecting its efficacy; in contrast, they have potential therapeutic antitumor action ***(Mukhopadhyay et al., 2012).***

In addition to being an antioxidant agent, Panax ginseng extract decreased the level of Cis-induced elevated inflammatory mediators, TNF-α and IL-1β, the apoptotic marker p53, and improved the depressed levels of ACh and GABA neurotransmitters in the Cis/Gin group of pups. Similarly, pretreatment with Korean red ginseng was found to downregulate the expression of the proapoptotic protein p53 ***(Cuong et al., 2015).*** Ginseng extract treatment with the same dose used in this study was found to significantly increase the GABA and Ach levels near control values in the cerebral cortex and hippocampus which were exposed before to a neurotoxicant substance ***(Al-Hazmi et al., 2015).***

Regarding postnatal development of the hippocampus, previously published data proved that the interneurons and pyramidal cells of CA increased at prenatal period E12–E19 but differentiated especially GABAergic cells at PD5-PD15, hence the value of hippocampal GABA assessment performed in this study, moreover, the granule cells in the DG increased postnatally up to PD25 ***(Danglot et al., 2006).***

Current histological examination of hippocampi in the control group showed that the hippocampus formed of two main parts the Cornu Ammonis (CA) and dentate gyrus (DG). each of these regions consisted of: the pyramidal cell layer (PCL) is the main cell layer in CA containing pyramidal cells with a large rounded vesicular nucleus with prominent nucleoli, tightly packed in CA1 (4-6 layers) and loosely packed in CA3 (2-5 layers), granular cell layer (GCL) is the main cell layer in DG contained many densely arranged granule cells with round pale vesicular nuclei, these data coped with the histological examination in previous studies ***(Mete et al., 2021).*** Comparing hippocampi histology on PD11 and PD17, the cornu ammonis and dentate gyrus increased in length by age and made more external bulging, granule cells, as well as pyramidal cells, increase in size at PD17 with more prominent apical dendrites, similar results were described by ***(Kamel et al.,***

2015). In comparison with the age-matched controls, histological examination of hippocampi revealed pathological changes in the Cis group at PD11 and PD17, which are more obvious at PD17. Different regions showed that pyramidal and granular cells were fewer, shrunken deeply stained, or swollen, moreover, some cells had pyknotic nuclei, pericellular vacuolations, and empty areas. These results were parallel with the study done by ***Piccolini et al., (2013)*** who found a significant increase in the number of shrunken cells in principal layers of CA3 and DG, at PD11 and PD17 of Cis groups compared to control groups.

Additionally, neuropil lost its normal homogeneity with the appearance of vacuolations and dilated blood vessels, this results parallel with ***Owoeye et al., (2018)*** who reported that structural damage induced by Cis was evident on different hippocampal regions but more in the DG and CA3 than in the CA1 of the treated rats. While, those results didn't completely match with another study ***(Piccolini et al., 2013)*** where authors reported no pathological alterations occurred in CA1 at PD11. Furthermore, those histopathological and morphometrical changes induced by Cis were improved with concomitant usage of Gin in the Gin/Cis group, as at PD11 and PD17, most pyramidal and granular cells gained their normal structure having large rounded vesicular nuclei with prominent nucleoli, but few shrunken or deeply stained cells and dilated congested blood vessels were still present. This improvement in Gin/Cis groups suggests the neuroprotective effect of Gin. These results were in line with ***Chen et al. (2019b)*** who proved the ameliorative effect of Gin against Cis toxicity in adult rats.

Calcium ion signaling plays a vital role in neurogenesis such as cell proliferation, migration, differentiation, and synaptogenesis. However, the elevation of its level is incorporated in neuronal degeneration and neurological disorders. CB (28 kDa) is a calcium-binding protein ***(Nägerl et al., 2000)*** present in main hippocampal cells (granule and pyramidal) and in interneurons of the hippocampal formation ***(Ábrahám et al., 2009).*** CB is believed to be important in the regulation of calcium-dependent activities of developing neurons. It inhibits excessive calcium influx in neurons, which would otherwise promote degenerative processes ***(Maskey et al., 2010).*** Interfering of the intracellular Ca2+ homeostasis may cause ionic imbalance and subsequent cell death ***(Liu and Fechter, 1996).*** At very early postnatal stages, CB is expressed in dentate granule cells while in the adult hippocampus; it is present mainly in dentate granule cells and also found in pyramidal cells and some interneurons ***(Freund and Buzsaki, 1996).*** In the current study, at PD11 and PD17, CB was progressively expressed in control and Gin groups and poorly expressed in Cis groups. Moreover, CB labeling intensity was increased at PD17 versus PD11. Additionally, CB expression in the granular layer of DG was in an outside-in pattern as explained by ***Ábrahám et al., (2009).*** While in Cis/Gin groups there was moderate CB expression, indicating preservation of CB by the neuroprotective effect of Gin against Cis neurotoxicity ***(Hussien and Yousef 2021)*** proving that Gin is counteracting the neurotoxicity caused by cisplatin via reducing oxidative stress and neuro-inflammation and restoring the neurological efficiency.

Individual types of isolated ginsenoside treatment had significantly decreased Cis-induced biochemical and histopathological features of neurotoxicity and the Panax ginseng extract was recently found to ameliorate Cis chronic neurotoxic effects in adult rat hippocampus ***(Hussien and Yousef 2021)***, but, to our knowledge, our study is the first assessing the effect of cisplatin on the function and structure of the immature developing hippocampus and demonstrating the protective effect of using the commercially available whole Panax ginseng C.A. Meyer extract, which can be a preclinical study for future application in infantile and pediatric cancer cases.

CONCLUSION

In conclusion, our findings indicate that despite cisplatin use being essential in the treatment of many cancer cases, it's associated with marked neurotoxicity, especially in early childhood exposure, that is reflected by the general physical condition and behavioral disturbance. This central neurological malfunction occurs due to several pathological pathways that involve both structural and biochemical neural disruption. Moreover, our results add new evidence for the ameliorative effects of Panax ginseng (Korean red ginseng) extract on these different pathological mechanisms played by cisplatin to induce its neurotoxic effect and emphasize the need for more scientific and clinical investigations to determine the therapeutic scope of ginseng as a single or adjunctive supplement treatment for cancer patients suffering from neurotoxic effects.

RECOMMENDATIONS

Further investigations of concomitant use of cisplatin and Ginseng whole extract (G115 100 mg) on cancerous cell lines either in vivo or in vitro are recommended to investigate the attenuation of cisplatin adverse effects balanced with the possible attenuation of its therapeutic effect.

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